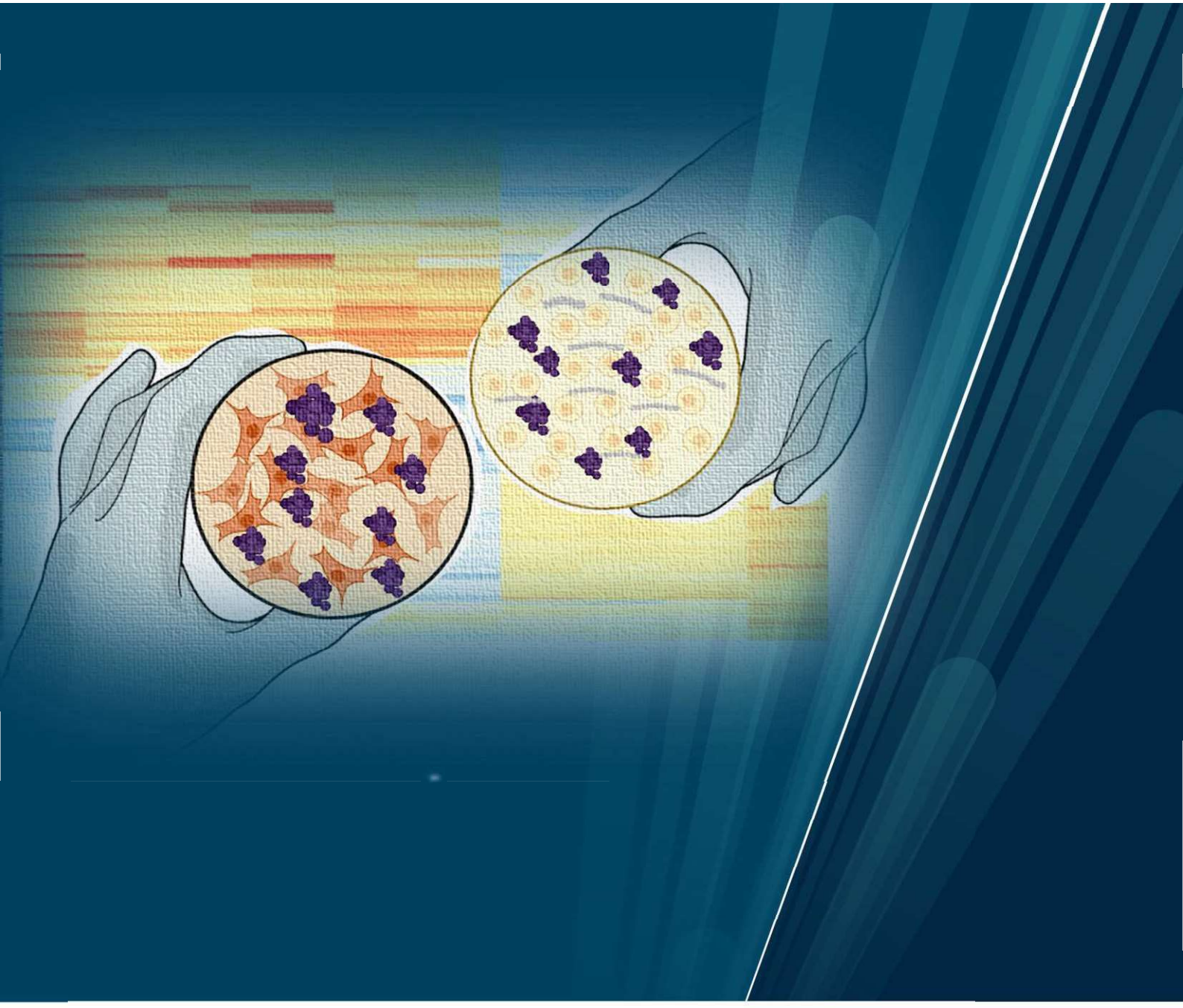
UiT The Arctic University of Norway

Faculty of Health Sciences

Exploring *Staphylococcus aureus* Adaptation in *In vitro* Colonization and Infection Models: A Transcriptomics Approach

Srijana Bastakoti Belbase

A dissertation for the degree of Philosophiae Doctor, March 2024



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SRIJANA BASTAKOTI BELBASE

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Research group of Host-Microbe Interactions
Department of Medical Biology
Faculty of Health Sciences
UiT – The Arctic University of Norway

To my beloved parents,

Shyam Prasad Bastakoti and Bhawani Bastakoti,

for their unwavering motivation, endless love and encoragement

To my husband, Shiva Belbase

with all my love,

Contents

Acknowledgments

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Summary

Staphylococcus aureus is both a commensal and a versatile pathogen, with approximately 30% of human adults carrying this bacterium in their nose and throat. Nasal colonization is a significant risk factor for infection, and *S. aureus* carriers can experience recurrent colonization even after therapeutic decolonization. There is limited knowledge on throat colonization, and therefore, it is vital to understand the mechanisms of *S. aureus* colonization and persistent carriage in the throat to develop effective preventive and therapeutic strategies. This study unravels *S. aureus*'s transcriptional responses to host cells, to factors that either mimic or that are derived from the host (or host-environment), as well as to other microbes. The objective is to enhance our understanding of transcriptomic adaptation of *S. aureus* during colonization and infection, with a particular focus on throat colonization.

We analysed the patterns of differentially expressed genes (DEGs) using an *S. aureus* throat strain and *S. aureus* USA300 LAC under colonization and infection mimicking scenarios. These conditions included exposure of *S. aureus* to tonsillar cells in an *in vitro* model (**Paper I**), co-culturing with another throat bacterium (**Paper II**), and exposure of MRSA USA300 to various host derived factors and human macrophages (Paper **III**). An RNA-sequencing (RNA-seq) based transcriptomics approach was employed to identify DEGs, followed by functional enrichment analysis to elucidate their biological significance.

Our findings revealed distinct gene expression profiles associated with *S. aureus* colonization and infection, highlighting essential virulence factors that are required for its adaptation to tonsillar cells, macrophages, and host factors. These virulence factors include those associated with adhesion, secreted proteins, iron-acquisition, and iron homeostasis. Transcriptomic profiling (**Papers I** and **II)** emphasized important gene expression changes related to virulence and adhesion as *S. aureus* adapts over time to its niche and competes with another bacterium. Furthermore, *S. aureus* exhibited distinct gene expression responses to professional phagocytes (Thp1 cells) compared to non-professional phagocytes (tonsillar cells) and different host factors (**Paper III**).

This study provides new knowledge on bacterial factors involved in *S. aureus* and MRSA colonization and infection. The most promising determinants are potential targets for development of new preventive measures and treatment, such as antivirulence therapy independent of antibiotics, to combat *S. aureus* colonization and subsequent infections.

Abbreviations

agr	Accessory gene regulator
BAM	Binary alignment map
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CDC	Centers for disease control and prevention
CFU	Colony forming units
CHG	Chlorhexidine gluconate
CHIPS	Chemotaxis inhibitory protein of *Staphylococcus*
chp	Calcineurin B homologous protein
ClfA	Clumping factor A
Cna	Collagen adhesion
Coa	Coagulase
CV	Core variable
DEGs	Differentially expressed genes
dps	DNA protection during starvation protein
ECM	Extracellular matrix
emp	Extracellular matrix protein-binding protein
FBS	Fetal bovine serum
FDR	False discovery rate
FnbP	Fibronectin-binding proteins
GO	Gene ontology
HAIs	Healthcare-associated infections
HA-MRSA	Hospital acquired methicillin-resistant *Staphylococcus aureus*
hla	Alpha toxin
hld	Delta toxin
HTEpiC	Human tonsil epithelial cells
icaA	Intracellular adhesion A
ICU	Intensive care unit
isaB	Immunodominant staphylococcal antigen B
isd	Iron-regulated surface determinants
KatA	Catalase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDH	Lactate dehydrogenase
LPSN	List of prokaryotic names withstanding in nomenclature
MGEs	Mobile genetic elements
MLST	Multi-locus sequencing typing
MOI	Multiplicity of infection
MRSA	Methicillin-resistant *S. aureus*
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
MSIS	Norwegian surveillance system for communicable disease
msrA2	Peptide methionine sulfoxide reductase MsrA 2
MSSA	Methicillin-sensitive *S. aureus*

NGS	Next generation sequencing
OD	Optical density
P/S	Penicillin/streptomycin
PAMPs	Pathogen-associated molecular patterns
PCA	Principal component analysis
PE	Paired end
PFGE	Pulse-field gel electrophoresis
PMA	Phorbol 12-myristate 13-acetate
PVL	Panton valentine leucocidin
qRT-PCR	Quantitative real-time polymerase chain reaction
RIN	RNA integrity number
RNA-seq	Ribonucleic acid-sequencing
rRNA	Ribosomal RNA
SAG	*Streptococcus anginosus* group
SAM	Sequence alignment map
SarA	Staphylococcal accessory regulator
SCC	Staphylococcal cassette chromosome
SCIN	Staphylococcal complement inhibitor protein
Sdr	Serine-aspartate repeat domain proteins
Spa	Staphylococcal protein A
SSL5	Staphylococcal superantigen-like protein 5
TLRs	Toll-like receptors
TNS	Trypsin neutralization solution
TSB	Tryptic soy broth

List of papers

Paper I

Srijana Bastakoti*, Clement Ajayi, Kjersti Julin, Mona Johannessen, Anne-Merethe Hanssen*: **Exploring differentially expressed genes of *Staphylococcus aureus* exposed to human tonsillar cells using RNA sequencing.** *BMC Microbiol* 2023 Jul 12;23(1):185. doi: 10.1186/s12866-023-02919-5

Paper II

Srijana Bastakoti*, Maiju Pesonen, Clement Ajayi, Kjersti Julin, Jukka Corander, Mona Johannessen, Anne-Merethe Hanssen*: **Co-culturing with *Streptococcus anginosus* alters *Staphylococcus aureus* transcriptome when exposed to tonsillar cells.** *Front Cell Infect Microbiol* 2024; 14:1326730. doi: 10.3389/fcimb.2024.1326730.

Paper III

Srijana Bastakoti*, Clement Ajayi†, Stephen Dela Ahator†, Mona Johannessen, Christian Stephan Lentz, Anne-Merethe Hanssen*: **Transcriptomic responses of *Staphylococcus aureus* USA300 LAC during exposure to human Thp1 macrophages and host factors.** Manuscript

1 INTRODUCTION

Studying colonization, adhesion, and invasion of bacteria in hosts, along with their interaction with host factors and commensals, allows for improved understanding of bacterial pathogenesis and offers new avenues for infection prevention and treatment. *Staphylococcus aureus* (*S. aureus*) is commonly found as a commensal bacterium in the upper respiratory tract (1), predominantly inhabiting the anterior nares (the front part of nasal passages) (2, 3) and to a lesser extent in the throat of healthy individuals (4, 5). Additionally, it can colonize various other regions of the human body, such as the skin, perineum, intestine, and vagina (6-11). This opportunistic pathogen is renowned for its colonizing ability across multiple human body sites (12). The investigation of bacterial factors involved in the colonization and infection stages could lead to the development of new antimicrobials, vaccines, and more effective therapeutic strategies.

Researchers are currently utilizing *in vitro* and *in vivo* models to explore the expression of bacterial genes and their roles in molecular pathways, biological processes, or cellular functions related to colonization and infection (2, 13). The interaction between *S. aureus* and its host has been investigated to elucidate the mechanisms underlying its colonization sites in humans (14-17). However, factors influencing *S. aureus* throat colonization have not been widely studied.

The relative importance of bacterial factors, host factors, and environmental factors involved in *S. aureus* throat colonization and carriage is largely unknown. We aim to address this knowledge gap by investigating *S. aureus* factors crucial for throat colonization. It has been proposed that recurrence of *S. aureus* colonization might happen due to its ability to originate from endogenous bacterial reservoirs within various parts of the human body. Within these reservoirs, *S. aureus* may hide within immune cells and establish interactions with the host, facilitating colonization (18). Nasal colonization is a significant risk factor for infection in both community and healthcare settings (15). Understanding the colonization dynamics of *S. aureus* is crucial for infection control and prevention strategies, and for developing targeted decolonization therapies to reduce the risk of infection in carriers, particularly before surgical procedures or in outbreak situations.

The introduction chapter encompasses topics relevant to *S. aureus*, including clinical relevance, virulence factors, colonization, adhesion and invasion, adaptation to host, strategies for prevention and decolonization, followed by the significance of transcriptomics analysis in understanding *S. aureus* adaptability and virulence.

1.1 *Staphylococcus aureus*

Staphylococcus aureus belongs to the *Staphylococcus* genus under the *Staphylococcaceae* family of the order Bacillales and the phylum Bacillota (formerly known as Firmicutes). It is gram-positive, non-spore forming, nonmotile, and a facultative anaerobe. Microscopically, *S. aureus* appears round and aggregates in grapelike clusters (19). Within the vast array of prokaryotic organisms catalogued in the List of Prokaryotic Names with Standing in Nomenclature (LPSN) database, a total of 88 species and 30 subspecies of *Staphylococcus* have been identified (20). Among these species, *S. aureus* and *S. epidermidis* hold particular significance in human health. While *S. epidermidis* typically acts as a mild pathogen, mainly causing opportunistic infections in immunocompromised individuals, *S. aureus* emerges as a predominant culprit behind joint and soft skin tissue infection, wound infection, boils and a wide range of skin infections and foodborne illness (19, 21).

A significant characteristic of *S. aureus,* distinguishing it from other staphylococci, is its unique ability to coagulate plasma by releasing the coagulase enzyme, which converts fibrinogen to fibrin within the host (22). This clotting mechanism shields *S. aureus* from the immune system and helps in the establishment of infections (23, 24). Coagulase is thus recognized as a key virulence factor of *S. aureus*, which produces both free and bound forms of the enzyme. Free coagulase enzyme is secreted extracellularly, while bound coagulase is a cell wall-associated protein that interacts with α- and β-chains of the plasma fibrinogens to form a coagulate (25, 26).

1.1.1 Clinical Relevance

S. aureus, particularly methicillin-resistant *S. aureus* (MRSA), is one of the leading causes of hospital-acquired infections (27). MRSA exhibits resistance to multiple beta-lactam antibiotics, including methicillin (28). This resistance is facilitated due to the presence of the *mecA* gene, encoding penicillin-binding protein 2a (PBP2a), which alters the cell wall structure. Also, MRSA strains carry a genetic element called Staphylococcal cassette chromosome *mec* (SCC*mec*), facilitating the mecA gene transmission between *S. aureus* strains (28).

S. aureus is renowned for its adaptability and possesses an array of virulence factors that contributes to its pathogenicity and clinical significance (29). These factors can be surface-associated and may be secreted. They enable *S. aureus* to colonize various hosts, evade the immune system, and cause a wide range of illnesses, from minor skin infections to life-threatening diseases (21, 29). Infections caused by *S. aureus* can be classified into three general types: 1) superficial wound infections; 2) systemic and

serious disease such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, bacteremia and sepsis; and 3) toxicosis, e.g., food poisoning, toxic shock syndrome, and scalded skin syndrome (27, 30, 31).

S. aureus infections typically occur either by autoinfection – a person develops an infection with their own carrier strain, or through cross-infection – where the infecting strain is transferred from another individual (32). The transmission dynamics of MRSA, especially in the case of isolated throat carriage, remains poorly understood (33). An estimated 20-30 % of healthy individuals are asymptotic carriers of *S. aureus* (i.e., colonized), indicating a precursor for infection (5, 30). Common risk factors for *S. aureus* infection are *S. aureus* carriage, poor hygiene, substance abuse, and specific comorbidities (e.g., cystic fibrosis, dermatological conditions) that can contribute to the infection (12). Additionally, risk factors for MRSA colonization include severe underlying illness, prolonged hospital stay, extensive antibiotic usage, use of invasive procedures such as central venous catheters, and frequent contact with the healthcare personnel (34).

It is alarming that globally, about 15% of intensive care unit (ICU) infections stem from *S. aureus*, with MRSA accounting for approximately one-third (31%) of these cases (35). This implicates the substantial burden of MRSA related mortality and morbidity (36, 37). Bloodstream infections rank as the second most common cause of mortality (second to lower respiratory tract infections), with *S. aureus* emerging as the second most prevalent pathogen (second to *Escherichia coli*) (38). Treatment of *S. aureus* infection is impaired because of the spread of resistant *S. aureus* strains, mainly MRSA (39). MRSA has become one of the main causes of nosocomial infections worldwide (40).

According to the Centers for Disease Control and Prevention (CDC), MRSA presents a serious threat to public health due to increasing prevalence in hospitals, communities, among animals (including transmission between humans and animals), high infection rates, resistance, and therapeutic challenges (41). There is an increased risk of mortality associated with MRSA bacteremia compared with methicillin-susceptible *S. aureus* (MSSA) bacteremia (39). In 2022, the Norwegian Surveillance System for Communicable Disease (MSIS) reported a total of 1,934 individuals diagnosed with MRSA, including 843 infected and 1,091 colonized (42). The incidence rate of MRSA infections peaked in 2017, decreased significantly until 2021, while a slight increase was noted in 2022 (42-44). According to the report from NORM/NORM-VET 2022, about 21% of people acquired MRSA during travel abroad or prior entering Norway, while 28% acquired MRSA within Norway (42). There is a relatively low prevalence of MRSA in Norway. Only 1% MRSA was detected in blood culture isolates, with the majority of MRSA cases reported as superficial wound infections (42, 45). Despite efforts to combat its spread, MRSA remains a serious pathogen in many European countries (46).

Recently, MRSA was detected in the throat of two healthcare workers in Norway without individual risk factors for long-term carriage. Interestingly, MRSA detection was negative in areas like wound, nose, and perineum. Even after MRSA eradication protocol (treatment with mupirocin nasal ointment, chlorhexidine body wash and use of oral antibiotics), MRSA was detected again in the throat within two weeks (47). The success rate of MRSA eradication is reported to be between 41-88% (48, 49), with throat carriage being the most difficult to eradicate (50).

1.1.2 Clonality and spread of *S. aureus*

The clonal spread of *S. aureus* refers to the proliferation of specific bacterial groups, where a small number of surviving bacteria are released, multiply and spread throughout the host. Understanding this clonal distribution is essential to comprehend *S. aureus* transmission strategy, including infection, adaptation, and its connection with antibiotic resistance, as well as spread of epidemics (51, 52). Epidemiological surveillance of MRSA is vital for mitigating the incidence of epidemic clones and preventing their transmission. Molecular methods such as staphylococcal protein A (*spa*) typing, multi-locus sequencing typing (MLST), pulse-field gel electrophoresis (PFGE), and classification of the mobile genetic element Staphylococcal cassette chromosome *mec* (SCCmec) are essential for identifying the relatedness, genetic diversity, and clonal distribution of *S. aureus* including MRSA clones (29). These typing methods play crucial roles in understanding the epidemiology, transmission dynamics, and clinical significance of *S. aureus* clones in both healthcare and community settings (29).

MRSA is responsible for global outbreaks, with emerging new clones (53). Hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) clones frequently circulated within clinical environments, affecting immunosuppressed individuals. In contrast, community-associated MRSA (CA-MRSA) clones can lead to infections in patients without previous history of hospitalization (54, 55). CA-MRSA has emerged as a pathogen (56), primarily causing skin and soft tissue infections that may progress to bacteremia (57). These clones exhibit greater genetic diversity and higher pathogenicity level compared to HA-MRSA (58). The global dissemination of epidemic MRSA clones is of concern as it restricts antibiotic options for treating MRSA infections (58).

MRSA clones typically originate from MSSA reservoirs through the acquisition of the SCC*mec* element, as described for the epidemic CA-MRSA clones such as ST8 (USA300) (59), and ST80 clone in Europe (60). Additionally, MSSA clones may secrete toxins like Panton valentine leucocidin (PVL), potentially leading to evolution into MRSA by enhancing virulence and facilitating the spread of antibiotic resistance (61). Notably, MSSA clonal complex (CC) 398 frequently colonizes nares and causes various

infections in people, suggesting this clone is well-adapted to humans (62). MRSA strains isolated from individuals exclusively carrying the bacteria in their nasal passages or throat reveal separate clustering of each group (63). In the United States, USA 300 (ST8-IVa) and USA 400 (ST1-IVa) are the main CA-MRSA clones (64). Similarly, CC8 (USA300), CC30 (South-West Pacific clone), and CC80 (European CA-MRSA clone), carrying genes for PVL, dominate CA-MRSA infections in the Nordic countries, possibly due to travel and immigration (65, 66).

1.1.3 Genomic Characteristics

Comprehensive whole-genome sequencing of diverse strains has provided insights into *S. aureus* genomics, facilitating a deeper understanding of the underlying mechanisms of antibiotic resistance and virulence (67). Comparative analysis of these sequences has revealed important genomic characteristics of *S. aureus* (68), including conserved metabolic and structural functions, as well as species-specific functions promoting virulence and host-specific adaptation (69). Typically, *S. aureus* has a circular genome in the 2 - 3 Mb range, encoding around 2,700 proteins with an overall GC content of 30-39% (70, 71). Approximately 80% of the *S. aureus* genome is well conserved across different lineages, also referred to as the core genome, and 20% of the genome is variable which is known as the accessory genome (dispensable genetic material) (68).

The core genes mainly encode essential housekeeping genes involved in metabolic and regulatory functions and some virulence-associated factors crucial for adhesion and invasion (71). Certain genes like *spa* and coagulase (*coa*) are located in regions linked to chromosomal inversion events that drive *S. aureus* evolution and differentiation (72). Within the core genome, 10-12 % of core variable (CV) regions exhibit more variability between strains and lineages. These variable regions contain genes (eg., *spa*, *fnbA, fnbB, cna, sasA-G*, *sdrD* to *sdrF*, *coa, clfA, clfB*) with a higher substitution rate compared to core genes. These genes encode surface proteins and key global virulence regulator (*agr, traP* and *sarT*) that regulate surface protein expression (69, 73). The gene variations in surface proteins that occur in response to the interaction between *S. aureus* and humans allow *S. aureus* to evade the host immune system (74-76).

The accessory genome consists of genes associated with virulence, adaptation, mobilization, and resistance to toxic compounds (77). These genes are scattered throughout the genome and are often associated with mobile genetic elements (MGEs) like staphylococcal cassette chromosome (SCC), bacteriophages, plasmids, transposons, and pathogenicity islands (78-80). Virulence genes are found in pathogenicity islands, while resistance genes are detected on SCC, plasmids and transposons (71). MGEs

are responsible for the transfer and exchange of genetic information between different bacterial species. The accessory genome plays a crucial role in the evolution and adaptation of clones, which are able to infect selected hosts and cause specific diseases (81, 82).

1.1.4 Virulence Factors

Virulence factors are defined as factors that facilitate niche adaptation, colonization, dissemination, and persistence of an infectious agent (29). Microbial virulence factors typically encompass a diverse range of molecules that enhances the ability of microorganisms to become pathogens. The activation of virulence factors to the cell surface and extracellular environment allows the microorganisms to evade host defenses and cause disease (83).

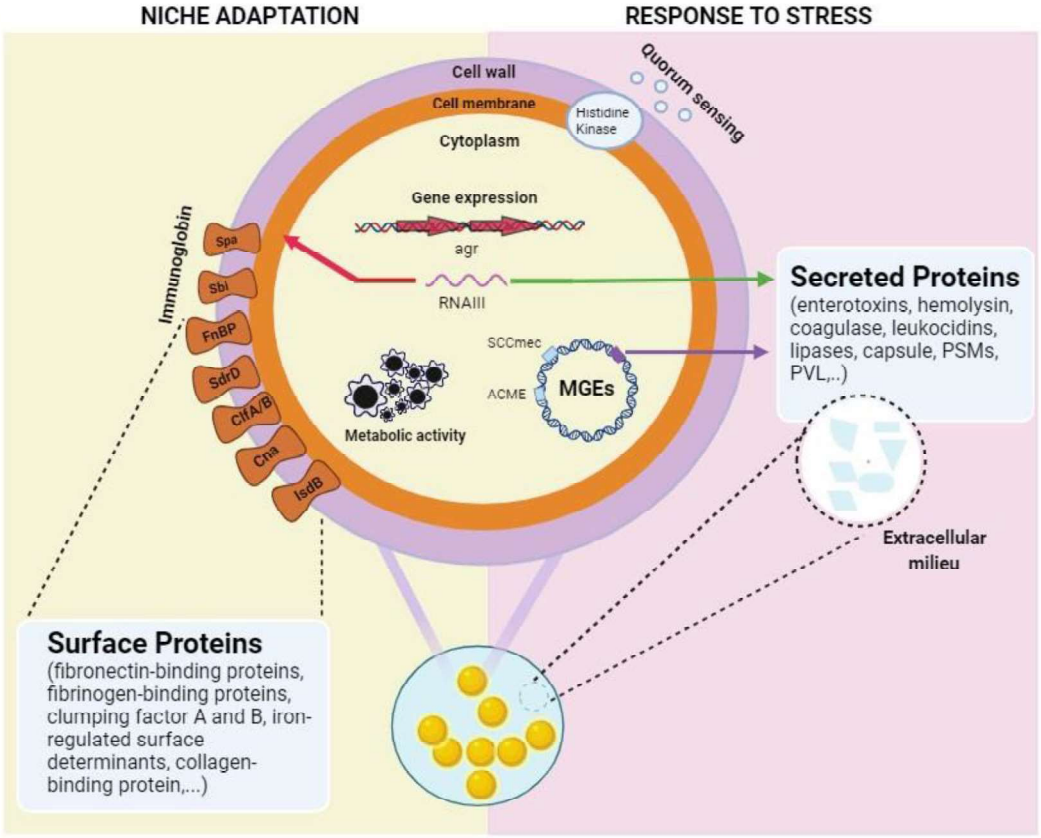

Figure 1: Schematic representation of key *S. aureus* virulence factors associated with the cell surface and secreted proteins. *Based on Jiang et al. (84). Created using BioRender.*

In *S. aureus,* virulence factors are often encoded on pathogenicity islands. Virulence factors play a vital role in the colonization and subversion of the human host in various host environment (29, 85). Several virulence factors expressed by *S. aureus* contribute to its pathogenicity (**Figure 1**, and summarized in **Table 1**), mainly displayed on the surface of staphylococcal cells or secreted into the host milieu (86, 87). Specifically, these virulence factors include **(i)** surface proteins that binds to the extracellular matrix, fibronectin, fibrinogen, hemoglobin and heme, which ultimately promote *S. aureus* adhesion and colonization; **(ii)** surface proteins involved in immune evasion (e.g., coagulase, clumping factor, protein A); **(iii)** iron-regulated surface proteins IsdB, IsdC and IsdH; **(iv)** surface factors that inhibit phagocytic engulfment (e.g., capsule, protein A); **(v)** membrane damaging toxins that disrupt the host cell membranes (e.g., hemolysins, leukotoxin); **(vi)** membrane invasins that are exported to an extracytoplasmic region and favor bacterial spread in tissue (e.g., leukocidin, kinases, hyaluronidase); **(vii)** superantigens that contribute to symptoms of septic shock (SEA-E, SEG, TSST, Eta, Etb); **(viii)** biochemical compounds that enhance survival of *S. aureus* in phagocytes (catalase production, carotenoids); **(ix)** several proteases; and **(x)** determinants for antibiotic resistance (88).

Moreover, some toxins (e.g., alpha-toxins, leukocidins, and phenol-soluble modulins) released by *S. aureus* can directly kill neutrophils and macrophages. *S. aureus* also releases proteases (e.g., Staphopain A and aureolysin metalloprotease) that break down immune proteins, eventually weakening the immune system (89), in addition to proteins (such as Staphylococcal Complement Inhibitor protein; SCIN, clumping factor A; ClfA, immunoglobin-binding proteins Spa and Sbi) that block the complement system and stop antibodies from binding (89, 90). This prevents immune cells from recognizing and destroying the bacteria (91).

Table 1: Virulence factors in *S. aureus*. Based on (92-94) and references therein.

Protein or other compounds	Ligand and Functions
Surface proteins promoting adhesion and colonization	
Serine-aspartate repeat domain proteins (Sdr) C, D, E	Bind to extracellular matrix; expressed during nasal colonization (95, 96).
Fibronectin-binding proteins A and B (FnbA/B)	Bind to fibronectin, fibrinogen, and cytokeratins; vaginal colonization (97, 98).
Iron-regulated surface determinant A (IsdA)	Binds to fibrinogen; enhances hydrophobicity; nasal colonization (99).
S. aureus surface proteins A–G (SasA– G)	Bind to extracellular matrix; involved in biofilm formation (100).
Clumping factors A and B (ClfA/B)	Bind to fibrinogen and type I cytokeratin 10: nasal colonization (101-103).
Extracellular adherence protein (Eap/Map)	Adhesion to host cell; damages angiogenesis and wound healing.
Elastin-binding protein (EbpS)	Promotes bacterial colonization facilitating pathogenesis (104, 105).
Laminin-binding protein (Eno)	Promotes adherence to laminin containing ECM leading dissemination (106)
Collagen adhesion (Cna)	Collagen; mediates adherence to collagenous tissue (107)
Surface proteins involved in immunological disguise	
Coagulase (Coa)	Activates host prothrombin; promotes infection establishment (108)
Clumping factor A and B (ClfA/B)	Binds to loricrin; skin infection (109)
Staphylococcal Protein A (Spa)	Binds to Fc/Fab part of IgG (110)
Serine-aspartate repeat domain proteins D, E	SdrD increases bacterial survival in blood (111); SdrE interferes with complement system (112)
Iron-regulated surface proteins	
Iron-regulated surface determinant B (IsdB)	Binds to hemoglobin and heme; relapsing reinfection.
Iron-regulated surface determinant C (IsdC)	Binds to heme; relapsing reinfection.
Iron-regulated surface determinant H (IsdH)	Binds to haptoglobulin.
Surface factors exhibiting phagocytic engulfment	
Core biosynthesis genes (CapA-P)	Capsular polysaccharide; inhibits phagocytic engulfment.
Staphylococcal protein A (Spa)	Protein A; inhibits phagocytic engulfment.
Membrane- damaging toxins and invasins	
Hemolysins (Hla, Hld, HlgA-C)	β-Hemolysin promotes skin colonization (113); δ- Hemolysins form pores and disrupt the host cell membrane
Leukotoxins (LukD, LukE)	Lyse the host cell membranes and spread bacteria
Leukocidins (LukF, LukS)	Invasins; Lyse the host cell membrane and spread bacteria
Hyaluronidase (HysA)	Invasin; Lyses the host cell membrane and spreads bacteria
Nuclease (Nuc)	Lyses the host cell membrane and spread bacteria
Lipase (Lip, Geh)	Hydrolyses lipids; contributes to skin colonization and invasion (114)
Exotoxins	
Staphylococcal enterotoxins (SEA to SEE, and SEG)	Reduce the immune response of TH_2 cytokines; contribute to symptoms of septic shock.
Toxic-shock syndrome toxin -1 (TSST-1)	Responsible for the symptoms of toxic shock syndrome.
Biochemical compounds	

Catalase (KatA)	Catalase production; contributes to survival in phagocytes.
Staphyloxanthin	Carotenoids; contribute to survival in phagocytes.
Proteases	
Serine protease (V8 protease)	*In vivo* growth and survival of *S. aureus* (115).
Staphylococcal cysteine proteases (staphopain A)	Colonization of the murine lung; intracellular killing of epithelial host cells (116).
Caseinolytic protease (Clp)	Responsible for elimination of misfolded/aggregated proteins (117).
Phenol soluble modulin (psm)	Induces neutrophil extracellular trap release.
Metalloprotease (aureolysin; aur)	Cleaves staphylococcal surface-associated proteins (FnbA and ClfB); inhibits the antimicrobial activity of cathelicidin
Exfoliative toxins A/B (Eta, Etb)	Bind to skin protein profilaggrin; cause staphylococcal scalded skin syndrome.
Resistance proteins	
Beta-lactamase Blaz, Penicillin-binding protein 2a (PBP2a/PBP2´)	MRSA; acquired resistance to antimicrobial agents
Vancomycin/teicoplanin A-type resistance protein (VanA)	VRSA; acquired resistance to antimicrobial agents
Esterase (FmtA)	Mediates biofilm formation, cell division, colonization; resistance to cell wall active antibiotics (118).

S. aureus microbial surface components recognizing extracellular matrix molecules (MSCRAMMs)

Many surface proteins found in gram-positive bacteria share a conserved C-terminal sorting signal. This signal typically consists of an LPXTG sequence followed by a hydrophobic domain and a tail containing mostly positively charged amino acids (119). Among these proteins are adhesins (listed in **Table 1**) capable of binding to the extracellular matrix (ECM) of the host. Most of these proteins recognize one or more of the large glycoproteins found in plasma and ECM, and allow *S. aureus* to adapt efficiently to diverse microenvironments, which include connective tissue, bone, bloodstream, and vascular tissue (120). Adhesins play a crucial role during colonization by facilitating the binding of *S. aureus* to ECM components, including fibrinogen, fibronectin, elastin, collagen, laminin, and vitronectin, through covalent or non-covalent interactions. This binding process is mediated by a group of *S. aureus* molecules i.e., MSCRAMMs (121, 122).

MSCRAMMs serve multiple functions in both infection establishment and host colonization. The initial attachment is augmented by the release of substances such as polysaccharides, proteins, and extracellular DNA, forming a protective extracellular matrix that shields the bacterial cells. Ultimately, it enhances bacterial survival in the bloodstream during invasive infection and contributes to the development of abscesses within both the skin and internal organs (123).

Importantly, MSCRAMMs are potential candidate molecules for vaccines and other immunotherapies to prevent colonization and infection (124, 125). For example, vaccines targeting MSCRAMMs have demonstrated promise in reducing virulence and colonization in animal models of *S. aureus* nasal colonization (126), and vaccines containing MSCRAMM antigens like IsdB protein have also shown effective reduction in virulence and colonization (127).

1.1.5 Regulation of Virulence Factors

The pathogenicity of *S. aureus* relies heavily on the presence and regulation of several virulence factors, whose expression is based on growth phases and environmental conditions (128). Complex regulatory systems regulate the expression of cell-surface-associated and secreted exoproteins in *S. aureus* (129, 130). Complex regulators, such as the accessory gene regulator (*agr*) system and the staphylococcal accessory regulator (SarA) protein family, play a pivotal role in controlling the expression of various virulence factors at the transcriptional level (1, 128) and the *agr* system enables *S. aureus* to facilitate colonization, cell invasion, and the establishment of infections (131).

The *agr* system, a quorum sensing regulatory system (**Figure 2),** influences biofilm formation and enhances bacterial adherence, invasion, and resistance in *S. aureus* (132, 133). The *agr* locus consists of two divergent transcriptional units, agrBDCA and RNAIII, encoding the *agr* signaling pathway components and effector molecule, respectively (132, 133) .Various genes within the *agr* operon are involved in upregulating potential virulence factors while simultaneously downregulating surface-binding proteins, which eventually leads to epithelial invasion and apoptosis (129). Consequently, the expression of the *agr* operon is associated with pathogenicity in *S. aureus* (134). Initially, the *agr* system exhibits reduced activity at the onset of infection when bacterial numbers are low, resulting in increased production of adhesins and surface proteins, promoting initial adherence. Additionally, it controls the expression of surface proteins like Protein A, coagulase, fibrinogen binding protein, and repressor of toxin (Rot) protein (135). Subsequently, upon successful colonization and depletion of nutrient resources, the *agr* system mediates the upregulation of destructive exoenzymes and toxins like *hld* and *hla* at both the transcriptional and translational levels (136). This upregulation facilitates nutrient acquisition and favors *S. aureus* to evasion of the immune system (137, 138).

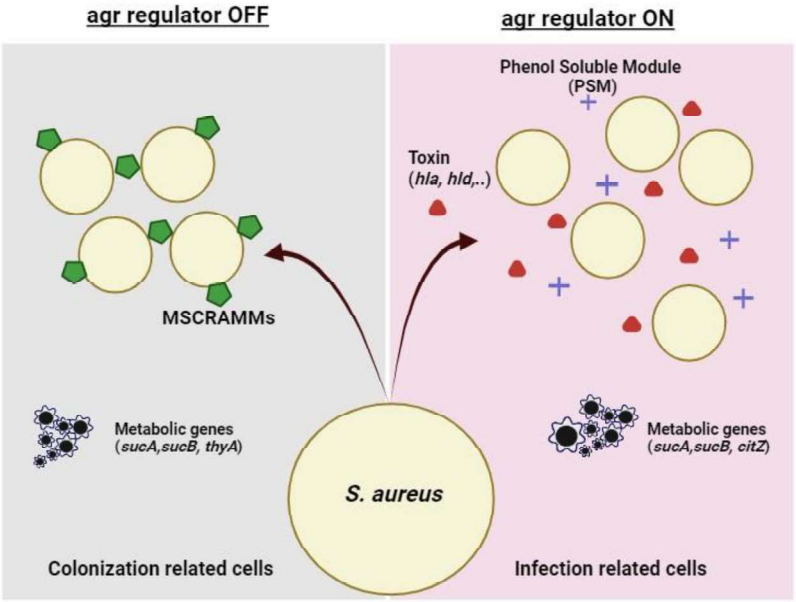

Figure 2: Visualization of the accessory gene regulator (*agr*) system in *S. aureus*. *Based on García-Betancur et al. (139). Created using BioRender.*

Conversely, SarA is a DNA-binding protein within a group of global transcriptional regulators controlling cellular processes and expression of nearly 120 genes encoding adhesions, toxins, and enzymes (140-142). *S. aureus* contains various SarA paralogs (SarR, SarS, SarT, SarU, SarV, SarX, Rot, MgrA and MarR), which indirectly enhances virulence, biofilm production, autolysis, resistance, and metabolic processes (143, 144). The *sarA* gene product activates the *agr* promoter and other promoters of specific virulence genes, regardless of agr involvement (145). In addition, SarA controls the post-transcriptional expression of *spa* and collagen adhesion genes of the exponential growth phase by binding to the mRNA target (146, 147). While the *agr* locus transcription occurs primarily in exponential growth phases, SarA proteins remain stable throughout growth (140, 148).

In bacterial infection, precise temporal coordination of gene expression is important for adaptation and infection establishment (128). Thus, understanding these regulatory networks is crucial for developing interventions to counter *S. aureus* infections by, for example, neutralizing the expression of virulence factors or resistance to antibiotics. Both Agr and SarA positively regulate *lrgAB*, controlling penicillin tolerance in strains (149, 150).

1.2 *S. aureus* Colonization, Infection and Prevention

The colonization process is dynamic; *S. aureus* must first be in contact with the host cell or tissue and subsequently adhere. It must overcome immune components, and proliferate within its new niche, and compete with other commensal microbes (151, 152). Both *in vitro* and *in vivo* studies have demonstrated direct roles for many *S. aureus* virulence factors, such as adhesins, making them attractive targets for therapeutic and preventive strategies against *S. aureus* infections (153). Consequently, there is a growing emphasis on employing advanced molecular techniques to determine the exact structures, functions, and regulatory mechanisms of these proteins. Such understanding facilitates the development of more targeted approaches for combating *S. aureus* infections (29). This knowledge is essential for identifying targets for antimicrobial therapy.

1.2.1 *S. aureus* Colonization

Humans are frequently exposed to *S. aureus*, with colonization occurring in most individuals for long or short duration throughout their lives. The nasal sites often serve as the point of origin for colonization to other sites via hand transfer (15, 154) with greater bacterial loads in the nares increasing the likelihood and persistence of colonization elsewhere (50, 155). The colonizing strain is responsible for about 80% of *S. aureus* infections (27, 156). Colonization is conferred to the presence of a permanently colonizing *S. aureus* strain in several reservoir sites of the human body (15, 157). To become a successful colonizer, the bacterium needs to attach to the lining of the airways, colonize the mucosal surface, evade immune response, and transmit to other susceptible individuals (158). This colonization process is pivotal in establishing individuals as *S. aureus* nasal carriers, thereby increasing the risk of infection transmission (5, 159, 160).

A well-described colonization mechanism is nasal colonization, in which *S. aureus* develops solid adherence capacity to the epithelial cells and overcomes host defence mechanisms (160). Although the nares have been considered the primary site of *S. aureus* colonization (5), several studies in adults indicate that pharyngeal carriage may be equally or more common (161-163). Understanding *S. aureus* colonization and adaptation to the host, as well as ways to prevent these processes, is essential for the effective control and treatment of associated diseases. Colonization with *S. aureus* constitutes a significant risk factor for recurrent episodes of disease, e.g., rhinosinusitis (164), tonsillitis (165), and osteomyelitis (166) after the successful adhesion and invasion of the host cell. Thus, colonization in the body is a crucial step for establishing *S. aureus* infection in humans (5). It is clinically relevant, as the risk of bacterial infection is linked directly to colonization (27, 156, 167), especially in

immunocompromised and hospitalized patients. For instance, many *S. aureus* colonized patients can later develop an infection caused by MRSA, such as pneumonia or soft tissue infections (168-171). While nose colonization is detailed, there is limited knowledge about throat carriage and colonization.

Bacterial colonization in the tonsillar surface and core is dominated by *S. aureus,* followed by *Haemophiles influenza* in the core (172). This finding underscores the challenge of eradicating *S. aureus*, especially when it resides deep within the tonsils, where current eradication methods might not reach. Moreover, exclusive throat carriage is increasingly identified as an additional *S. aureus* reservoir particularly in young populations (155, 156, 161). The risk factor for exclusive throat carriage is age 30 years or younger, and absence of exposure to the health care system (156). The throat may also be an important reservoir for MRSA (162, 173-176). Some strains of *S. aureus* regularly colonize the throat of healthy people and can persist for years (63), and among MRSA strains, 32.1% are isolated from exclusive throat carriers. Both MSSA and MRSA can persistently colonize the throat for four years or more (177). In MRSA carriers, colonization is most commonly observed in the nasal vestibule, followed by the perineum and the throat (178). The success rates of eradication range from 41% to 88% (48), with eradication from the throat area being the most challenging (50).

There is also evidence suggesting a link between throat colonization and *S. aureus* colonization of the rhinopharynx (upper part of the throat behind the nose), shedding light on the pathogenesis of MRSA throat colonization (50). This link presents potential implications for managing MRSA throat colonization and shows the importance of novel treatment methods (50). Thus, the throat is considered an important colonizing site for *S. aureus.*

1.2.2 Microbiome in the Nose and Throat

Microbiota comprises a diverse range of microorganisms, including bacteria, viruses, and fungi, that inhabits in a specific environment like the human body, soil, or water. The microbiome encompasses the collective genetic information contained within the microbiota interacting with each other and with the host (179). Functional links between the human microbiota and infectious or inflammation-related diseases have been established (180). Many studies reveal that an imbalance in the microbiome can lead to formation of disease (181). The mucosal and epithelial surfaces, including the oropharynx, harbors a plethora of both aerobic and anaerobic bacteria, creating niches for microbial colonization (182).

The nasal microbiome of healthy individuals primarily comprises genera like *Bifidobacterium, Corynebacterium, Staphylococcus, Streptococcus, Dolosigranulum* and *Moraxella* (183). In the throat or pharynx (comprising nasopharynx, oropharynx, and hypopharynx), predominant genera include *Staphylococcus, Streptococcus* and *Veillonella* (184). Importantly, the prevalence of *S. aureus* in the throat appears higher than previously reported (185). However, the precise relationship between the throat microbiota and *S. aureus* infections remains poorly understood. Bacterial reservoir sites, such as the anterior nares of the host, serve as a means for spreading human pathogens (186).

The composition of microbiota from the surface and the core of tonsillar tissue is not well defined. Limited studies have been conducted for tonsillar microbiota, despite its essential role in the human immune defense system and exposure to numerous microorganisms all the time. Genera abundant in tonsillar crypts include, *Streptococcus* (21.5%), *Neisseria* (13.5%), *Prevotella* (12.0%), *Haemophilus* (10.2%), *Porphyromonas* (9.0%), *Gemella* (8.6%), and *Bacillota* (6.4%) (187), some of which are associated with the recurrence of tonsillar hypertrophy (188, 189). There is an association between tonsillar hypertrophy and microbial interactions; a higher bacterial load in tonsil tissue correlates with larger tonsil size (190).

Changes in the microbiome composition or disruption of the microbial balance in the body can increase the likelihood of *S. aureus* colonization and subsequent infection. Factors such as antibiotic exposure, existing medical conditions, and environmental exposures (such as, saliva, temp, pH, nutrients, and competitors) can disrupt the microbiome (191), creating opportunities for *S. aureus* to colonize and cause infection. The composition and diversity of the microbiome in the throat and nose can influence both the *S. aureus* colonization and infection by altering various physiological processes and immune functions. Opportunistic bacteria like *Streptococcus pneumonia*, *Haemophilus influenza,* and *S. aureus* often cause pneumonia together with local and systemic infections. These infections usually occur when these bacteria successfully colonize the upper airways (192-194).

Interaction Dynamics of Colonizing Strains

During colonization, *S. aureus* exists within a polymicrobial community, yet knowledge of the roles of commensals and interspecies interactions impacting *S. aureus* colonization and infections remains limited (195). Opportunistic pathogens that are well-adapted to the host appear to have a greater capacity for displacing the host's microbiota (158, 196). Moreover, the interaction between two commensals can mutually benefit colonization and enhance the survival of both in specific body sites (197). Such interaction enables bacteria to adhere to host cells and can even increase their resistance to the host's innate immune system (198). *S. aureus* typically exists alongside different species of staphylococci, *Corynebacterium* and *Cutibacterium* on the skin (199) and this pattern is also observed in the human

nose (16). These interspecies interactions and the influence of non-pathogenic commensals on *S. aureus* colonization are intricate (156). Nonetheless, advancement in genomics and metabolomics technologies have made understanding of microbial interactions possible. These interactions can influence the response of *S. aureus* to antibiotics and can swing from preventing colonization to promoting *S. aureus* transition to invasion (87).

Adhesins produced by *S. aureus* play a pivotal role in facilitating interactions with various components such as salivary, serum, and ECM, as well as host cells and other microbes. This initial interaction is crucial as it marks the primary step towards colonization, the formation of complex microbial communities, and potential invasion of host tissues (200). The success of *S. aureus* depends not only on adhesins, virulence genes and the ability to escape antibiotic treatment, but also on the coordinated and timely expression of genes upon host infection (201), a dynamic process that may change in the presence of other microbes.

1.2.3 *S. aureus* Invasion and Infection

S. aureus not only adhere to cell surfaces during colonization but also invade both phagocytic and non-phagocytic cells (**Figure 3**) where they can escape antibiotic treatment (202, 203). Here, they constitute a significant risk factor for recurrent episodes of associated diseases, highlighting the potential benefits of strategies aimed at local eradication of carriage to prevent *S. aureus* infections. Following invasion, the activation of virulence genes triggers the production of toxins that play role in the progression of disease. Eventually, initially upregulated adhesion genes undergo downregulation (130, 204).

Professional phagocytes, such as macrophages and neutrophils, play a major role in clearing *S. aureus* infection (205). Neutrophils are integral to the immune system and play a crucial role in the host's defense against bacteria. Their primary function is to recognize, engulf, and eliminate invading bacteria through a process called phagocytosis (206). To discriminate between "self" and "non-self" agents, mammalian professional phagocytes employ a variety of phagocytic receptors. One example is the family of Toll-like receptors (TLRs) (207), which are expressed on the surface of phagocytes. TLRs recognize specific pathogen-associated molecular patterns (PAMPs) that are commonly found on the surface of microbes. The binding of a PAMP to a TLR triggers a signaling cascade within the phagocyte, activating various defense mechanisms against the invading pathogen.

However, *S. aureus* undergoes several mechanisms to reduce phagocytosis efficiency, and it has been found to survive within host cells, including professional phagocytes and non-phagocytic cells (208). For example, *S. aureus* thrives in host due to the action of the Staphylococcal Protein A (SpA), known

to bind to Fc region of immunoglobulins, which prevents opsonization and subsequent phagocytosis by neutrophils, further aiding in the evasion of the host immune response (209).

S. aureus also produces a protein called Staphylococcal Superantigen-like Protein 5 (SSL5) that stops neutrophils from sticking to the walls of blood vessel walls and moving to the infection site (210). It also secretes other proteins like SSL10 and Chemotaxis Inhibitory Protein of *Staphylococcus* (CHIPS), which inhibit chemotaxis and interfere with neutrophil receptors and activation, reducing their ability to fight the infection (211, 212). Many *S. aureus* strains produce a polysaccharide capsule that acts as a protective layer, making it hard for immune cells to recognize and attack them effectively (145, 213). Moreover, *S. aureus* can also release toxins (**Table 1**) that can kill neutrophils and macrophages (214-216). During infection, *S. aureus* competes with the host's immune cells for nutrients, leading to changes in metabolism that favor the bacterium and allow it to persist (217).

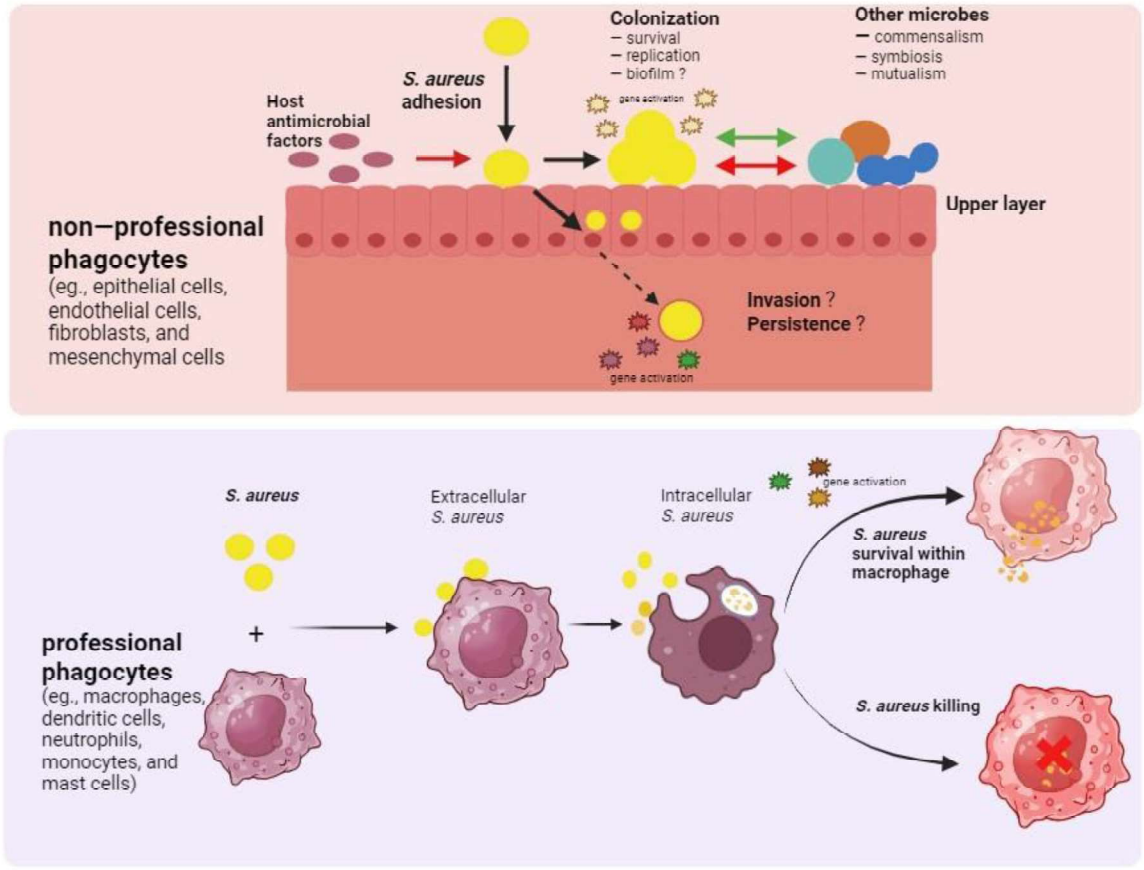

Figure 3: Illustration of *S. aureus* adherence to cell surfaces during colonization and invasion by both phagocytic and non-phagocytic cells. This adherence and internalization activate of virulence genes within S. aureus, enhancing S. aureus adaptation in colonization and infection. *Based on and modified from Edward et al. (151). Created using Bio render.*

1.2.4 *S. aureus* Adaptation to Host

Understanding host adaptation provides insights into host-specific immune evasion mechanisms, which in turn enhances understanding of disease (218). Both host and microbe determinants contribute to *S. aureus* colonization, with the bacterium's capacity to adhere to host cells being crucial for initiating colonization and, subsequently, adaptation (219). Host adaptation can arise through genetic drift, neutral diversification, or adaptive evolution, where advantageous mutations are favored, or detrimental ones are purged in the new host environment (220). Genetic drift often occurs alongside a reduction in effective population size (221). For instance, during the transition from colonization to invasive infection by *S. aureus*, a small subset of a more diverse initial population may survive within a host cell, subsequently expanding to cause disease (**Table 2**) (51). Conversely, adaptive evolution leads to an increased frequency of beneficial variants. Both mechanisms likely play a role in infection and adaptation (222). To identify host-specific factors that promote bacterial survival, comparative population genomics across human and animal isolates must be integrated with functional genomic studies (223). Such insights will help in developing interventions to prevent the rise of new pathogenic strains and limit cross-species transmission.

Studies on *S. aureus* genomics have greatly enriched the understanding of *S. aureus* adaptation to different host species. The accessory genome of *S. aureus* has been found to contain specific genetic elements that are unique to certain host species and help *S. aureus* to invade the hosts' immune system (224). Large-scale analysis of *S. aureus* populations across diverse hosts reveals humans as the main reservoir, facilitating the bacteria's transfer between hosts. Interestingly, cows seem to harbor *S. aureus* strains that could potentially cause widespread outbreaks in humans (218).

Studying the movement of genes across different environments has revealed sets of genes specific to each host, providing insights into *S. aureus* adaptation. Even diverse strains of *S. aureus* originating from the same host species tend to possess similar sets of extra genes (87). Furthermore, analysis of antimicrobial resistance genes within the *S. aureus* accessory genome has revealed unique patterns among different host species. For example, human isolates are more likely to be resistant to antibiotics like β-lactams commonly used in humans, while pig isolates are more likely to be resistant to tetracycline and antiseptics (218).

Table 2: Major genetic mechanism of *S. aureus* adaptation to switch from colonizer to pathogen to persistence. *Based on Howden et al. (87).*

Functional category	Gene/ Operon	Description	Ecological niche*			References
			Colonization	Colonization to invasion switch	Early invasion to persistence	
Global regulators	*agr*	Accessory gene regulator	+++	+++	+++	(225-227)
	rsp	Repressor of surface proteins		+		(228, 229)
	purR	Purine biosynthesis regulator			++	(226)
	yjbH	Adaptor protein, negative regulator of Spx			++	(226)
	gdpP	cyclic-di-AMP phosphodiesterase			+	(230)
	rsbU	sigma-B regulation protein	+++			(227)
Metabolic genes	*sucA-sucB*	Two components of the α-ketoglutarate dehydrogenase (tricarboxylic acid cycle [TCA])	+	+	++	(226, 231)
	citZ	Citrate synthase			+++	(231)
	thyA	Thymidylate synthase	+++			(227)
Resistance determinants	*walRKHI*	Two-component system, cell-wall regulator (resistance to vancomycin)			+++	(226, 227, 232)
	mprF	Phosphatidylglycerol lysyltransferase (resistance to vancomycin, daptomycin)			+++	(226, 233)
	rpoB	RNA polymerase subunit b (resistance to rifampicin, vancomycin)			+++	(226, 227, 234)
	fmtB	Methicillin resistance determinant	+++			(227)
	rpsJ	Ribosomal S10 protein (resistance to tigecycline)	+++			(227)
	dfrB	Dihydrfolate reductase (resistance to trimethoprim)	++			(235)

*It is important to consider whether the evolution of *S. aureus* infections has occurred within colonizing strains, between colonizing and invasive strains, or within invasive strains (+ suggestive enrichment, no statistical support; ++ statistical support, did not reach genome-wide significance; +++ strong statistical support).

1.2.5 *S. aureus* Decolonization

Nasal colonization is a significant risk factor for infections in both surgical and hospitalized patients, with MSSA colonizing between 12-30% of humans and MRSA around 1-2%, mostly in the nose and throat (236, 237). Both MRSA and MSSA produce a wide spectrum of surgical site infection, ventilator-associated pneumonia, and bloodstream infections (238, 239). Therefore, establishment of infection

control measures, including effective throat decolonization strategies, is urgently needed to prevent and manage *S. aureus* colonization and infections. Some of the common prevention strategies involve following infection control guidelines, including hand hygiene, environmental cleaning, and the appropriate use of personal protective equipment. Additionally, implementing screening and decolonization strategies is essential to identify and treat individuals colonized with *S. aureus*, particularly those at high risk of infection or transmission (240).

The fundamental principle of decolonization is to eradicate or reduce the bacterial load at the colonized site, thereby decreasing the host's likelihood of infection or transmission of *S. aureus* to others (241). MRSA decolonization typically involves administering topical antimicrobials or antiseptics to eradicate or suppress the carrier state to reduce clinical infection rates (242). This approach is widely used as a preprocedural intervention to reduce the risk of surgical site infections and as a routine measure to prevent healthcare-associated infections (HAIs), especially among intensive care unit (ICU) patients (243). Decolonization interventions include strategies such as chlorhexidine gluconate (CHG) bathing, chlorhexidine mouthwash, and applying antiseptic or antibiotic agents such as mupirocin, iodophor antiseptics, and alcohol-based products intranasally to target the anterior nares niche for *S. aureus* (244-247). Among these, mupirocin and iodophors have the strongest effect. A significant reduction in MRSA colonization and infection has been observed after decolonization and nasal mupirocin (248, 249). However, recurrence of colonization may further lead to repeated decolonization for patients undergoing further surgery (250).

While current decolonization agents may be effective, there is a risk for development of resistance to mupirocin and chlorhexidine over time. The failure of decolonization of *S. aureus* is due to the frequent recurrence of *S. aureus* even after the use of mupirocin treatment (251). A reduction in mupirocin's effectiveness for MSSA and MRSA decolonization poses a risk (252). Thus, alternative approaches to nasal decolonization, particularly alternatives to mupirocin, are needed to ensure effective eradication of *S. aureus* colonization in patients. The control and prevention of *S. aureus* infections have been complicated by the spread of multidrug-resistant strains, particularly MRSA (253, 254).

Decolonization of the nasal reservoir is necessary and sufficient for infection reduction among *S. aureus* carriers (194, 255-257). However, examining other reservoir sites such as the throat, is crucial for reliably detecting *S. aureus* carriers (155, 156, 258). Throat colonization poses a significant challenge in eradication efforts, often leading to treatment failure. Recent findings from Denmark have shed light on the association between *S. aureus* throat colonization and rhinopharynx colonization, which could be an important risk factor for prolonged MRSA throat carriage (50). Eradication of MRSA from the throat of patients is even more challenging than in other locations since the use of standard topical treatment

supplemented with systemic antibiotic therapy does not work (48, 259). The use of systemic antibiotic therapy can cause adverse effects such as gastrointestinal symptoms and allergic reactions, while also potentially promoting antibiotic resistance (260). Since MRSA throat colonization is often asymptomatic, the risks associated with systemic antibiotic treatment may be less tolerable for individual patients (50). The ability of *S. aureus* to colonize the throat region makes it difficult to reach by mupirocin treatment. Hence, new antimicrobial compounds for *S. aureus* decolonization are urgently needed.

1.3 Gene Exploration: A Transcriptomics Perspective

The emergence of 'omics' technologies has revolutionized research by producing high-throughput data, effectively connecting genome and transcription to the phenome (261, 262). Omics technologies provide powerful tools for investigating microbe-host interaction across various experimental setups, including cell cultures, animal models and human samples (14, 263). Recent advances in omics techniques enable comprehensive assessment of DNA information content (genomics), its temporal transcription into RNA (transcriptomics), translation into proteins (proteomics) and metabolite production (metabolomics) by bacteria in their native environments (264-266). This thesis focuses on transcriptomics in detail.

Transcriptomics explores the complete "transcriptome" of a cell, tissue, organ, or organism under different physiological or pathological conditions (267). Unlike the genome, the transcriptome is dynamic and actively changes due to many factors such as the organism's development stage and environmental conditions. The term transcriptome, coined by Charles Auffray, refers to the entire set of ribonucleic acid (RNA) expressed in a biological entity (268). It includes various aspects of RNA biology, including transcription and expression levels, functional roles, subcellular localization, trafficking mechanism, and degradation processes (267). Additionally, transcriptomics elucidates the structures of transcripts and their parent genes, including starting sites, 5' and 3' end sequences, splicing patterns and post-transcriptional modifications (269). The transcriptome encompasses all types of transcripts, including messenger RNAs (mRNAs), microRNAs (miRNAs), and various long noncoding RNAs (lncRNAs) expressed by an organism (267), also referred to as total RNA.

At the transcriptional level, a significant portion of bacterial adaptation occurs, where molecules like RNA polymerase sigma factors, transcription factors, and regulatory RNAs modulate mRNA synthesis, processing, and degradation through diverse mechanisms, particularly under infection-mimicking conditions (13, 270). These regulatory factors control the expression of genes that are necessary for the bacterium to survive and reproduce in different environmental conditions. By adapting gene expression

patterns, the bacterium can effectively respond to changes in the surroundings, enhancing the ability to survive and thrive. Moreover, DEGs analysis provides valuable insights into the genes and pathways that are activated or suppressed during interaction with the host, host factors and competitors. Thus, by identifying and analyzing DEGs, we can uncover key strategies used by the bacterium during adherence, host immune invasion, and antibiotic resistance (271).

1.3.1 RNA-seq based Transcriptomics.

Today, RNA-seq is an indispensable tool, widely recognized as the primary technology for transcriptome analysis (272). It enables high-throughput profiling of both coding and non-coding RNA at single-nucleotide resolution. RNA-seq offers several advantages, including simultaneous analysis of gene expression and sequence feature, unbiased selection of sequences, identification and annotation of novel transcripts, profiling of the entire transcript, and good technical reproducibility, enhancing our understanding of gene regulation mechanisms (273).

The RNA-seq workflow initiates with the extraction of total RNA from cells followed by purifying the RNA molecules of interest. While several next-generation sequencing (NGS) platforms are available for RNA-seq, Illumina's short-read sequencing has gained popularity due to its high throughput, accuracy, cost-effectiveness, and widespread availability (274, 275). Typically, the transcriptomics library preparation protocol involves removal of highly abundant ribosomal rRNA (in both host and bacterial transcriptomics) prior to sequencing or poly(A) selection (during host transcriptomics) (275). Single-strand RNA is then converted into double-stranded complementary DNA (cDNA) strands through a reverse transcription reaction. Sequencing adapters and barcodes are then incorporated to construct RNA-seq libraries, which are subsequently subjected to next-generation sequencing (NGS). The resulting data, termed "reads", undergo filtration and trimming based on sequence quality, mapping to reference genomes, and normalization to correct for biases arising from transcript length, composition, and replicates (275, 276). The number of reads aligning to a particular region serves as an indication of the transcriptional activity of the corresponding gene. RNA-seq enables the simultaneous measurement of gene expression of thousands of genes under a single condition or facilitates comparison across multiple conditions, a process known as differentially expressed genes (DEGs) (271).

Currently, there has been a shift from traditional bulk RNA-seq (RNA-seq) to the widely embraced single-cell RNA sequencing (scRNAseq) and the newly introduced spatial RNA sequencing (spRNAseq) (277). Bulk RNA-seq is renowned for its global gene expression analysis. scRNAseq investigates RNA biology at the single-cell level by examining up to 20,000 individual cells

simultaneously, while spRNAseq enables the spatial resolution of RNA activities (277). Moreover, there has been an improvement in the analyses of smaller amounts of sample material or low-quality material, such as clinical formalin-fixed paraffin-embedded (FFPE) samples (278, 279). Furthermore, continuous development efforts are also directed towards inventing new sequencing kits, including improved methods for rRNA depletion, the selection of non-poly(A) species (especially in prokaryotes), a specialized protocol for targeting classes of non-coding RNAs, longer reads sequencing kits, and multi-species RNA-seq (280). Similarly, standardized processing and data analysis pipelines are essential for all aspects of transcript analysis, particularly in prokaryotes systems (281).

1.3.2 Application of Transcriptomics in Microbiology

The emergence of high throughput RNA-seq has revolutionized the field of biological research by offering a novel means for quantitative measurement of transcription, a highly dynamic process that regulates numerous cellular functions (282). This technology has significantly contributed to the analysis of infection, together with host interference and different treatments (283-285). RNA-seq offers a comprehensive or targeted characterization of the transcriptome, allowing researchers to determine the timing and location of gene expression. Broadly, RNA-seq gives two types of information (**Table 3**): qualitative, such as genome annotation, and quantitative, providing insights into gene expression levels (286).

Table 3: Types of information facilitated by RNA-seq.

Qualitative information	Quantitative information
• Genome annotation • Transcripts orientation • Transcriptional start sites • Exon/intron boundaries (in eukaryotes) • Polyadenylation sites (mainly in eukaryotes) • Isoforms (alternative splicing) (in eukaryotes) • Gene fusions • Variant discovery	• Gene expression levels - *either relative or absolute* • Differential expression - *comparing levels across two or more conditions* • Isoforms expression levels

Bacterial transcriptomics analysis provides insights into regulatory networks, metabolic changes, and potential virulence factors present in bacteria at different treatment settings. It is widely used to understand bacterial response mechanisms to environmental changes, stress conditions, and antimicrobial treatments (47, 48, 263, 287).

1.3.3 Transcriptomics in *S. aureus*

S. aureus exhibits a remarkable ability to thrive as both a colonizer and a pathogen. It adapts to diverse biological environments, such as the human skin, nasal mucosa, gastrointestinal and reproductive tracts, and upon invasion, nearly all human tissue (5, 84). Surviving in such a broad niche requires adaptive responses that impact regulatory mechanisms, from gene expression to transcriptomic, proteomic, and metabolic levels (263).

Transcriptomics analysis of *S. aureus* offers valuable insights into the gene regulation and expression dynamics of genes involved during infection or colonization. Some of the recent *S. aureus* transcriptomics studies are listed in **Table 4**. They can be used to gain insights into the molecular, biological, and cellular responses of *S. aureus* to different stimuli. A better understanding of *S. aureus* in its natural human niche may support strategies for limiting the carriage of *S. aureus* (288, 289). *S. aureus* has been studied under several *in vitro* conditions describing virulence factors (95, 130), attachment mechanisms (95), global regulation (270, 290), stress response (291, 292), nutrient acquisition (293, 294) and adaptation (295, 296).

Overall, transcriptomics analysis can reveal changes in gene expression profiles of both the pathogen and the host cells under infection-related conditions and during colonization (263). For instance, gene expression analysis during persistent *S. aureus* nasal colonization has been studied using quantitative RT-PCR and, more recently, via RNA-seq techniques (14, 297, 298). Advances in the regulatory adaptation of *S. aureus* in nasal colonization have demonstrated distinct transcriptional changes associated with adhesion genes and wall teichoic acid biosynthesis genes (14). Several studies have also explored global *S. aureus* gene expression *in vitro* in response to various stimuli such as neutrophil (299, 300), mild acid (301), biofilm growth (302), blood exposure (303), phagocytosis (299), host adaptation (295), and infection model (270, 304).

RNA-seq based transcriptomics analysis offers a broader and unbiased view of transcriptome by quantifying all genes present within an organisms transcriptome, providing deeper understanding of gene expression patterns and complexities compared to RT-qPCR, which is limited in analyzing a selected number of genes (305, 306). By elucidating the transcriptional landscape of pathogens and host cells, transcriptomic studies contribute to a better understanding of disease mechanisms, paving the way for the development of novel diagnostic and therapeutic strategies (263).

Table 4: Examples of recent transcriptomics studies on *S. aureus* colonization, infection, and adaptation.

Type of study	Host Model/strains	Host Cells/medium	Year; reference
Colonization and/or Infection			
In vivo and ***ex vivo***	Human	Skin and mucus membrane	2022; (14)
In vitro and ***in vivo***	Pediatric patients and Murine model	Skin and soft tissue cells	2021; (307)
In vivo	Murine	Vaginal tract	2019; (6)
In vivo	Human	Nasal swab	2016; (298)
In vivo and ***in vitro***	Cotton rat for colonization, Murine for Bacteremia	Nasal for colonization Blood for Bacteremia	2015; (13)
Co-culture			
In vivo	*S. aureus* and *P. aeruginosa*	Cystic fibrosis lungs	2022; (308)
In vitro	*S. aureus* and *P. aeruginosa*	Brain-heart infusion (BHI)	2020; (309)
In vitro	*S. aureus* and *P. aeruginosa*	Epithelial pulmonary cells	2019; (310)
In vitro	*S. aureus* and *S. epidermidis*	Nasal Swab	2018; (311)
Infection and/or adaptation			
In vitro	Patient with *S. aureus*	Peripheral blood mononuclear cells	2023;(312)
In vitro and ***in vivo***	Children diagnosed with osteomyelitis	-	2023; (313)
In vitro and ***in vivo***	Mice	Liver	2022; (304)
In vitro	*S. aureus* strains	-	2022; (314)
Ex vivo	Human	Skin	2021; (315)
In vitro and ***in vivo***	Human	Nasal epithelial cells	2021; (305)
In vitro	-	Cystic fibrosis lungs	2019; (266)
In vitro	-	S9 bronchial epithelial cells, Thp1 macrophages, Human plasma	2016; (270)

2. AIMS OF THE STUDY

The primary aim of the study was to understand *S. aureus* adaptation to the host environment. This included 1) to investigate the factors driving *S. aureus* throat colonization, with focus on interaction with tonsillar cells and competing organisms; and 2) to investigate which *S. aureus* factors and pathways are activated upon exposure to conditions mimicking nasal environment and infection. By identifying specific bacterial differentially expressed genes (DEGs) during these interactions, our goal is to inform targeted interventions for prevention of colonization and infection.

The hypotheses of this study included:

1) There are differences in total DEGs associated with virulence factors in *S. aureus* when exposed to tonsillar cells for different durations.
2) The *S. aureus* transcriptomic profile is different if exposed to tonsillar cells as a single bacterial species or in a two-species condition.
3) Unique DEGs are expressed in *S. aureus* during exposure to macrophages, nasal medium, plasma or bacterial medium.

Specific objectives:

Paper I

- To establish a tonsillar cell culture model for studying the *S. aureus* transcriptome *in vitro.*
- To explore the *S. aureus* transcriptome when exposed to tonsillar cells.
- To identify key virulence factors exhibited by *S. aureus* when exposed to tonsillar cells.

Paper II

- Perform transcriptomic profiling of *S. aureus* during *in vitro* co-culturing with an interacting throat bacterium and tonsillar cells.
- To identify *S. aureus* key virulence factors in the presence of another throat bacterium.
- To compare *S. aureus* DEGs in a single bacterial species condition (Paper I; monoculture) and a two-species condition (Paper II; co-culture).

Paper III

- Explore the *S. aureus* transcriptome when exposed to macrophages, plasma, nasal medium, and bacterial medium.
- To identify key virulence factors in intracellular and extracellular *S. aureus* when exposed to macrophages.
- To determine virulence factors and functionally enriched pathways in *S. aureus* exposed to plasma and nasal medium versus bacterial medium.
- To identify essential DEGs in *S. aureus* irrespective of exposure conditions.

3. METHODOLOGY

The following section offers an overview of the experimental design used for *in vitro* setups, RNA-seq workflow and data analysis, including optimization and rationale for the choice of methods employed in this study.

In vitro experimental design

To uncover putative advantageous factors which promotes *S. aureus* throat colonization, we developed an *in vitro* tonsillar model using human tonsil epithelial primary cells (HTEpiC; tonsillar cells). To understand *S. aureus* adaptation and response in conditions mimicking nasal colonization as well as infection, we utilized nasal medium, plasma, human monocyte Thp1 cells and the bacterial medium (Tryptic Soy Broth; TSB). Our methodology involved *in vitro* infection assays, RNA-sequencing (RNA-seq) and quantitative real-time polymerase chain reaction (qRT-PCR). Furthermore, we performed functional analyses of significantly differentially expressed genes (DEGs) using gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

The attached **papers I**, **II,** and **III** contain detailed descriptions of the *in vitro* experimental materials and procedures, as well as pipeline for RNA-seq data analysis. The overall experimental design utilized in this thesis is illustrated in **Figure 4.**

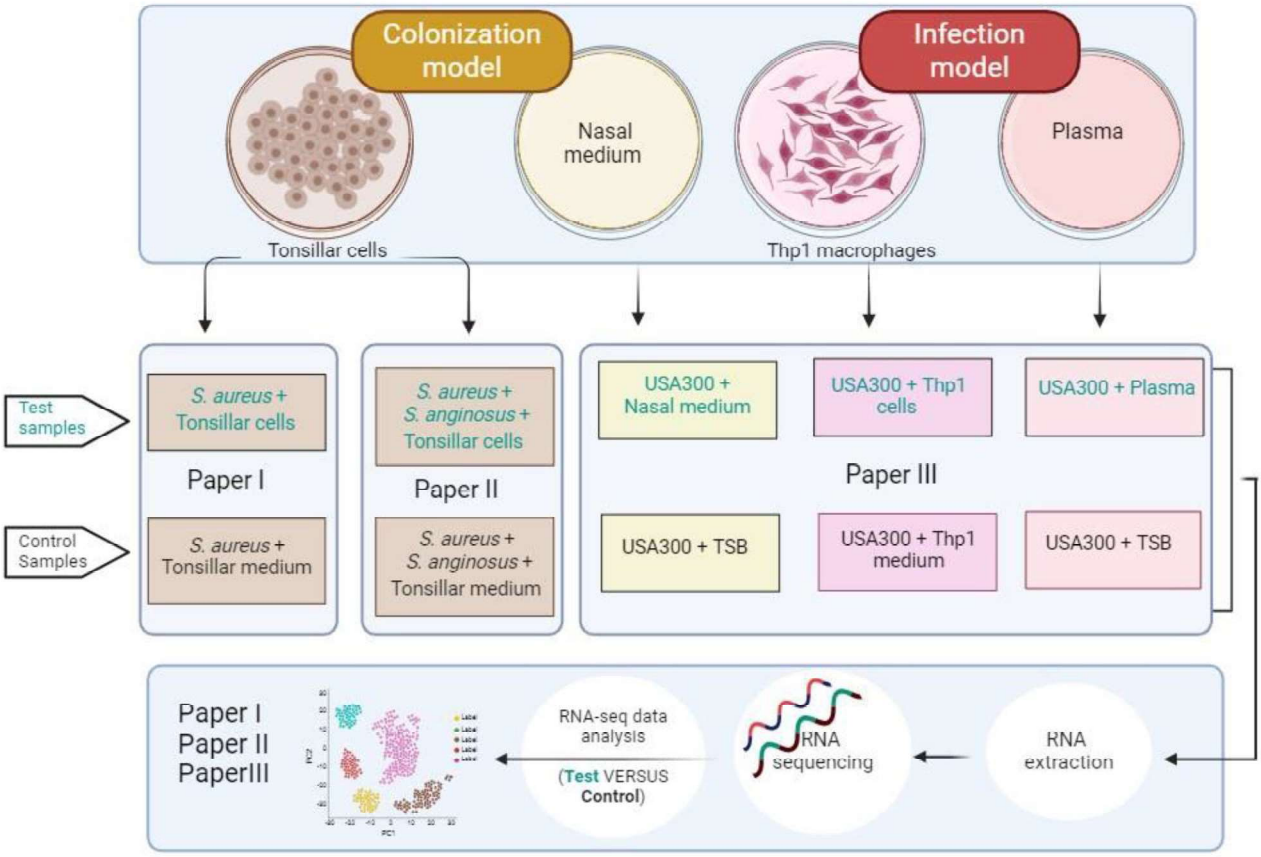

Figure 4: Experimental design employed in papers I to III. *Created using BioRender.*

*Exposure of tonsillar cells to S. aureus (**Paper I**)*

Before considering the *in vitro* host-pathogen interaction study using HTEpiC (tonsillar cells) (316, 317), the cells were cultured for several weeks. The media was changed every 2-3 days until passage 4, to achieve a confluent monolayer. At first, cell culture plates were coated with poly-L-lysin. Tonsillar cells were seeded at a density of approximately (~) 4×10^5 viable cells per well in 6 well plates for the adhesion assay or ~ 7×10^4 viable cells per well in 24 well plates. To prepare the seeding mix, cells were harvested from a culture flask and resuspended in the tonsillar medium containing fetal bovine serum (FBS), Trypsin/EDTA and trypsin neutralization solution (TNS), as described in **Figure 5**.

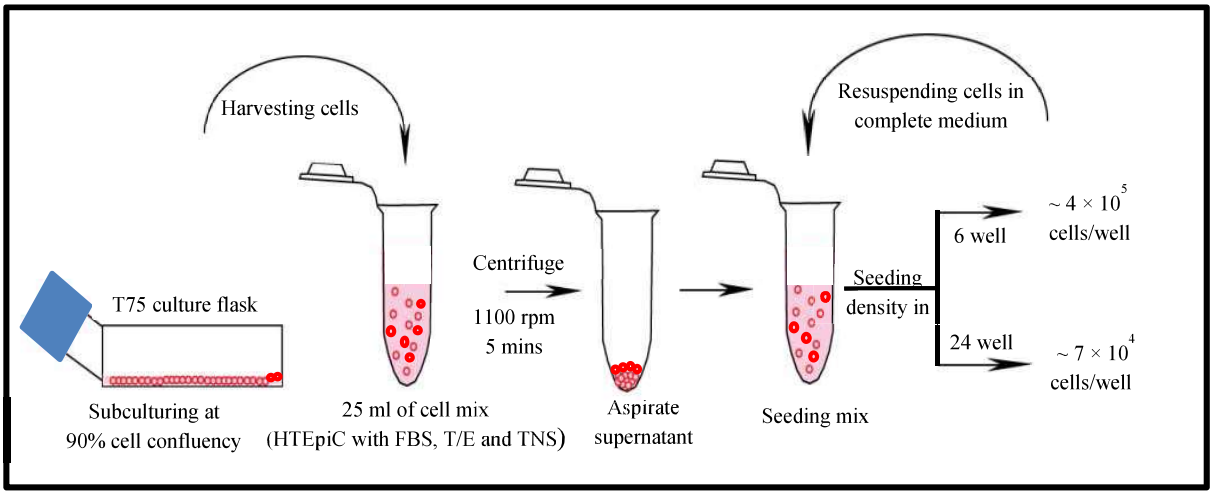

Figure 5: Experimental set up for harvesting and resuspending cells prior exposer with bacteria. Human tonsil epithelial cells (HTEpiC, tonsillar cells) with confluent monolayer growth (> 90% confluency) at passage 4 were processed for subculturing and harvested. After counting cells, the seeding mix was prepared according to the calculation needed for 6 or 24-well plates. The seeded well plate, at appropriate cell density, after confluent monolayer growth was ready to be exposed to bacteria for performing adhesion assay.

S. aureus TR145 at OD600nm of 0.4 was added to tonsillar monolayers at MOI 5 in complete tonsillar medium (without Penicillin/Streptomycin (P/S)) or complete medium (without P/S) and incubated for 1 and 3 hours. Bound *S. aureus* from both time durations (representing the collection from the adhesion assay) was collected using a previously outlined method (96, 318). The harvested bound *S. aureus* (also referred to as Test samples) and *S. aureus* without tonsillar cells (also referred to as Control samples) underwent RNA extraction followed by RNA-seq analysis. Three independent experiments were considered for *S. aureus* with tonsillar cells.

*Co-culture of S. aureus, S. anginosus and Tonsillar cells (**Paper II**)*

We performed co-culturing assays in a similar setup as described above. RNA-seq was performed on a mixed bacterial sample to identify interaction-induced transcriptional alterations in surface-associated populations (bacteria harvested from the surface of the host cell). *S. aureus* and *S. anginosus* were incubated separately (monoculture, only for CFU plate enumeration) and together (co-culture, for RNA-seq) on the 6-well plate with/without tonsillar cells. Following incubation, bound bacteria were recovered, plated, and prepared for downstream processing by preparing lysate of mixed bacteria, which was collected after the infection assay. The harvested bound mixed bacteria (also referred to as Test samples) and mixed bacteria without tonsillar cells (also referred to as Control samples) were subjected to total RNA extraction and RNA-seq. Three independent experiments were considered for *S. aureus* and S. *anginosus* with tonsillar cells.

*Exposure of macrophage and host factors to S. aureus (**Paper III**)*

Thp1 cells were differentiated into macrophage-like cells using phorbol 12-myristate 13-acetate (PMA). *S. aureus* MRSA USA300 was exposed to the macrophages for 1 hour. Afterwards, the bacteria were recovered from both inside the cells ("intracellular") and outside the cells ("extracellular"), along with MRSA USA300 grown in the absence of Thp1 cells. The harvested *S. aureus*, extracellular and intracellular, when had been exposed to Thp1, was labeled as the test sample. This was individually compared with *S. aureus* in Thp1 media, referred to as the control samples. RNA extraction and RNA-seq were then performed on all samples. Two biological replicates were conducted for each condition.

S. aureus USA300 cultures were exposed to different host factors (synthetic nasal medium, human plasma, and TSB) for 1 hour. After incubation, bacteria were harvested and processed for RNA extraction and RNA-seq. Two independent experiments with three technical replicates were conducted for each condition.

Optimization of the experimental protocol

The procedures implemented in **papers I**, **II** and **III** (Briefly described above) resulted from optimization experiments as follows:

Selection of bacterial strains

An adhesion assay pilot experiment was performed as described previously (96), including four different *S. aureus* strains exposed to tonsillar cells. These included a throat isolate *S. aureus* TR145 (spa-type t084, clonal complex 15), a nose isolate *S. aureus* NA196 (spa-type t065, clonal complex 45) from healthy carriers in the Tromsø 6 Study (Røkeberg et al. unpublished), the laboratory strain *S. aureus* MSSA476 (clonal complex 1) (319), and the community-associated CA-MRSA USA300 strain LAC (320), *S. aureus* TR145 (BioSample accession SAMEA112465883), MSS476 (NC_002953.3) and MRSA isolates (accession no. NC_007793.1) had already been whole genome sequenced and were individually co-cultured with human cells. Among these, the highest percentage of adhesion to host cells was shown by *S. aureus* TR145, and this strain was most relevant for exposure to human primary tonsillar cells. Thus, *S. aureus* TR145 was selected for the main *in vitro* experiment and RNA-seq analysis (**Papers I** and **II**).

ATCC 33397 *Streptococcus anginosus* was selected for the co-culturing study as it was identified as a human throat colonizer (321-323). It is ß-hemolytic, belongs to Lancefield's group G, and originates from human throat tissue. This strain was also whole genome sequenced

(https://www.atcc.org/products/33397). *Streptococcus anginosus* belongs to the *Streptococcus anginosus* group (SAG) and is part of the commensal microbiota, mainly colonizing areas such as the oral cavity, oropharyngeal, gastrointestinal, and genitourinary tracts (324, 325). It is known to cause infections like dental abscesses and is linked to throat infections like pharyngitis and tonsilitis, but the role of *S. anginosus* in infection is controversial. Although *S. anginosus* colonizes without harming the host, it may play a role in pathogenesis during synergistic interactions with other oral commensals (326-328).

Additionally, we conducted an agar-based competition assay between *S. anginosus* ATCC 33397 and *S. aureus* TR145, following a previously described method (310), demonstrating their ability to co-exist. Our aim was to perform *in-vitro* co-culturing experiments in the presence or absence of tonsillar cells, using two bacterial species that would not eliminate each other. *S. aureus* was our primary focus, and we sought to observe its transcriptomic response in the presence of another colonizing bacterium, rather than an infectious one. If the other chosen strain were infectious, it would most likely outcompete *S. aureus*, which would prevent us from gaining accurate insight into the transcriptomic profile.

In **Paper III**, we evaluated the adaptation of *S. aureus* to a synthetic nasal medium (mimicking the nasal niche) and to Thp1 cells and plasma, mimicking an infection condition by choosing MRSA USA300 LAC (320). This clonal subtype, classified as pulsed-filed type USA300, is associated with community outbreaks and commonly found in skin infections (329-331). USA300 has acquired mobile genetic elements encoding both resistance and virulence factors, including the Panton–Valentine leukocidin gene and the SCC*mec* type IVa resistance gene complex (320). This selection allows us to investigate the adaptive and responsive mechanisms used by *S. aureus* to adhere or invade the host cell, and the response to different host factors.

Selection of host and host factors

Primary human tonsil epithelial cells (*Sciencell 2560*; referred to as tonsillar cells) were chosen to mimic the throat colonization condition due to their relevance in the tonsil environment. The tonsillar cells, isolated from a tonsil donor, consist of a mixture of stratified squamous and reticulated epithelial cells. The selected *S. aureus* throat strain TR145 was exposed to tonsillar cells, establishing an *in vitro* host-microbe interaction, representing a model that closely resembles throat conditions (**Papers I** and **II**). Previously, a similar transcriptomics study was performed using this cell line with Group A streptococci (317).

Our approach in **Paper III** aimed to understand how *S. aureus* responds to different niches during colonization and infection. We utilized a synthetic nasal medium to mimic nasal secretions (4), representing a primary nasal colonization site. Plasma was chosen to simulate an infection-like condition

and the bloodstream environment (270), as *S. aureus* is a common cause of bacteremia. In addition to these host factors, we included professional phagocytes (macrophages Thp1) (270) to explore the *S. aureus* interaction with the host defense. This allowed us to mimic an infection-like condition and gain insight into *S. aureus* colonization and adaptation to macrophages.

Determination of Optical density (OD)

To determine the optimal OD for exposing the bacterial strains (*S. aureus* and *S. anginosus*) to the tonsillar cells, we initially prepared a fresh bacterial culture (1:10 dilution from overnight culture) in TSB for up to 1 h, representing an OD_{600nm} of 0.8. Subsequently, the bacteria were centrifuged, washed twice in phosphate-buffered saline (PBS) and resuspended in 1 ml filter sterilized PBS. The bacterial suspensions were adjusted to an OD_{600nm} of 0.4 (corresponding to approximately 1×10^8 CFU/ml), in the host culture medium (tonsillar cells medium). For the infection assay, the overnight *S. aureus* MRSA USA300 culture was diluted 1:50 in TSB and grown to optical density 600 nm (OD_{600nm}) = 0.8. This culture was then centrifuged, washed with PBS, and serially diluted. The adjusted bacterial suspension (OD_{600nm} of 0.4) was prepared in the host culture medium (Thp1 cell medium).

Number of cells plated per well

The aim was to achieve conditions where the viability of the monolayer growth of the host cells was optimal for the exposure time points 1 h and 3 h. Experiments were conducted in which tonsillar cells were seeded at varying numbers per well, and a seed density of $\sim 4 * 10^5$ cells/well in six-well plates gave the best results (in terms of viability and monolayer growth in less time) in a 6 well plate. Similarly, in Thp1 cells, a seeding density of $9*10^6$ cells in T175 cell culture flasks was selected.

Multiplicity of infection (MOI)

To determine the MOI required for achieving maximum infection without affecting cell viability, host cells (tonsillar and Thp1) were infected at varying MOIs of 1, 5, 10, 50 and 100. In the tonsillar cells experiments, 1 h and 3 h after infection, cell viability was monitored with lactate dehydrogenase (LDH) release, and the proportion of infection cells was determined. The best results were obtained at a MOI of 5, where less than 3% cytotoxicity was detected in the case of tonsillar cells. A MOI of 5 also allowed us to collect enough bacteria (that were bound to the host cells), for use in the RNA-seq workflow. Similarly, we tested MOI of 50 and 150 for Thp1 cells at both 1 h and 2 h time points during infection. The cytotoxicity assay performed (*Ajayi et al., unpublished*) at MOI 50 after 1 h of exposure of Thp1 cells to MRSA USA300 indicated a higher bacterial recovery with minimal stress to host cells.

Selection of time points

The selection of specific cell types and time points enabled us to investigate *S. aureus*' adaptation in colonization and infection in response to different host-derived factors and host cells. The two main time points were selected in the experiments: 1 h for the infection model, and 1h and 3h for the colonization model. In the colonization model, the aim was to harvest only extracellular *S. aureus* and understand the mechanisms employed by *S. aureus* to remain in the host cell before being internalized, and further analyze transcriptional changes occurring during the duration of exposure to the host cell. This is in accordance with another study, inferring that *S. aureus* maintains nasal colonization within these time frames (332). Additionally, study have shown low number of *S. aureus* internalization into keratinocytes, indicating a colonization stage (333).

Thus, in this study (**Papers I** and **II**), the 1 h time point represents the early colonization stage, while 3 h represents the late colonization phase. After 3h, *S. aureus* would start invading the cells and become intracellular. Thus, the conditions for recovery of bound bacteria after incubation of 1 h and 3 h with human cells (described above) were selected, and adhered bacteria were collected for RNA isolation/RNA-seq and plated for CFU determination.

On the other hand, we explored both extracellular and intracellular *S. aureus* within Thp1 macrophages to retrieve a transcriptome profile from 1 h of infection (**Paper III**). The choice was made considering Thp1 macrophages are professional phagocytes (270), indicating early infection.

RNA-seq Transcriptomics Workflow and Data Analysis

RNA-seq data analysis enabled us to understand gene expression patterns in *S. aureus* elucidating key virulence determinants and adaptation to different environments. This section encompasses an overview of the RNA-seq workflow, including the bioinformatics pipeline. **Figure 6** highlights some points to consider before finalizing RNA-seq workflow and data analysis pipeline.

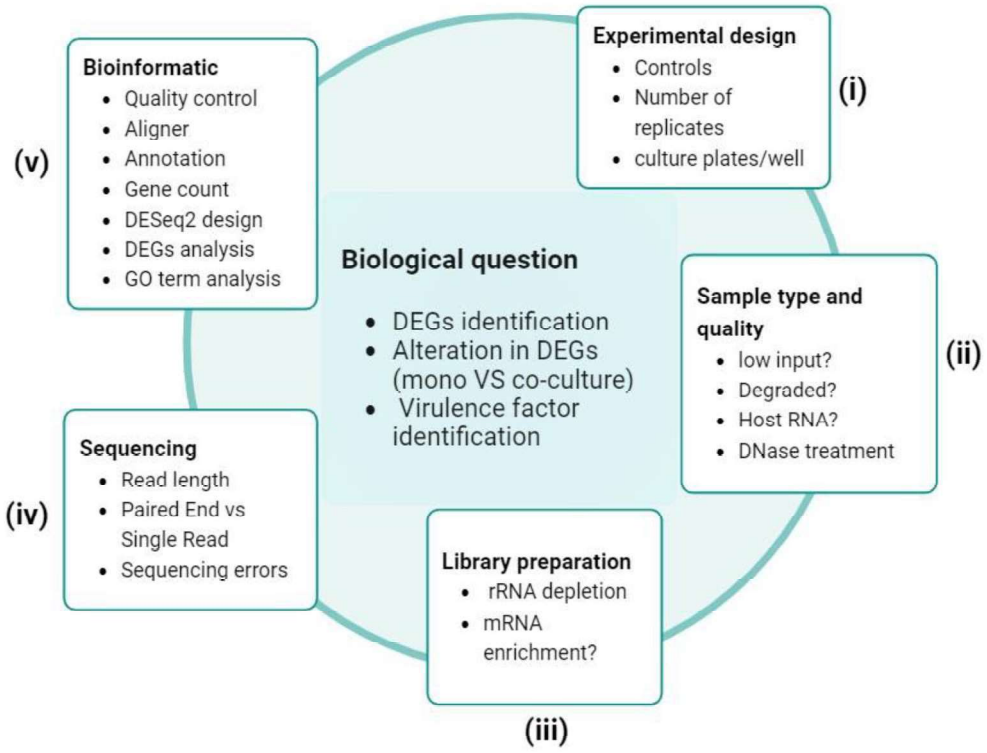

Figure 6: Points of considerations during RNA-seq workflow and data analysis.

RNA-seq workflow

A transcriptome analysis using RNA-seq reveals the specific gene clusters in *S. aureus* that undergo down- or up-regulation when co-cultured with host cells and host factors. The RNA-seq methodology included: **i**) experimental design (defining the aims and designing assay accordingly); **ii)** Extraction (Isolate and purify RNA); **iii**) Library preparation (mRNA enrichment, convert RNA to cDNA, and add sequencing adapters); **iv)** Sequencing (Sequence libraries using NGS platform); and **v)** Data analysis (Trimming, filtering, alignment of reads/mapping, DEseq2 analysis)). Throughout the RNA seq workflow, several questions arose that warranted consideration during the experimental design, and they are briefly described below (**Figure 7**).

Experimental design

The design stage of an experiment is undeniably the most critical step in ensuring the success of an RNA-seq experiment. These decisions include crucial factors such as the type of RNA-seq assay, number of samples, replicates, inclusion of controls, and test conditions (275). We included a minimum of 2-3 biological replicates to ensure valid biological interpretation of the results, and up to 3 technical

replicates (pooled together) per condition for statistical analysis of the data. This is in accordance with best practices for RNA-seq data analysis (275).

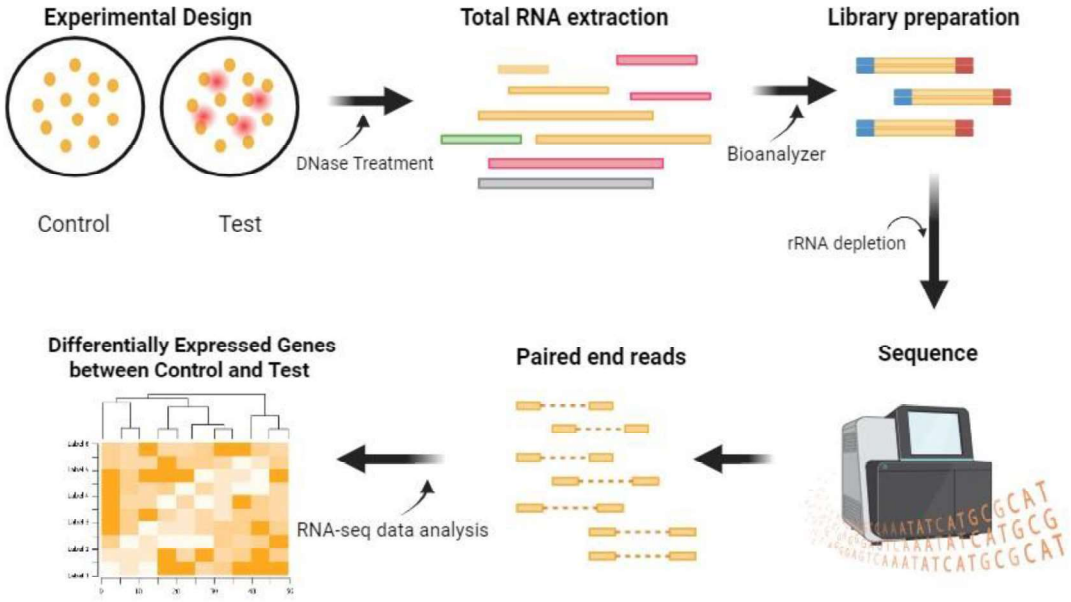

Figure 7: Things to be considered during RNA-seq workflow and data analysis. *Created from BioRender.*

Extraction

The first step in characterizing the transcriptome involves isolating and purifying RNA. To achieve this, we established a protocol for extraction and pre-processing of high-quality total RNA from monoculture, co-culture, and infection experimental samples. This involved utilizing the RNeasy minikit (Qiagen) for RNA isolation, a common method for isolating high-quality RNA from various sample types, including cells, tissue and bacteria. Thereafter, DNase treatment using Heat and Run kit (ArcticZymes) was performed for all RNA samples, to exclude genomic DNA contamination (334). Then, RNA quantitation and quality assessment were performed using a NanoDrop 1000 spectrophotometer (ThermoScientific) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively. The RNA integrity number (RIN) value (range of 1 to 10 for RNA sample) obtained from the bioanalyzer assay served as a crucial indicator

of RNA quality. Maintaining the integrity of RNA molecules is important to capture a snapshot of genes expression at the moment of RNA extraction (335). A RIN of greater than 8 is considered good, indicating fully intact RNA.

The quality and quantity of the input material have a significant impact on data quality; therefore, meticulous care was taken while isolating and preparing RNA for sequencing. Consequently, following the collection of samples from *in vitro* assays, the bacterial pellet was preserved with RNAprotect (Qiagen) to safeguard RNA integrity and protect gene expression profiles at the time of sample collection. Freezing bacterial samples at -70°Cand using the RNAprotect reagent helps stabilize RNA molecules until the RNA extraction process. The RNA-protected samples underwent enzymatic lysis using lysozyme and lysostaphin, and mechanical disruption using a homogenizer, employing an optimized disruption cycle and duration according RNAprotect Bacteria Reagent Handbook guideline. The total RNA with DNase treatment was utilized for RNA-seq library preparation.

Library preparation

This step involves generating a collection of RNA fragments compatible with sequencing (336). The process involved depletion of rRNA, a major RNA constituent of cells, so that only mRNA remained to sequence. This was achieved using the RiboCap depletion kit (Lexogen) followed by reverse transcription, and addition of sequencing adapters and amplification using Lexogen's CORALL™ Total RNA-seq Kit with RiboCop. Opting for bulk RNA seq (coding and non-coding) or total RNA sequencing, ensures comprehensive sequencing coverage, even with low concentration of RNA input. Ribo depleted RNA was used directly as the template for CORALL reverse transcription. Rather than employing enrichment methods targeting specific transcripts (e.g. mRNA, lncRNA, miRNA), we pursued rRNA depletion and performed bulk RNA seq. The enrichment process enables direct mRNA sequencing, which would reduce the cost. If rRNA removal has been excluded, deeper sequencing would be required to compensate for the sequencing reads wasted on rRNA (337). Additionally, the cDNA synthesis step was performed in a manner that preserved the original strand orientation of the transcript, thereby generating "strand-specific" or "directional" libraries.

Sequencing

The choice of sequencing platform and its configurations, along with other parameters such as read length, and output- depends on the goals of the study. NGS technologies can generally be grouped into two main categories: short-read (also called second generation) sequencing and long-read (also called third generation) sequencing, each offering distinct advantages for RNA-seq applications (338). In our study, we chose the Illumina MiSeq™ 550 sequencing platform with short-read sequencing mode

primarily due to its cost-effectiveness per base and its capability to generate billions of reads in a massively parallel manner. We selected the 2x150 bp setup, commonly referred to as "paired-end 150 bp sequencing" or "PE150", as it is one of the most prevalent setups for short-read sequencing. The sequencing runs generated approximately 350-400 million reads per flow cell, resulting in a data output of 100-120 gigabytes (Gb) per flow cell.

The selection of the sequencing platform (and flow cell), together with the number of multiplexed samples, determines the sequencing depth. Sequencing can be conducted in two modes. Each cDNA fragment can be sequenced either from only one end, called single-end sequencing, or both ends, called paired-end (PE) sequencing. Typically, the former is more cost-effective and faster than the latter. However, paired-end sequencing offers advantages in detecting genomic rearrangements and repetitive sequence alignment more effectively than the single-end configuration, as it gathers more information from each fragment (339).

Data analysis

The ultimate and most fulfilling step in an RNA-seq experiment involves assessing data quality and extracting biologically relevant insights. Generally, data generated from an RNA-seq platform are termed "reads". These raw reads were then processed in an optimized prokaryotic RNA-seq data analysis pipeline, which included steps such as trimming, filtering, mapping reads, DEseq2 analysis and visualization of DEGs analysis. Each step is elaborated upon below.

RNA-seq bioinformatics pipeline

The retrieved bacterial data were analyzed to explore DEGs present in different conditions of interest. An "in-house" bioinformatic pipeline was developed for this study, based on various existing software and guidelines. The detailed RNA-seq bioinformatics pipeline (**Figure 8**) is described below:

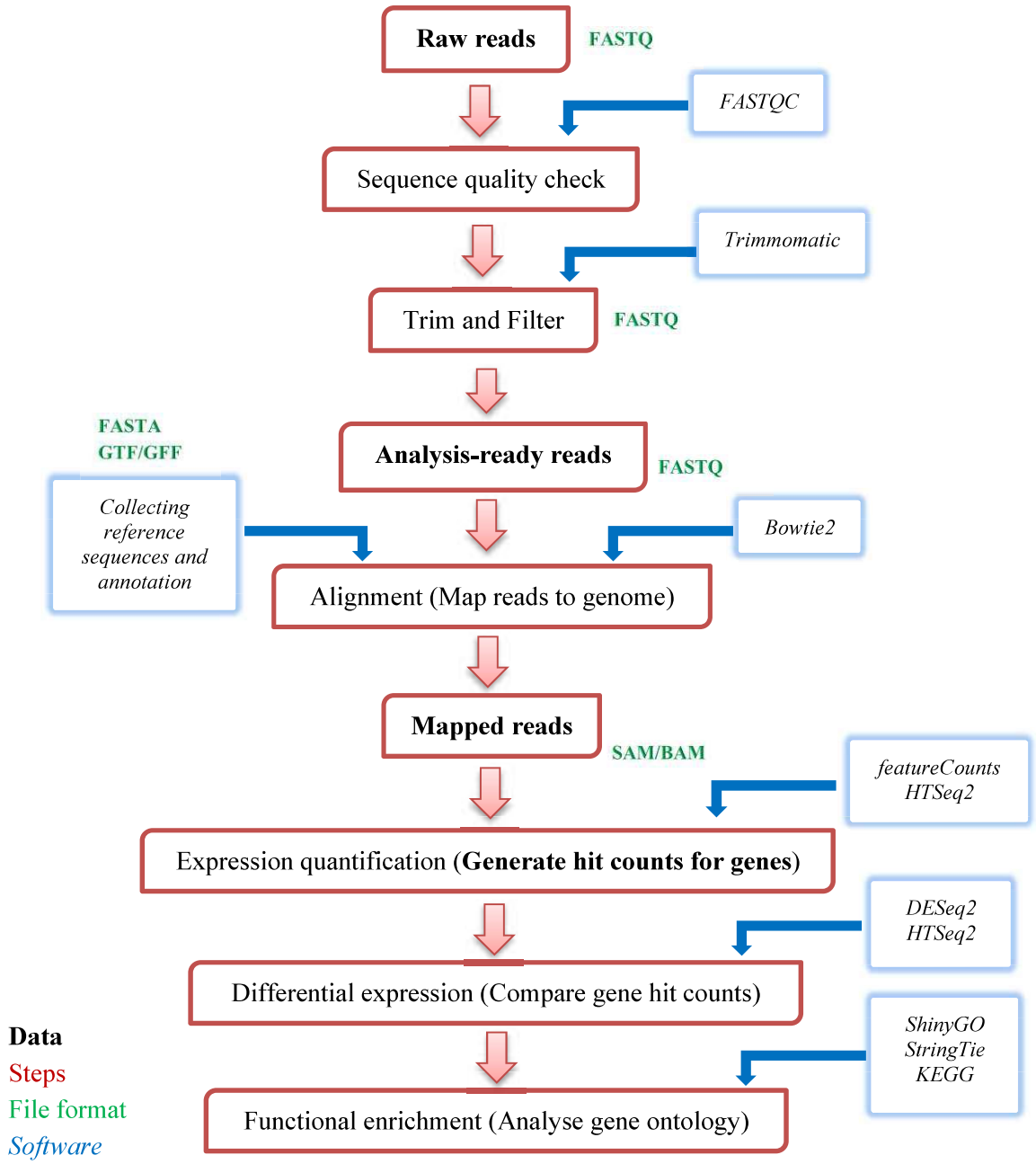

Figure 8: RNA-seq bioinformatics pipeline and analysis software for prokaryotes. Following read quality assessment and trimming of adapter and low-quality reads, reads are aligned to a reference genome. Next the expression level of each gene is estimated by counting the number of reads that map to each gene or full-length transcript by featureCounts or HTSeq2. Downstream analyses with RNA-seq data include DEGs analysis using DESeq2, and functional enrichment analysis using ShinyGO, StringTie, and KEGG.

Quality Check

The quality control process involves analyzing the sequence quality of RNA seq raw reads, including checking of GC content, presence of adaptors contamination, low quality reads at 3 or 5 prime ends, and over-represented or duplicated reads (275). This is done to identify sequencing errors or PCR artifacts, or contamination. Acceptable ranges of duplication and GC contents may vary based on the experiment and organism, but consistency among samples within the same experiment is crucial. FATSQC is one of the popular tools for quality assessment, especially for analyzing Illumina-generated reads (275). We implemented pre-processing of our raw reads from each library using FASTQC/0.11.9-Java-11. Filtering (removal of adaptor dimer reads) and trimming (removal of low-quality bases) were performed by Trimmomatic/0.39-Java-11 (340). Following this step, only sequences with a quality score (Q) greater than or equal to 20 and a minimum of 55 nucleotide sequence length were retained in the dataset. A final quality check was performed on the trimmed files to visualize the clean raw sequencing data for alignment.

Alignment

The clean reads are typically mapped to either a genome or a transcriptome. We chose to map to genome because mapping to a transcriptome may have reduced the mapping efficacy, as reads coming from unannotated transcripts would be lost (275). In our data analysis pipeline, we quantified transcript levels using RNA Seq data by aligning the reads to a reference genome, counting the reads per feature, and then conducting DEGs analysis. Numerous free software packages and read mapping algorithms are available for this purpose, such as Bowtie2, Star, HTSeq, Tophat, and Cufflinks.

Two *S. aureus* strains, TR145 and USA300 LAC, had already been whole genome sequenced (fasta file), and their corresponding annotated file (GTF) was available. The alignment of clean raw reads was performed by Bowtie2/2.4.4-GCC-10.3.0. For all the samples, percentage of mapping efficacy was retrieved from Bowtie2 (**Papers I, II** and **III**) along with the Sequence Alignment Map (SAM) file. Samtools/1.14-GCC-11.2.0 was used to convert the SAM file into a Binary Alignment Map (BAM) file. The BAM file was sorted by name, and an index file (.bai) was generated. These processed files were utilized for further downstream analysis.

Expression quantification

Before conducting gene expression analysis, the reads mapped to each gene were quantified using the HTSeq2 counting tool (**Paper I**) and featureCounts (**Papers II and III**) to count the number of reads assigned to each gene (341, 342). This process required the utilization of a GTF file containing gene models and a sorted bam file to generate counts for each gene. The same GTF file employed during the

alignment step was utilized for gene counting. HTSeq count and featureCounts generated folders with gene count files and gene matrix, respectively. In **Papers II** and **III**, featureCounts was preferred due to its superior speed compared to HTSeq, along with its ability to produce a gene matrix. The gene matrix can be used directly for DESeq2 analysis whereas gene count files generated from HTSeq2 should be converted to a gene matrix before performing DESeq2.

DESeq2 to analyze count data

After aligning reads to a reference and generating count files, DESeq2 in R was used to explore any DEGs present in the sample (343), as explained below:

- *Creating DESeq2 object:* DeSeq2 object creation involved use of count matrix and metadata table, and design formula. The design formula specifies the column(s) in the metadata table and how they should be used in the analysis. This tells DESeq2 that for each gene evaluation of gene expression, change was performed with respect to which different levels.

- *Normalization of read counts*: DeSeq2 offers internal normalization, where several factors influencing read counts were tackled. One source of variation among samples is the difference in library size, representing the total number of reads generated for a given sample. Library size normalization methods were used to correct for sequencing depth and RNA composition. Likewise, the difference in biological replicates was also normalized. This allows meaningful comparisons of gene expression across samples (344).

- *Remove genes with missing p-value adjusted and gene names:* The result table retrieved from DE tests contains the padj column which represents the p-value adjusted for multiple testing and it is the most important column of the results. The DE was further curated to give only those genes which shows p- value adjusted (padj) along the indication of gene name.

- *Define DE genes by setting threshold on padj (and lfc)*: Like padj, another important result column in the DE test is log2foldchange (lfc). Threshold for padj was adjusted to less than 0.05 (padj < 0,05) and for log2foldchange greater than 2 (lfc > 2). Any genes that followed these two thresholds were a good starting point for identifying significant genes.

Exploratory visualization of DEGs in R

The DEGs were visualized in R using plots such as PCA, heatmaps, and volcano plots. This visualization enabled us to discard any samples that was an outlier to the sample clustering, gave us a confident that our biological replicates were consistent, and revealed several gene expression signatures.

Functional Enrichment Analysis

Gene ontology (GO) enrichment analysis using ShinyGO and KEGG analysis using String Tie was performed to identify genes involved in significantly enriched pathways after exposure to host and host factors. Only pre annotated genes were processed for functional enrichment analysis. Under the thresholds of $p < 0.05$ and false discovery rate (FDR) < 0.05, genes involved in biosynthetic, metabolic, and cellular process were revealed.

Significance of Gene Expression Analysis

Expression analysis of relevant determinants enables us to look at how or why *S. aureus* specializes in the throat niches and how variation in RNA sequence correlates with the degree to which *S. aureus* can cause disease or virulence. Differential gene expression analysis offers insights beyond whole genome composition, revealing differences in gene expression patterns that may be crucial for understanding *S. aureus* adaptation and responses. Similarly, the exploration of DEGs identified in USA300 when exposed to human and host factors will provide an understanding of the mechanisms employed by *S. aureus* to adhere and persist within the host and different environments. By identifying specific genes expressed and enriched biological pathways in *S. aureus* exposed to human macrophages, this investigation sheds light on the strategies employed by *S. aureus* to evade host defenses and thrive within a host environment. Likewise, studying *S. aureus* cultured in host-derived media such as human plasma and nasal medium, reveals the significant genes that are expressed by *S. aureus* to adapt to host factors, allowing it to be viable and flourish in these specific host environments. By elucidating the significant genes expressed under these conditions, we will better understand the molecular mechanisms underlying *S. aureus* pathogenesis and host adaptation.

Validation by qRT-PCR

Validating RNA-seq results through qRT-PCR is considered best practice in confirming candidate gene expression to enhance the reliability of RNA-seq data and reduce potential errors stemming from diverse sequencing platforms. In **Paper I**, we employed qRT-PCR validation to confirm findings from RNA-seq. This validation mainly requires utilization of the same RNA material used for RNA-seq and selection of targeted genes (*ilvC, metI, emp, icaA, rpsT*, and *metE*) identified from DEGs analysis. The genes were selected based on expression level, and functions involving cellular process, molecular functions and/or biological process. This validation approach augments confidence in the accuracy of estimated gene regulation, at least its expression pattern. Since we used three biological replicates in our RNA-seq experiments and the datasets generated from these replicates were consistent with each other, further validation via qRT-PCR may not be mandatory. Moreover, the RNA-seq method and data analysis approach are considered robust enough even without validation by qRT-PCR (345).

4. SUMMARY OF RESULTS

Paper I: Exploring differentially expressed genes of *Staphylococcus aureus* exposed to human tonsillar cells using RNA sequencing.

In this paper, we identified essential factors exhibited by *S. aureus* TR145 during exposure to human primary tonsillar cells using an *in vitro* tonsillar model and RNA-seq to investigate their interaction.

- We demonstrated the suitability of using HTEpiC as an *in vitro* model for investigating key factors in *S. aureus* essential during exposure to human tonsillar cells.

- *S. aureus* multiplied both in the absence and presence of the tonsillar cells without inducing significant cytotoxicity level after 1h, with a slight increase observed after 3 h.

- RNA samples from the 3 h time point revealed over 55% of RNA reads uniquely mapped to the reference genome (*S. aureus* TR145), whereas samples collected after 1 h of exposure showed less than 20% mapping efficacy in most cases.

- Principle Component Analysis (PCA) plot displayed a clear clustering of the three biological replicates of *S. aureus* harvested at 1h and 3 h in the presence or absence of tonsillar cells.

- We found a total of 508 (300 upregulated + 208 downregulated) significantly differentially expressed genes (DEGs) in *S. aureus* with tonsillar cells versus *S. aureus* alone at 1 h and 3 h.

- We found *S. aureus* expressing several essential factors when exposed to tonsillar cells. Upregulation of genes such as *isdACEFHI*, *sbn*ABCDE, *dps, sle1* and *icaA* were exclusively expressed at 3 h of exposure to tonsillar cells.

- In early colonization phase (1 h), the genes *metE, sdrD* and *msrA2* were upregulated while *emp, chp* and *sbnH* were downregulated.

- Gene ontology analysis revealed that upregulated genes after 3 h exposure to tonsillar cells were involved in biological adhesion, biosynthesis, as well as iron acquisition and transport.

- There was a good correlation between RNA-seq and RT-qPCR, validating the RNA-seq data.

Paper II: Co-culturing with *Streptococcus anginosus* alters *Staphylococcus aureus* transcriptome when exposed to tonsillar cells.

In this paper, we explored the transcriptomic profile of *S. aureus* TR145 in the presence of *Streptococcus anginosus* during exposure to human primary tonsil cells. We employed the same experimental set-up as described in paper I to make it comparable to paper II.

- We observed minimal cytotoxicity to tonsillar cells (below 5%) after co-culturing of *S. aureus* and *S. anginosus* for 1 h or 3 h.

- The recovery of surface-bound bacteria increased over time, and thus, all samples were suitable for RNA-seq.

- Most reads mapped to the *S. aureus* reference genome.

- A PCA plot showed clear clustering of RNA reads from the three biological replicates.

- A total of 332 significant DEGs at 1 h and 279 significant DEGs at 3 h were identified in *S. aureus* co-cultured with *S. anginosus* in the presence of host cells compared to *S. aureus* co-cultured with *S. anginosus* in the absence of host cells.

- Of the 297 obtained DEGs, 185 genes were exclusively present in *S. aureus*, while the remaining 112 DEGs could result from the expression of both *S. aureus* and *S. anginosus* genes.

- We identified upregulation of specific adhesion factors (such as *isaB, sdrD* and *icaA)* and virulence genes (such as *lukDv_1, lukDv_2, aur, hlgA, hlgB* and *sbi*).

- Gene ontology (GO) analysis revealed enriched terms such as defense response and response to bacterium after 3 h of co-culturing, along with riboflavin biosynthesis after 1 h. Additionally, the overlapping DEGs at 1h and 3h indicated enrichment in branched chain amino acid biosynthetic processes, as well as cell adhesion.

- The *S. aureus* transcriptomic profile was significantly altered in the monoculture condition compared to co-culture with *S. anginosus*, especially in adhesion, virulence, iron-acquisition, and iron homeostasis genes.

Paper III: Transcriptomic responses of *Staphylococcus aureus* USA300 LAC during exposure to human Thp1 macrophage and host factors.

In this manuscript, we investigated the MRSA USA300 LAC transcriptome *in vitro* when exposed to Thp1 cells (referred to as "host"), plasma and nasal synthetic medium (referred to as "host factors") as well as TSB, for 1 h.

- Our results showed differences in gene expression patterns in USA300 exposed to host (Set 1) and host factors (Set 2), impacting virulence, metabolism, and stress response.

- Thp1 remained viable at MOI 50 as evaluated by measuring the release of LDH.

- Set 1 showed a greater number of DEGs in intracellularly located USA300 compared with extracellularly located USA300. More than 100 genes show increased expression in intracellular USA300 within Thp1.

- In Set 2, USA300 exposed to plasma and nasal medium triggered numerous DEGs, after 1 h of culturing. Exposure to nasal medium resulted in a significant upregulation of >200 genes in USA300 LAC, while exposure to plasma resulted in upregulation of 150 genes.

- Our study of USA300 exposed to host cells did not reveal any significant DEGs involved in any heme transport system (*hts*) genes or iron surface determinant genes (*isd*).

- Exposure to plasma resulted in upregulation of virulence factors related to capsular polysaccharide in USA300.

- A notable downregulation was observed in 45 ribosomal protein genes in USA300 when exposed to nasal medium.

- Genes that were continuously expressed in USA300 regardless of culturing conditions were identified, including genes encoding adhesion factors and siderophore-producing genes, which showed upregulation across all conditions.

5. GENERAL DISCUSSION

Why this kind of study?

S. aureus throat carriers face a higher risk of *S. aureus* infections compared to non-carriers, and they are prone to recurrent *S. aureus* colonization even after decolonization treatment with intranasal antimicrobials like mupirocin (251, 346-351). The problem with MRSA eradication and recurrence is exemplified in a recent publication on MRSA throat carriage in two healthcare workers, where unsuccessful eradication attempts resulted in tonsillectomy. However, it is essential to note that removing tonsils is not considered a definite solution for persistent MRSA throat carriage (47). This highlights the challenges associated with managing MRSA carriage in the throat. Studies have shown a higher prevalence of *S. aureus* throat/oropharyngeal carriage than nasal carriage among healthy individuals, including those within the health care system (155, 185). The high recolonization ability, particularly in the oropharynx, suggests a need for alternative decolonization protocols (185). Thus, a growing importance is placed on investigating *S. aureus* throat colonization, especially in cases of prolonged carriage and challenges with recolonization (47).

One strategy employed to identify specific *S. aureus* colonization factors is exploring its transcriptome under different conditions and over various time durations. Transcriptomics has emerged as a leading approach in microbial pathogenesis research and vaccine development, providing valuable insights into the complex interplay between pathogens and hosts in both health and disease (284). The transcriptome of *S. aureus* in the throat has not been well characterized. The mechanisms involved in *S. aureus* attachment and colonization in the throat are unclear, highlighting the necessity for *in vitro* and *in vivo* studies (151, 298, 352). Our utilization of an *in vitro* tonsillar model designed to simulate *S. aureus* throat colonization demonstrates distinct features associated with *S. aureus* colonization of tonsillar cells as single species and in co-culture with *S. anginosus* (**Papers I** and **II**). The interactions between *S. aureus* and *S. anginosus* in the throat are poorly understood, including their influence on growth and metabolism during throat colonization. Bacterial co-culture can induce interspecies competition for nutrients, space, and attachment sites, thereby enhancing antibiotic resistance and virulence (353).

By characterizing the *S. aureus* transcriptomic profile during adaptation to synthetic nasal medium, mimicking the nasal environment, and to professional phagocytes Thp1 macrophage and plasma, mimicking infection conditions (**Paper III**), we can detect which genes show significant expression changes under conditions resembling an *in vivo* situation. This is a prerequisite for exploring alternative treatment strategies, including development of novel drug targets and vaccine alternatives (354).

Overall transcriptomic response of *S. aureus*: monoculture versus co-culture

This thesis represents the first comprehensive exploration of the *S. aureus* transcriptome during the establishment of throat colonization, mimicked by an *in vitro* tonsillar cell model, both in mono (**Paper I**) and co-culture (**Paper II**) conditions. Our approach ensures comparability with other *in vivo* and *in vitro* data from studies involving non-professional phagocytic cells such as keratinocytes, osteoblasts, fibroblasts, epithelial, and endothelial cells (355). We have shown the adaptability of *S. aureus* in tonsillar cells and revealed that using a colonizing throat strain during co-culture conditions led to expression of essential virulence factors, including genes encoding secreted proteins that remain intact in tonsillar cells. A similar finding was reported in a study using invasive strains focusing on intracellular bacteria, and no bacterial killing was observed despite strong activation by *S. aureus* in host cells like osteoblast and keratinocytes (355). This ensures the ability of *S. aureus* to remain viable in the host cells models. We have used several stressors in this study, such as two different host cells, *S. anginosus*, and various host factors, to identify *S. aureus* responses. There are also other studies focusing on the *S. aureus* transcriptome performed under environmental stressors in cell culture such as aerobic versus anaerobic growth, low temperature, and hydrogen peroxide (356, 357), indicating that *S. aureus* adapts to stress through various mechanisms, including adjustment in energy metabolism, activation of stress resistance-related genes and *sigB* regulatory systems to facilitate stress response and environmental adaptation (314, 354).

Our transcriptomic profiling of *S. aureus* exposed to tonsillar cells (**Papers I** and **II),** demonstrates essential bacterial factors required to adhere to the host cell. We identified 300 upregulated and 208 downregulated genes that were differentially expressed in the throat strain *S. aureus* TR145 adhered to tonsillar cells (**Paper I**). This finding is consistent with previous research on *S. aureus* transcriptional adaptation during colonization in the nose, revealing the importance of variation in gene expression patterns in response to niche adaptation and physiological conditions (14). However, during the co-culturing of *S. aureus* with S. *anginosus* in the presence of tonsillar cells, the number of significantly upregulated genes was reduced (~ 150 genes), along with a similar reduction in downregulated genes (**Paper II**). This observation suggests that in the presence of a competing bacterium, *S. aureus* probably does not need to express many genes for adherence and survival in tonsillar cells, indicating a potential adaptive response where *S. aureus* and *S. anginosus* co-exist.

Moreover, some bacterial species can generate substances that give them with a competitive advantage when co-cultured with another bacterium, but not necessarily kill the other bacterium. In line with this, another study shows that *S. pneumoniae* can influence *S. aureus* growth through the secretion of hydrogen peroxide, suppressing *S. aureus* growth and influencing expression patterns and downregulation of virulence genes (352). Several specific competitors can significantly affect *S. aureus*

colonization, indicating a complex interplay among different bacterial species in the human microbiome. Taken together, our finding suggests that although the number of DEGs in co-cultured *S. aureus* is less than in monocultured *S. aureus*, the presence of *S. anginosus* has affected *S. aureus* for better adaptation and expression of essential virulence factors in the co-culture setting. This could also suggest that *S. aureus* is able to use the nutrients either from the host or another bacterial species.

Transcriptional alteration at different time points

Transcriptomic data may elucidate whether the early response between two bacteria involves competition for resources, metabolic adaptation and expression of host-directed virulence factors (296).We observed an increase in the expression of toxin-producing genes in *S. aureus* with prolonged exposure (3 h) to tonsillar cells, especially in the presence of *S. anginosus* (**Paper II**), which was not activated when *S. aureus* was alone interacting with tonsillar cells (**Paper I**). This suggests enhanced virulence traits in *S. aureus* in the late phase of colonization. Other studies have shown that *Pseudomonas aeruginosa* and *S. aureus* compete for resources and metabolic adaptation (358), and in an *in vivo* co-culture setting, *S. aureus* can act as a source of iron for *P. aeruginosa* (359). Additionally, an *ex vivo* investigation has indicated significant alterations in the *S. aureus* transcriptome during early adaptation to the lung environment, with extensive changes observed within 30 minutes of infection followed by fewer changes 2 h post-infection (295). We have not evaluated the internalization efficacy of *S. aures* into tonsillar cells because, given their non-professional phagocytic nature, a low internalization rate was anticipated (360). Therefore, the chosen time points have given insights into the transcriptional changes required for *S. aureus* to attach tonsillar cells in the early (1 h) and late (3 h) colonization state.

The unique gene expression patterns at certain time points, suggest *S. aureus'* ability to adhere and adapt to the changing host environment over time. Our results show that, specific genes are upregulated when *S. aureus* interacts with tonsillar cells (monoculture setting) (**Paper I**). In the early phase (after 1 h), genes related to methionine biosynthesis (*metE*), adhesion (*sdrD*) and cell division are activated. The activation of *sdrD* was not observed in 3 h of exposure to host. Mainly at 3 h, genes associated with iron acquisition (*isdACEFHI*), iron hemostasis (*sbnABCDE*), cell attachment, and stress response (*katA*) are upregulated. Studies on *S. aureus* colonization have detected the expression of specific genes involved in adhesion, including *clfB, fnbA, sdrCDE, isdA, sasF, ebpS*, and *atlA* (14, 103, 130, 361) at various time points. For instance, in a skin colonization model, early colonization spans from 15 mins to 74 h, while late colonization is characterized as 5-8 days (315). Expression of adhesion molecules (*clfA* and *fnbA*) increases during colonization, and immune evasion genes activate during late colonization (315). Our findings validate the identification of genes crucial for early adaptation, detected within 1 h of host

exposure, with variation observed within only 3 h. Additionally, this temporal pattern confirms the time sensitive nature of adhesion gene activation.

Another study on the transcriptional response of *S. aureus* to its natural niche indicated activation of specific genes, including *metE, sdrD, isdAB, sbnABC* and *katA*, in both *in vivo* (nasal swab) and *in vitro* (synthetic nasal medium) conditions, collected at least four times in one year period (298). The activation of these genes also shown in our studies (**Papers I** and **II**), underscore their significance not only in nasal colonization but also in throat colonization during early colonization phase.

In the late phase (3 h) of exposure to *S. anginosus* and tonsillar cells, *S. aureus* showed expression of hemolysin toxin; however, it was absent at 1 h exposure (**Paper II**) and when *S. aureus* was exposed alone to tonsillar cells at 1 h and 3 h (**Paper I**). Similarly, another study focusing on virulence gene expression in *S. aureus* during a cutaneous infection model at different stages reported alpha-hemolysin expression as early as 2 h after infection (362). This suggests that in our study, *S. aureus* produces certain secreted proteins in the presence of *S. anginosus*, and this effect is temporal, becoming activated only at 3 h of exposure.

S. aureus key determinants in a tonsillar model

We identified several virulence factors in *S. aureus* during the encounter with the host and competing bacterium. These include genes encoding virulence factors such as adhesins, surface proteins, and secreted proteins, along with genes involved in iron-acquisition, iron-homeostasis, amino acid metabolism, metabolic pathways, intracellular adhesion, and riboflavin biosynthesis. Studies have demonstrated that *S. aureus* can regulate the expression of virulence factors depending on environmental cues, allowing it to adapt to various microenvironments by expressing virulence factors such as protein A, coagulase, staphylokinase and toxins that contribute to its pathogenicity (363).

With the presence of *S. anginosus* and tonsillar cells, the transcriptome of *S. aureus* is significantly altered compared with *S. aureus* in presence of tonsillar cells without *S. anginosus* (**Paper I**). For instance, upregulation of iron-regulatory genes primarily occurs in *S. aureus* in the presence of tonsillar cells without *S. anginosus* **(Paper I)**, along with an increase in virulence factors encoding secreted proteins (sbi, lukDv, aur, hlgAB and slpF) during co-culturing (**Paper II**). Our findings on virulence gene expression align with prior co-culture studies involving *S. aureus* and *Pseudomonas. aeruginosa* (364, 365). Additionally, the interaction between MRSA USA300 and *P. aeruginosa* leads to the expression of virulence factors that reduce metabolism in *S. aureus* (366-368), and further enhanced virulence and antibiotic tolerance (369). *S. aureus* can enhance the virulence of *P. aeruginosa* through the release and assimilation of peptidoglycan component N-acetyl glucosamine (GlcNAc) (364, 370)

Additionally, studies demonstrate the promotion of *S. aureus* colonization of lung tissue in the presence of *P. aeruginosa* due to the upregulation of cell receptors in the lung tissue, which are absent in *S. aureus* infection alone (371). This implies that the presence of another bacterium influences the adaptation capacity of *S. aureus*.

In our co-culture setting, certain virulence factors are inactivated, while others exhibit altered expression levels compared to a monoculture environment. Co-culture serves as a better representation of *in vivo* biological conditions, and can reveal differential expression related to fitness, virulence, and the transcription of virulence-associated genes. In **Paper II**, we explored the responses triggered by the encounter of *S. aureus* and *S. anginosus*, in the presence of tonsillar cells for 1 h and 3 h. Here we detected several unique virulence factors compared to our monoculture study (**Paper I**). For instance, during the initial hour of co-culturing, we observed an increase in the expression of genes encoding adhesion proteins such as *isaB*. Additionally, the upregulation of *sdrD*, typically observed at 1 h in monoculture settings, was expressed at both 1 h and 3 h of co-culturing. Importantly, we observed upregulation of genes such as *hlgA, hlgB*, *aur, lukDv_1, lukDv_2* and *splF*, particularly related to encoding secreted proteins, while iron-regulatory genes remained inactive. This upregulation is influenced by iron-limiting conditions encountered by *S. aureus* upon contact with tonsillar cells and in competition with *S. anginosus*. Free iron concentration *in vivo* is limited compared to laboratory conditions. Activation of the *agr* system triggers the expression of toxins, facilitating invasion of the host, while downregulation of the *agr* system indicates the colonization state (362). Overall, transcriptomic profiling in **Papers I** and **II** highlights the significance of variability in gene expression related to virulence and adhesion in response to niche adaptation, competing bacterium, including time durations.

Enriched pathways in a tonsillar model

The upregulated genes identified in *S. aureus* in the absence of *S. anginosus* (**Paper I)** indicate pathways enriched in biological processes, including iron-ion transport. Similarly, several genes identified in *S. aureus* during co-culturing with *S. anginosus* and tonsillar cells (**Paper II**) after both 1 h and 3 h of exposure, resulted in pathways highly enriched in biosynthesis and metabolic pathways including adhesion. This is in line with another transcriptomic study demonstrating a significant impact on metabolic interactions between *S. aureus* and *P. aeruginosa*, where *S. aureus* exhibits a notable influence on gene expression related to carbon and amino acid metabolism, promoting survival of *P. aeruginosa* (309). Thus, the significant alterations in gene expression of *S. aureus* observed when co-cultured with *S. anginosus,* suggests a dynamic bacterial interaction driving towards coexistence and adaptation to the host. Dual transcriptomic analysis investigating the early response of *P. aeruginosa* and *S. aureus* during *in vitro* co-culture, has identified transcripts in each bacterium that are significantly affected by the

presence of another bacterium, leading to differential expression of numerous genes related to metabolism, transport functions, stress-related mechanism (358). A study on co-infection in cystic fibrosis airways with *P. aeruginosa* and *S. aureus*, gives insight into the substantial changes in transcriptional responses, mainly in metabolic pathways, offering a deeper understanding of bacterial interaction and adaptation within the complex environment of infection (372).

Moreover, the upregulation of genes exclusively identified at 1 h and 3 h of co-culturing (**Paper II**) are highly enriched in "Riboflavin biosynthesis" and "Defense response", respectively. In addition, downregulation of genes associated with "Ribosome" and "Pyrimidine metabolism" is observed (**Paper II**). In contrast, a recent transcriptomics study on *S. aureus* co-cultured with *Malassezia restricta* during skin colonization, indicates increased expression of genes encoding ribosomal proteins and downregulations of genes involved in riboflavin metabolism (373). Riboflavin (vitamin B2) is a precursor to essential co-enzymes. Expression of riboflavin is crucial for various oxidative-reduction processes and energy metabolism, exhibiting antimicrobial properties against a range of pathogens by inhibiting the growth (374). Thus, it is possible that in our co-culture study, there is a potential competition between *S. aureus* and *S. anginosus* for riboflavin uptake. The upregulation of genes involved in riboflavin biosynthesis could further contribute to the enhanced growth of *S. aureus* (373). These findings suggest that the transcriptional response of *S. aureus* to co-culture with different species can vary, highlighting the dynamic nature of bacterial interaction in enhancing colonization and infections.

S. aureus transcriptional response in tonsillar versus Thp1 model

Most bacterial adaptation occurs at the transcriptional level, where molecules like RNA polymerase, sigma factors, transcription factors, and regulatory RNAs modulate mRNA synthesis, processing, and degradation via a various mechanism (270). These factors control the expression of genes that are necessary for the bacterium to survive and reproduce in different environmental conditions. They help the bacterium to adapt to changes in the environment by changing its gene expression.

The response of *S. aureus* to non-professional phagocytes, such as tonsillar epithelial cells, differs from its response to professional phagocytes such as Thp1 cells (**Paper III**) in terms of gene expression and interactions with the host cells. When comparing genes differentially expressed in intracellular *S. aureus* (MRSA USA300 LAC) in Thp1 cells versus extracellular *S. aureus*, a significant difference in the number of DEGs were identified. This number is higher in *S. aureus* when exposed to tonsillar epithelial cells (**Papers I** and **II**). This variation in gene expression number suggests that *S. aureus* may trigger different signaling pathways and responses depending on the specific host cell type it encounters. This is supported by the findings showing that the *S. aureus* infection response is unique depending on the

host cell types, with certain cells showing greater susceptibility to bacterial uptake and cytotoxicity than others (355). Likewise, a study on *in vivo* infection displays that variability among hosts affects the transcriptomic profile of *S. aureus*, highlighting the importance of considering host factors in the analysis of *S. aureus* virulence (375).

Intracellular *S. aureus* in Thp1

We observed that most genes exclusively upregulated in intracellular *S. aureus* (**Paper III**) were highly enriched in KEGG pathways such as "Histidine metabolism", "Valine, leucine and isoleucine biosynthesis", "Biosynthesis of amino acid" and "Metabolic pathways." Notably, some of these pathways were affected in *S. aureus* exposed to tonsillar cells (**Papers I** and **II**). A previous study characterized the transcriptional response of *S. aureus* to professional phagocytes, such as macrophages, revealing specific patterns: the downregulations of genes encoding ribosomal proteins and the upregulation of amino acids biosynthesis genes (376). Similarly, comprehensive genome expression analyses of *S. aureus* upon internalization in human epithelial cells have shown its ability to survive in various non-phagocytic cells, both *in vitro* and *in vivo* (202). This adaptability of *S. aureus* highlights its versatility and ability to evade host defenses by persisting within a broad range of host environments (202). Interestingly, the identification of highly enriched "Histidine metabolism" in intracellular *S. aureus* may serve as a precursor for various metabolic pathways including central carbon metabolism and catabolism. These metabolic pathways are crucial for bacterial adaptation to host cells, biofilm formation, antibiotic resistance, and virulence (377, 378) and highlighting the multiple strategies employed by *S. aureus* in evading host defense mechanisms and establishing infection.

S. aureus in a colonization model

Our colonization mimicking study utilizing tonsillar cells (**Papers I** and **II**) and nasal medium (**Paper III**) shows high upregulation of methionine biosynthesis genes such as *metC* and *metE*. This finding highlights the role of amino acid biosynthesis, in *S. aureus* during adaptation in a colonization setting. Inhibitors that target methionine biosynthesis have shown antimicrobial activity against *S. aureus* in synthetic nasal medium, indicating the importance of this pathway for bacterial growth in the nasal environment (4, 379). Similarly, the iron-homeostasis genes (*sbnBCD*) are highly upregulated in monoculture setting (both 1 h and 3 h; **Paper I**), co-culture (only at 3 h; **Paper II**), and nasal medium (at 1 h; **Paper III**). Some exclusively expressed genes at high level in nasal medium, are *spn, serA, lrgB,* and *ilvD.* In contrast, other genes are uniquely expressed in the tonsillar model, namely *adlH1*, *katA, sdrC, sdrD, dps, sle1*, and *icaA*. This suggests that these genes may play a role in the immune response or other functions specific to the adaptation in tonsillar cells and the nasal environment.

Furthermore, the gene encoding ribosomal protein was downregulated in most of the *in vitro* conditions, such as in monoculture, co-culture, and nasal medium (**Papers I, II** and **III**). However, we observed an increase in various biological processes, particularly iron-ion acquisition. Similar observations have been reported in studies showing that the bacterium can exhibit changes in gene expression related to iron transport and biosynthesis, in response to iron limitation. For example, genes involved in iron transport are upregulated while ribosomal proteins are downregulated during iron-limited conditions (380, 381). This suggests that the bacterium prioritizes maintaining iron homeostasis to ensure its availability for various cellular processes, including protein synthesis. This dynamic response reflects the importance of iron-homeostasis for bacterial survival and growth. Nevertheless, in our study, the iron-regulatory genes are not expressed in *S. aureus* co-cultured with *S. anginosus* and tonsillar cells. This absence may indicate a shift in the regulatory mechanisms governing iron-acquisition and utilization in response to competing bacteria and the host environment. Moreover, bacteria that do not express iron-regulatory genes may become more susceptible to host defenses and have reduced ability to adapt to environmental changes (382).

S. aureus transcriptional response to host factors

To understand the transcriptional response of MRSA USA300 LAC, it was exposed to different host factors, such as human plasma and nasal medium (Paper **III**). A higher number of DEGs were identified in nasal medium (456 DEGs) compared to plasma (281 DEGs) suggesting a more pronounced response in nasal conditions. Several other environmental factors such as pH, nutrient availability, or oxygen tension, can also induce *S. aureus* to express a variety of transcripts (4, 301, 383). Another study shows the upregulation of genes involved in amino acid biosynthesis, iron transport, and other metabolic processes essential for bacterial survival in the nasal niche (384). Most importantly, inhibitors targeting methionine biosynthesis have been shown to have antimicrobial activity against *S. aureus* in nasal medium, resembling an *in vivo* nasal secretion setting, which is promising for treating systemic infections caused by this bacterium (379). This implies that some of the genes identified in our study, which are involved in highly enriched amino acid biosynthesis pathways, can also be further investigated as potential antimicrobial targets.

Additionally, we detected upregulation of genes involved in betaine biosynthesis pathways in *S. aureus* exposed to human plasma (**Paper III**). A previous study has shown enhancement of *S. aureus* growth rate in the presence of medium supplemented with biotin (385). Additionally, activation of betaine biosynthesis pathways by *S. aureus* under high salinity stress has been reported, which enables *S. aureus* to protect intracellular biomacromolecules (386-388). Besides BioA, other betaine biosynthesis genes (*bioB* and *bioD*) were also markedly upregulated in *S. aureus* exposed to human plasma in our study. The upregulation of betaine biosynthesis pathways in *S. aureus* can regulate osmotic stability (389) and

protect the active structure of proteins and nucleic acids (390). Comparison of *in vivo* and *in vitro* gene expression profiles of *S. aureus* across different human hosts has shown that limitation of iron strongly controls survival in the nose, and adhesins are important for colonization of the anterior nares (298). *S. aureus* overcomes environmental stress by the expression of compatible solute biosynthetic pathways, changes in the cell wall composition and the synthesis of general stress proteins (4, 361). Taken together, our findings indicate that *S. aureus* survival in nasal medium and plasma is commonly influenced by iron limitation alongside unique pathways.

Challenges in RNA-seq

Despite its popularity, RNA-seq workflow and data analysis pose certain challenges when characterizing the transcriptome of a bacterial species under diverse conditions (273). These challenges typically include low input of RNA concentration for RNA-seq, and low-level transcript may be overshadowed by more abundant ones. Ambiguous sequence mapping and differences in sequencing protocols can also hinder reproducibility between experiments (273, 391). Selecting the optimal RNA-seq platform and implementing an optimized RNA-seq data analysis pipeline is crucial for accurately generating and interpreting biological data (275).

In this study, we developed a new (in-house) bioinformatic pipeline for the RNA-seq data analysis, as no standard pipeline was available. This process involved evaluating different aligner and counting tools and optimizing the pipeline to effectively identify genes that were differentially expressed in *S. aureus*. Additionally, there were some challenges during our RNA-seq based transcriptomic workflow. Firstly, we experienced low bacterial RNA retrieval, especially from intracellular *S. aureus* after 1 h of exposure (**Papes III**) and in the colonization model using tonsillar cells (**Papers I** and **II**). Thus, for the samples with low RNA concentration, a higher amplification cycle number was preferred during RNA-seq library preparation, and deep sequencing was performed for all samples to retrieve genes also at low expression level. Although there was variation in mapping efficacy, the gene counts were normalized within DESeq2 before DEGs analysis.

Secondly, in our co-culture study, we acknowledge the genetic similarities between *S. anginosus,* and *S. aureus* and it is possible that some of the identified DEGs in *S. aureus* may also be present in *S. anginosus*. Therefore, we have differentiated DEGs exclusive to *S. aureus* and noted genes potentially expressed in *S. anginosus.* This similarity suggests the existence of common pathways during co-culturing. However, obtaining complete DEGs information of *S. anginosus* requires separate RNA-seq analysis, which was beyond the scope of our present study.

Lastly, some test samples (exposed to host cells or host factors) indicated very low mapping efficacy compared to control samples (only *S. aureus*). This could be due to the recovery of a low number of bound bacteria in the host cells, use of a short time frame (1h) for bacteria to adhere and that they were collected from a 6 well plate (**Papers I** and **II**) and T175 culture flasks (**Paper III**). A similar low mapping efficacy has been reported in a previous study, especially in co-culture samples (392).

We also performed microbial enrichment on the samples for the pilot run to remove rRNA before RNA-seq, utilizing the MICROBExpress Kit (Thermofisher). Even though, we observed approximately a 5 % increase in mapping efficacy compared to samples without an enrichment process, we opted not to utilize MICROBExpress in our study, because: **i)** the enrichment process necessitated high RNA concentration, which was lacking in most of our samples; and **ii)** our library preparation involved removal of rRNA before RNA-seq.

S. aureus virulence factors as potential targets in therapy?

Recent research suggests that novel antimicrobial strategies can be directed towards cell membrane lipids and virulence factors, offering alternatives to conventional antibiotics. This includes the use of anti-virulence treatment, which serves as an alternative to directly targeting bacterial growth (88). Anti-virulence strategies have gained attention as potential alternatives to traditional antibiotic treatments, primarily due to their perceived lower risk of developing resistance (29).

S. aureus produces several virulence factors which plays a role in the pathogenicity of *S. aureus* infections and treatment failure (393, 394). Understanding the role of these factors is essential for improving treatment options, other than antimicrobial, by developing new anti-virulence strategies with long-lasting efficacy against *S. aureus* strains (395-397). Our transcriptomics analyses have indicated the expression of several key virulence factors during tonsillar colonization, such as genes responsible for iron-ion transport (*isdC, isdE, isdF, isdI*), alpha amino acid biosynthesis process (*sbnA, argH, argG, trpF, trpB*), cell adhesion (*sdrC, sdrD, icaA*) and defense response to bacterium (*sle1*).

Similarly, potential genes identified from the infection-mimicking conditions are proteins involved in cell wall inhibition responsive protein (CwrA), iron-ion transport (high expression of *IsdB*), methionine (MetC, MetE and MetK) and tryptophan biosynthesis proteins (TrpC and TrpB), and type 8 capsular polysaccharide (cap8B, cap8F, cap8G). Several studies have indicated the potential of targeting virulence factors in *S. aureus* as a novel approach to combat infections (397). By targeting specific virulence factors, such as adhesins, pore-forming toxins, immunomodulators, and quorum sensing systems, a new treatment therapy using anti-virulence could be developed to effectively treat *S. aureus* infection (88, 397, 398). For example, vaccines containing MSCRAMM antigens like IsdB protein have

been shown to effectively reduce the virulence and colonization of *S. aureus* (127). We confirm its importance in vaccine development in infection condition, during exposure to macrophages and plasma (it was upregulated with high expression level), while in colonization mimicking condition, it was downregulated.

6. CONCLUDING REMARKS

Our research is based on establishment of models for studying *S. aureus* colonization and infection, and the use of transcriptomics to identify the bacterial factors involved. The utilization of tonsillar cells in an *in vitro* model has been demonstrated to be suitable in simulating *S. aureus* throat colonization when co-cultured with host cells and in the presence of another competing microbe. Our analysis of differentially expressed genes from *S. aureus* monocultures and co-cultures, demonstrates both unique and overlapping sets of DEGs following exposure to tonsillar cells for two different durations. The DEGs gives an insight into the adaptation strategy of *S. aureus* during early and late phase of tonsillar colonization. The genes were mainly associated in essential biological processes such as adhesion, iron-regulation, defense response, and amino acid biosynthesis and transport, crucial for *S. aureus* adherence and survival. Significantly, our findings indicate a substantial upregulation of virulence genes encoding secreted proteins, while iron-acquisition genes remain inactivated when *S. aureus* is co-cultured with both *S. anginosus* and tonsillar cells. This suggests that *S. aureus* can thrive and survive even in conditions where iron may not be abundantly available or may be utilized by competitors. Further investigation into the roles of the identified genes in the host immune response within context of a throat commensal landscape could provide valuable insights for future research and therapeutic development. Moreover, our transcriptomics data analysis highlights significant differences in gene expression between *S. aureus* exposed to professional phagocytes and those exposed to host factors. This allows us to identify virulence factors and metabolic changes in extracellular and intracellular *S. aureus.*

Taken together, this enhances our understanding of *S. aureus* transcriptional response and adaptation to the host during colonization and infection, as well as to various host factors. By elucidating the complex interactions between *S. aureus*, host cells, and host factors, we contribute to identifying potential targets for therapeutic interventions. This knowledge could, in the long run, provide us with tools for development of new preventive measures and treatments, among e.g. patients susceptible to infections, patients undergoing prophylaxis prior to surgery, and patients with recurrent infection. Both hospital patients and healthcare workers would benefit from new strategies for local *S. aureus* throat decolonization

7. FUTURE ASPECTS

A better understanding of how *S. aureus* colonizes the human throat can be revealed by conducting metatranscriptomic analysis on tissue from tonsils. Metatranscriptomics provides a deeper insight into the microbial community transcriptomic response over time in changing environmental conditions (399). This can provide a comprehensive understanding of the gene expression profiles of both *S. aureus* and other throat microbiota. Further, an organoid host model, closely mimicking *in vivo* organ properties and responses (400), can be utilized for studying disease and drug development (401). Comparison of findings from *in vitro* model studies with specific factors identified using metatranscriptomics and organoids models can validate the essential bacterial factors and deepen our understanding of S. *aureus'* throat carriage and the regulatory networks involved. Additionally, investigation of the host transcriptome may provide enhanced insight into the host response to *S. aureus* colonization and infection. Understanding host immune response and signaling pathways activated during host-microbe interaction could reveal potential targets for therapeutic intervention.

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Paper I

Exploring differentially expressed genes of *Staphylococcus aureus* exposed to human tonsillar cells using RNA sequencing

Srijana Bastakoti*, Clement Ajayi, Kjersti Julin, Mona Johannessen, Anne-Merethe Hanssen*

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RESEARCH Open Access

Exploring differentially expressed genes of *Staphylococcus aureus* exposed to human tonsillar cells using RNA sequencing

Srijana Bastakoti[1*], Clement Ajayi[1,2], Kjersti Julin[1], Mona Johannessen[1,2] and Anne-Merethe Hanssen[1*]

Abstract

Background The nose and the throat are the most predominant colonizing sites of *Staphylococcus aureus*, and colonization is a risk factor for infection. Nasal colonization is well described; however, we have limited knowledge about *S. aureus* throat colonization. The main objective of this study was to explore differentially expressed genes (DEGs) in *S. aureus* throat isolate TR145 exposed to human tonsil epithelial cells (HTEpiC) by using RNA sequencing (RNA-seq) and pathway analysis. DEGs in *S. aureus* at 1 or 3 hours (h) interaction with its host were explored.

Results *S. aureus* was co-cultured in absence and presence of tonsillar cells at 1 or 3 h. Over the 3 h time frame, the bacteria multiplied, but still caused only minor cytotoxicity. Upon exposure to tonsillar cell line, *S. aureus* changed its transcriptomic profile. A total of 508 DEGs were identified including unique (1 h, 160 DEGs and 3 h, 78 DEGs) and commonly shared genes (1 and 3 h, 270 DEGs). Among the DEGs, were genes encoding proteins involved in adhesion and immune evasion, as well as iron acquisition and transport. Reverse transcription qPCR was done on selected genes, and the results correlated with the RNA-seq data.

Conclusion We have shown the suitability of using HTEpiC as an in vitro model for investigating key determinants in *S. aureus* during co-incubation with host cells. Several DEGs were unique after 1 or 3 h exposure to host cells, while others were commonly expressed at both time points. As their expression is induced upon meeting with the host, they might be explored further for future targets for intervention to prevent either colonization or infection in the throat.

Keywords *Staphylococcus aureus*, Human tonsil epithelial cells, Throat colonization, Transcriptomics, RNA sequencing

*Correspondence:
Srijana Bastakoti
srijana.bastakoti@uit.no
Anne-Merethe Hanssen
anne-merethe.hanssen@uit.no
[1]Department of Medical Biology, Host-Microbe Interaction (HMI) research group, UiT – The Arctic University of Norway, Tromsø, Norway
[2]Center for Research and Education, University Hospital of North Norway (UNN), Tromsø, Norway

Background

Staphylococcus aureus is an opportunistic pathogen that can cause life-threatening diseases such as endocarditis, osteomyelitis, pneumonia, and bacteremia [1]. Besides being a human pathogen, *S. aureus* asymptomatically colonizes 20–30% of a healthy adult population [2, 3]. Colonization is a risk factor for infection, as the colonizing strain is responsible for approximately 80% of *S. aureus* infections within its host [1, 4].

The predominant and frequent colonizing sites for *S. aureus* are the vestibulum nasi (anterior nares) followed by skin, perineum, and pharynx [3, 5]. *S. aureus* encodes various adhesive proteins, including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), that are involved in adhesion to the host cells [6]. Several of these are expressed during nasal colonization [7]. During colonization, *S. aureus* not only adhere to cell surfaces but is also intracellularly [8], which may protect the bacteria against antibiotic treatment [9]. In addition, the pathogen also expresses proteins to overcome host immune defence mechanisms and retrieve iron from the host [7]. Although the nares are considered the primary site of *S. aureus* colonization [5, 8], pharyngeal *S. aureus* carriage has also been equally or more commonly observed [4, 10–12]. Both methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) can persistently colonize the throat of healthy people for years [13, 14], and 32.1% of colonizing MRSA strains have been exclusively isolated from throat carriers [13].

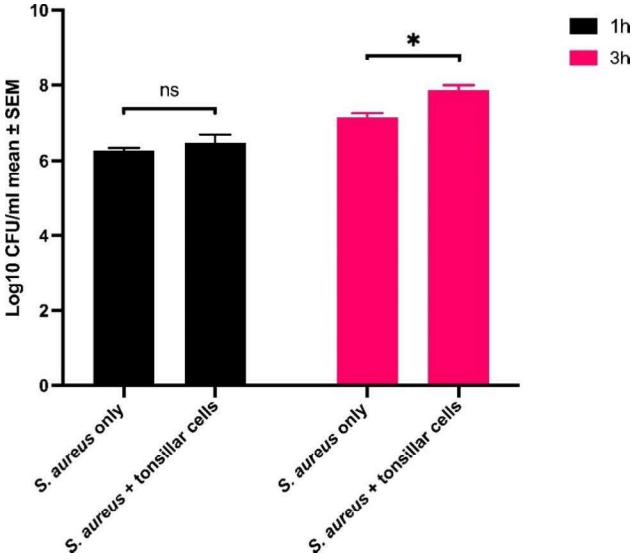

Fig. 1 The growth of *S. aureus* TR145 in absence or presence of human tonsillar epithelial cells. The results are presented as log10 CFU/ml from three independent experiments. The different colors show the *S. aureus* grown at different time points (1 and 3 h) either alone or together with tonsillar cells. Paired-t test was performed separately for two different time points to compare growth of *S. aureus* only with *S. aureus* mixed with tonsillar cells. At 1 h of exposure, there was no significant (ns) difference between *S. aureus* alone and *S. aureus* with tonsillar cells. Whereas, at 3 h of exposure, significantly difference ($p < 0.005$, *) was observed

Prevention and elimination of the *S. aureus* colonization carrier state may contribute to reduce the *S. aureus* infection burden and prevent the spread of MRSA [2, 15]. Mupirocin is widely used for the de-colonization of *S. aureus* in the nasal cavity. It interferes with the synthesis of bacterial proteins by reversibly binding to bacterial isoleucyl-tRNA [16]. However, *S. aureus* colonization frequently reoccurs after mupirocin treatment [17]. The ability of *S. aureus* to colonize the throat region makes it difficult to be reached by mupirocin treatment, and throat colonization has been shown to be linked to reduction in the eradication efficacy [18]. Hence, new antimicrobial compounds for *S. aureus* de-colonization, especially in throat, are urgently needed.

This study aimed to identify key determinants differentially expressed by *S. aureus* in presence of primary human tonsil epithelial cells using RNA sequencing (RNA-seq) and pathway analysis.

Results

S. aureus multiply in presence and absence of tonsillar cells

S. aureus TR145, isolated from a healthy adult that was exclusively throat colonized, was chosen as a representative throat strain in our study. To determine the effect of exposure to tonsillar cells on the growth of *S. aureus* TR145, bacteria grown to log phase OD_{600nm}=0.8–1.2 were diluted to OD_{600nm} 0.4 and seeded into wells with or without human tonsil epithelial cells at a MOI of 5. The CFU of the *S. aureus* inoculum used to infect either host media or the host cells were ~1.4×10^6 CFU/ml, corresponding to 6.2 log10 CFU/ml.

There was no significant difference in the recoverable CFU between *S. aureus* TR145 exposed to tonsillar cells compared to those grown without the host cells at 1 h; however, there was almost 1 log10 difference in bacterial growth when grown in presence of host cells for 3 h (Fig. 1).

As we aimed to analyze bacterial transcriptome in presence of human tonsil epithelial cells, we next evaluated the host cell viability after exposure to *S. aureus*. The bacterial effect on host cell viability was evaluated by measuring the lactase dehydrogenase (LDH) release by the tonsillar cells in presence or absence of *S. aureus*. As shown in Fig. 2, there was no significant difference in the LDH release from host cells after 1 h growth in absence or presence of *S. aureus*. The LDH release increased slightly after 3 h growth in absence (1.3%) or presence of *S. aureus* (1.7%).

Thus, *S. aureus* can multiply in absence and presence of the host cells, causing only minor cytotoxicity.

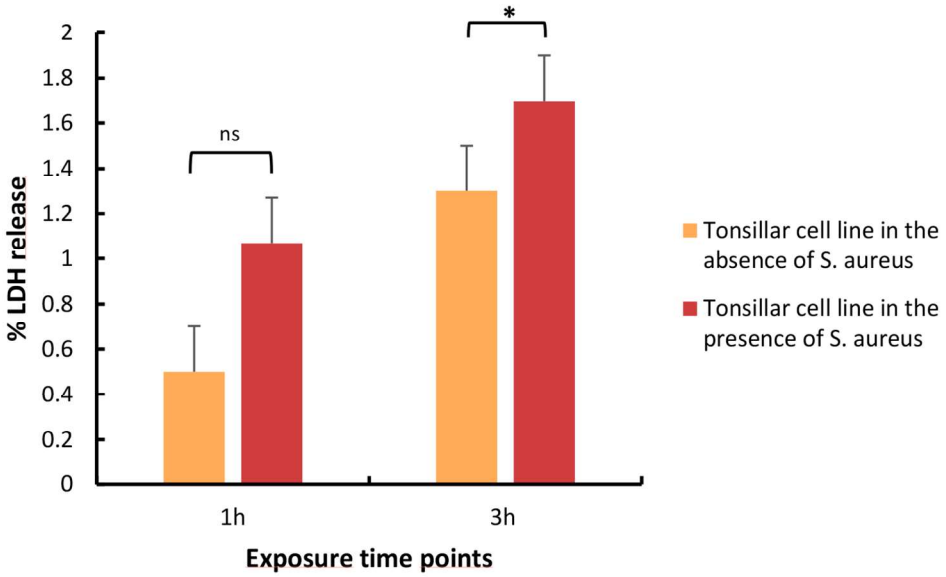

Fig. 2 *S. aureus* shows minor cytotoxicity to the tonsillar cell line. LDH release from tonsillar cells into the supernatant was measured following exposure with/without *S. aureus* for 1 and 3 h. The orange bar represents the percentage (%) of LDH released by the tonsillar cell line in the absence of *S. aureus* (negative control) whereas red bar presents the LDH % release by the tonsillar cell line in the presence of *S. aureus*. The bacterial cytotoxicity was calculated as a percentage of maximum LDH release control (positive control). The results are based on three independent experiments

RNA sequencing and analysis of RNA-seq data

Total RNA from *S. aureus* exposed to growth medium or host cells were isolated and used for RNA-seq library preparation. The quantity of RNA measured by Nanodrop1000 spectrophotometer detected RNA concentrations ranging from 1.5 ng/µl −80.6 ng/µl in the samples of *S. aureus* in absence of host cells and 23.2 ng/µl – 876 ng/µl in samples of *S. aureus* exposed to host (Additional file 1, Table S1). For most of the RNA samples, the RNA quality and integrity analyzed by Agilent 2100 Bioanalyzer detected an RNA integrity number (RIN) score of >7.0.

RNA-seq generated 34.2–53.6 million total reads per library. Only sequences with quality score Q≥20 were retained in the dataset. The reads remaining after trimming and filtering of low-quality bases and adaptor contaminants were 33.3–53.5 million reads per library (Additional file 2, Table S2). The filtered reads were then mapped with reference genome *S. aureus* TR145 (SAMEA112465883). All the RNA samples for the 3 h time point showed more than 55% of RNA reads uniquely mapped with its reference genome. In comparison, at 1 h of exposure condition, most of the samples showed less than 20% of mapping efficacy although one sample (C1h_Rep1) showed 93% of mapping efficacy (Additional file 2, Table S2). In summary, high-quality RNA was retrieved and used for RNA-seq, and only quality reads mapped against *S. aureus* TR145 were accessed for RNA-seq data analysis.

Data normalization and visualization of sample variance

The normalization of read counts was used for gene count comparisons between *S. aureus* alone (1 h and 3 h) or exposed growth for 1 h and 3 h. After normalization, both sequencing depth and RNA composition were corrected, including the difference in biological replicates (Fig. 3).

The principal component analysis (PCA) plot (Fig. 4) was used to visualize the sample variation between *S. aureus* grown in presence or absence of host cells. The results showed a clear clustering of the three biological replicates of *S. aureus* harvested at 1 or 3 h in presence or absence of tonsillar cells (Fig. 4).

These analyses indicate that all the variations which might have occurred due to biological replicates and sequencing depth/cycle have been normalized, and clear clustering of different sample groups are visualized, making it ready for DEGs analysis.

Differentially expressed genes (DEGs) in *S. aureus* in presence and absence of tonsillar cells

To investigate the changes in *S. aureus* gene expression after exposure to tonsillar cells, computational comparisons analysis of the DEGs from *S. aureus* exposed or not exposed to tonsillar cells was performed. The DESeq2 analysis of the HTseq generated gene count files revealed a total of 508 significant DEGs with adjacent p value (padj)<0.05 and log2fold change (lfc) ≥ |2|) from *S. aureus* TR145 exposed to tonsillar cells at 1 and 3 h compared to those grown in the absence of tonsillar cells at these time points (Fig. 5).

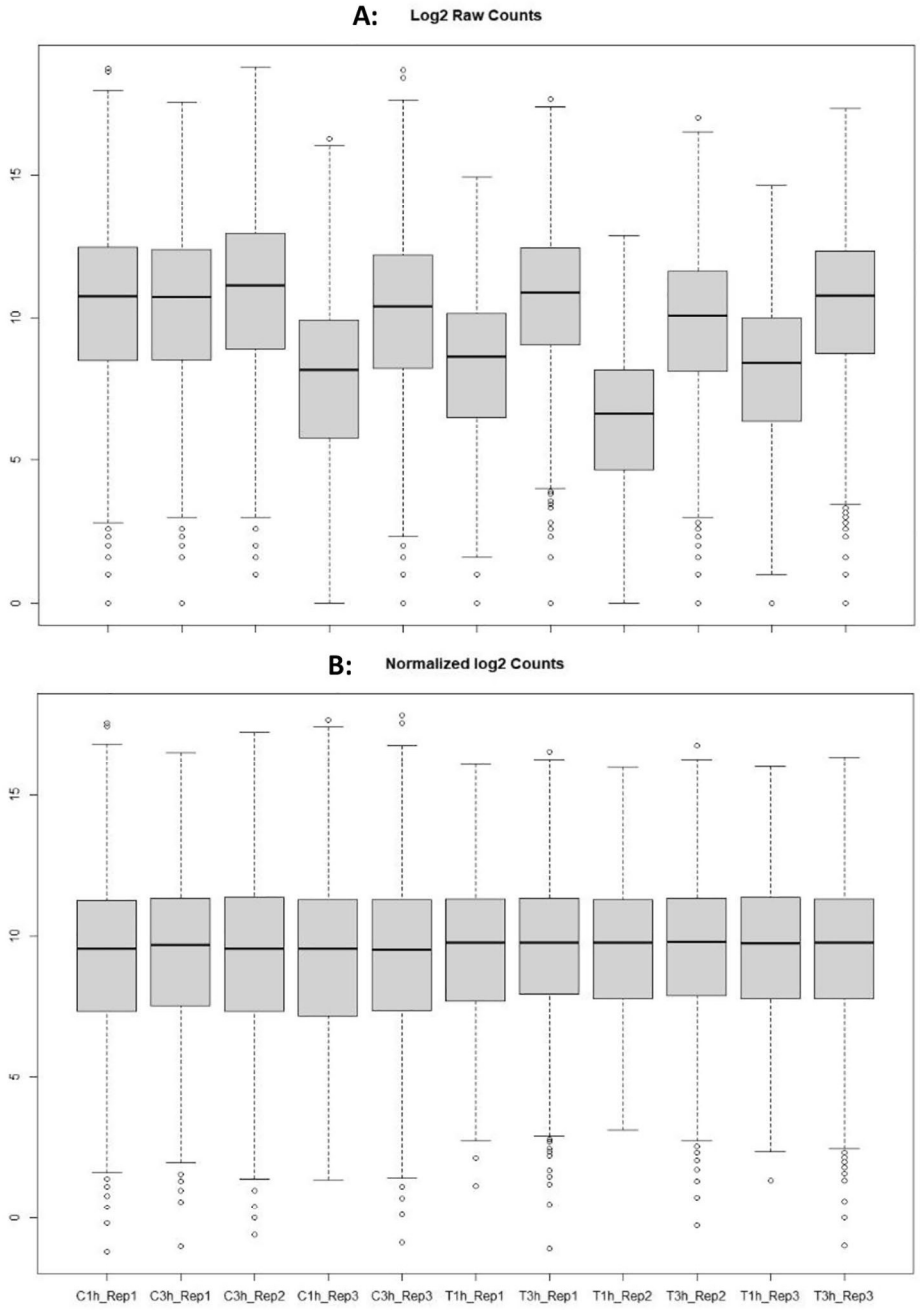

Fig. 3 Quality control of RNA-seq transcriptomics data presented in box plot before differentially expressed gene analysis in *S. aureus* exposed to tonsillar cells. The three biological replicates consist of *S. aureus* only 3 h (C3h), *S. aureus* exposed to tonsillar cells 1 h (T1h) and *S. aureus* exposed to tonsillar cells 3 h (T3h). There were two replicates for *S. aureus* only 1 h (C1h), as one sample was lost under preparation ahead of RNA-seq. The DESeq2 uses the median of ratio method for normalizing the counts and is depicted in log2 fold change. **A**: The distribution of raw counts before normalization. **B**: The counts after performing DESeq2 normalization

Two hundred and seventy DEGs were expressed both after 1 and 3 h exposure to host cells, while 160 and 78 DEGs were uniquely expressed at 1 and 3 h, respectively (Fig. 5A). Three hundred DEGs, (59%) were significantly upregulated in presence of host cells, whereof 156 were commonly expressed, while 105 and 39 DEGs were uniquely expressed at 1 and 3 h, respectively (Fig. 5A1). A total of 208 DEGs (41%) were significantly downregulated, whereof 114 were commonly expressed, and 55 and 39 were uniquely expressed at 1 and 3 h respectively (Fig. 5A2).

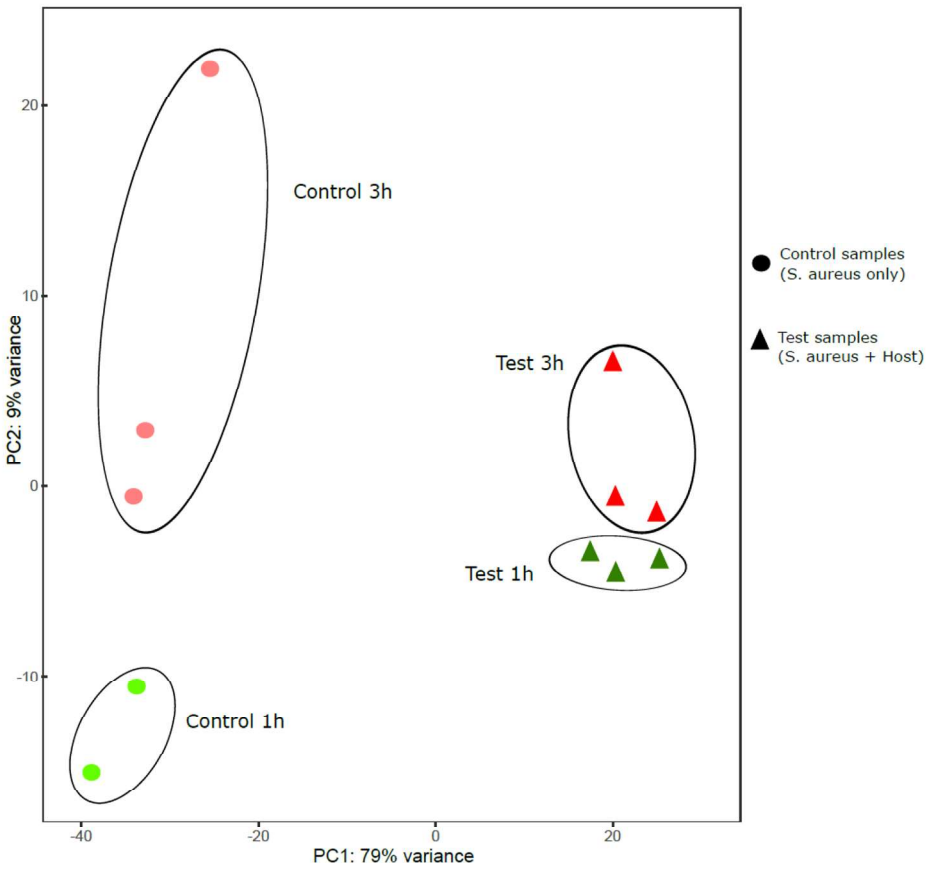

Fig. 4 Principal component analysis (PCA) analysis of RNA-seq data to visualize sample to sample variation. The PCA plot depicted clear clustering control (*S. aureus* only, shape- circle) versus test samples (*S. aureus* + tonsillar cells, shape-triangle). The test samples, *S. aureus* after 1 h (n = 3; dark green) and 3 h (n = 3; dark red) exposure to tonsillar cells cluster separately to that of control samples (n = 2, light green) at 1 h and (n = 3, light red) at 3 h. The first two components PC1 and PC2 explained 79% and 9% of the variability in the expression data, respectively

For *S. aureus* exposed to host cells for 1 h, 430 genes (160+270 Fig. 5A) were significantly differentially expressed, and among these 310 were pre-annotated. Of these, 105 genes were uniquely expressed at 1 h, and their identity and lfc expression are presented in Additional file 3, Table S3 including commonly shared DEGs (205 pre-annotated). After 3 h exposure to host cells, 348 (270+78, Fig. 5A) significant DEGs were found, and among these, 53 pre-annotated genes were uniquely expressed after 3 h. Their identity and lfc are presented in Additional file 4, Table S4 including commonly expressed genes. A complete list of DESeq2, without any threshold cutoff is presented in additional file 5, Table S5. In summary, the *S. aureus* transcriptome changed upon exposure to tonsillar cell line. Some transcripts were unique for the tested time points, while others were expressed at both time points.

Enriched gene ontology (GO) terms in *S. aureus*

Sets of all upregulated (300) and downregulated (208) genes derived from RNA-seq data analysis were separately applied in gene ontology (GO) enrichment analysis to identify enriched pathways involved during *S. aureus* exposure to tonsillar cells. The most enriched upregulated and downregulated GO terms identified are presented in Fig. 6A and B, respectively. Enriched GO terms were first filtered based on false discovery rate (FDR) cutoff (<0.05), then the significant pathways were selected by FDR and sorted by Fold Enrichment.

The upregulated genes identified significantly (FDR<0.05, fold enrichment≤10) enriched pathways involved either in biological process or molecular function (Fig. 6A1). About 10 genes (FDR<0.05, 8≥fold enrichment≤10) were involved in the highly enriched biological processes such as amino acid biosynthesis process, iron ion transport, neat domain, etc. (Fig. 6A1; small circles in purple). Only two GO terms, lyase and hydro-lyase activity, were involved in molecular function (Fig. 6A2).

GO term analysis from all downregulated genes revealed several enriched GO terms involved during cellular process followed by biological and molecular

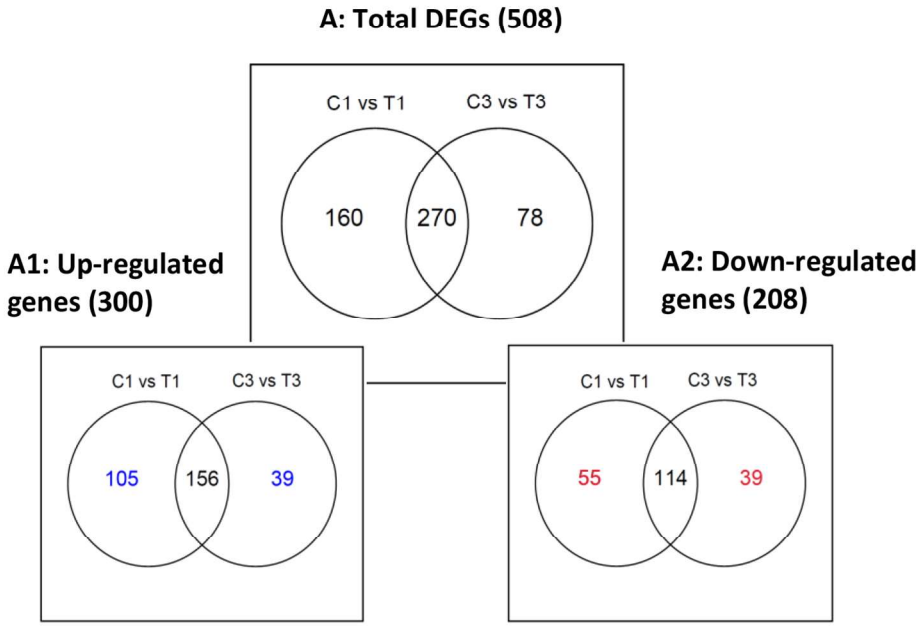

Fig. 5 Venn diagram of differentially expressed genes (DEGs) in *S aureus* in the presence of tonsillar cells identified by RNA-seq. A: Represents the total DEGs (508), thereof uniquely expressed at 1 h (160) or 3 h (78) exposure to host cells or expressed at both time points (270). **A1**: Representation of 300 up-regulated genes, uniquely expressed 105 and 39 genes identified at 1 and 3 h, respectively. **A2**: Representation of 208 down-regulated genes, uniquely expressed 55 and 39 genes identified at 1 and 3 h, respectively

activity (Fig. 6B1). About 45 genes (FDR < 0.05, 3 > fold enrichment ≤ 5) belong to the topmost enriched down-regulated GO terms including cytosolic ribosome, ribosomal subunit/protein, rRNA binding, and organelle (Fig. 6B1; circles in dark to light purple from the left). The correlation among the significant pathways for the up and downregulated genes are presented in hierarchical clustering tree diagram (Fig. 6A2, 6B2).

Taken together, GO analysis performed for DEGs were found to be highly involved in biological processes followed by cellular and in molecular pathways.

Further, to visualize the major pathway differences on uniquely regulated DEGs after 1 and 3 h exposure to tonsillar cells, the 10 topmost significantly enriched GO terms were analyzed. It resulted in 9 enriched GO terms from 3 h, and 10 enriched GO terms from 1 h (Fig. 7). One of the highly enriched pathways (FDR < 0.05, fold enrichment 35) detected from upregulated genes at 3 h was the iron ion transport (Fig. 7A). In contrast, no significantly enriched pathways were found at 1 h among highly upregulated genes using GO analysis with FDR < 0.05. Uniquely downregulated genes at 1 h revealed significantly enriched pathways (FDR < 0.05, fold enrichment ≤ 10) such as small ribosomal subunits, translation, and biosynthetic process (Fig. 7B). No significant enriched pathways were found among uniquely down-regulated genes at 3 h using GO analysis with FDR < 0.05.

Overall, this indicates that the bacteria downregulate transcription of genes encoding proteins involved in translational process after 1 h exposure to host. After 3 h exposure, the bacteria might be faced with competition for ions, and upregulate transcription of genes encoding proteins involved in iron acquisition and transport.

The uniquely upregulated genes at 3 h were further explored to identify several other significantly enriched biological processes involved during *S. aureus* exposure to tonsillar cells. The 20 topmost biologically enriched pathways analysis resulted in 16 significantly enriched GO terms (Additional file 6, Figure S1).

Most of the biologically enriched pathways were involved in biological adhesion, biosynthesis in addition to iron acquisition and transport. DEGs associated with iron acquisition and transport and biological adhesion are listed in Table 1.

Expression of *S. aureus* survival factors when exposed to tonsillar cells

The genes presented in Fig. 8, were selected with respect to gene expression level, and functions involved in adhesion, iron acquisition/transport and amino acid synthesis, as these are relevant when the bacteria meet the host, identified from GO enrichment analysis. Among the commonly shared upregulated genes were *leuB, ilvC, metC, metN, sdrC, katA, aldH1, msrA1* and *spa.* Some of the downregulated genes were fibronectin binding genes *fnbA* and *fnbB* as well as staphylococcal antigen A (*isaA*) (Fig. 8A).

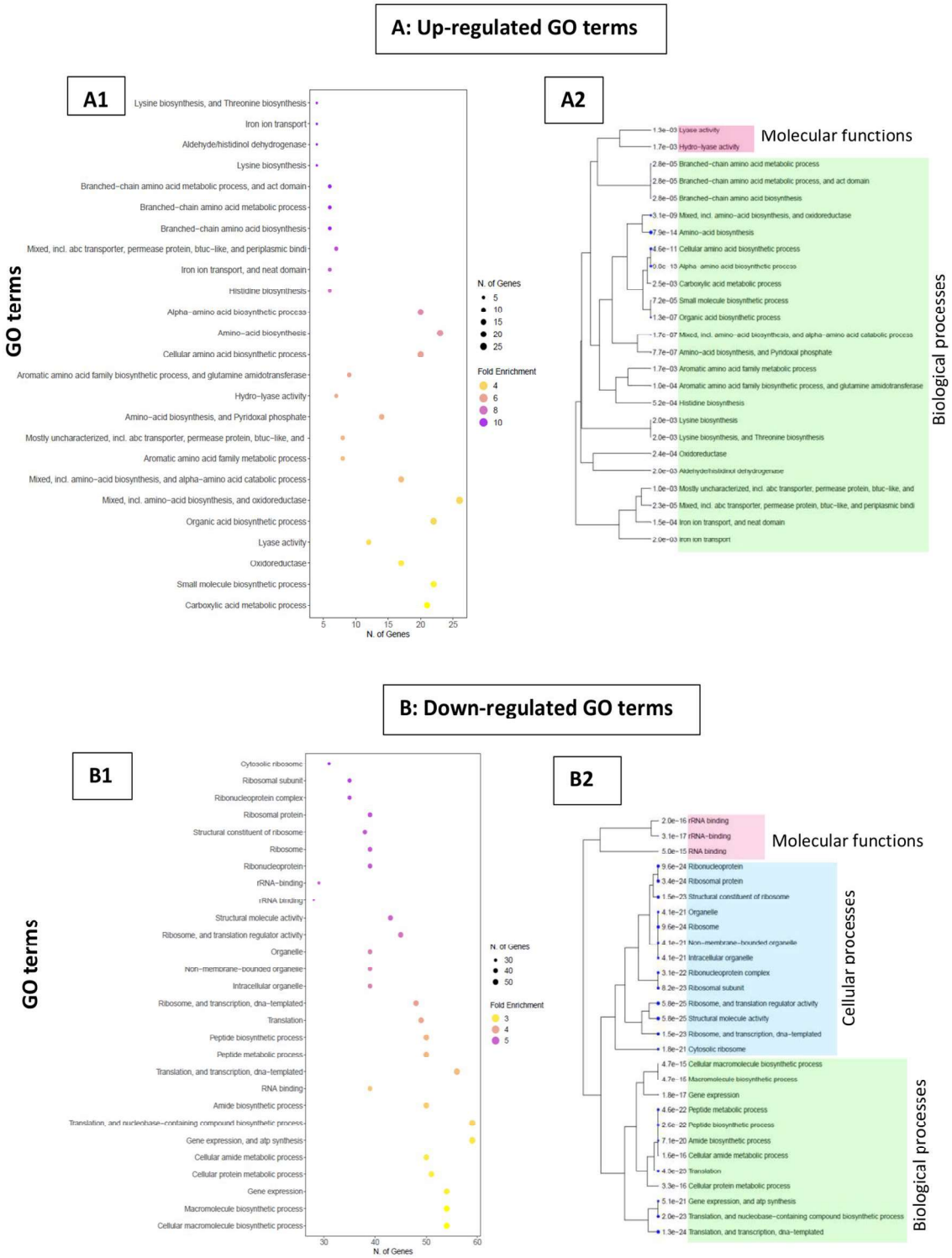

Fig. 6 Gene Ontology (GO) analysis of identified differentially expressed genes (DEGs) in *S. aureus* after 1 and 3 h of exposure with tonsillar cells. **A1** and **B1** represent the GO-enriched scatter plots, identified within top 30, from upregulated and downregulated genes The size of the dot represents the number of DEGs, and the color represents level of fold enrichment of the identified GO terms. **A2** and **B2** represent the hierarchical clustering tree diagram from GO analysis, showing different clades of GO-enriched terms divided into biological processes, cellular processes, or molecular components

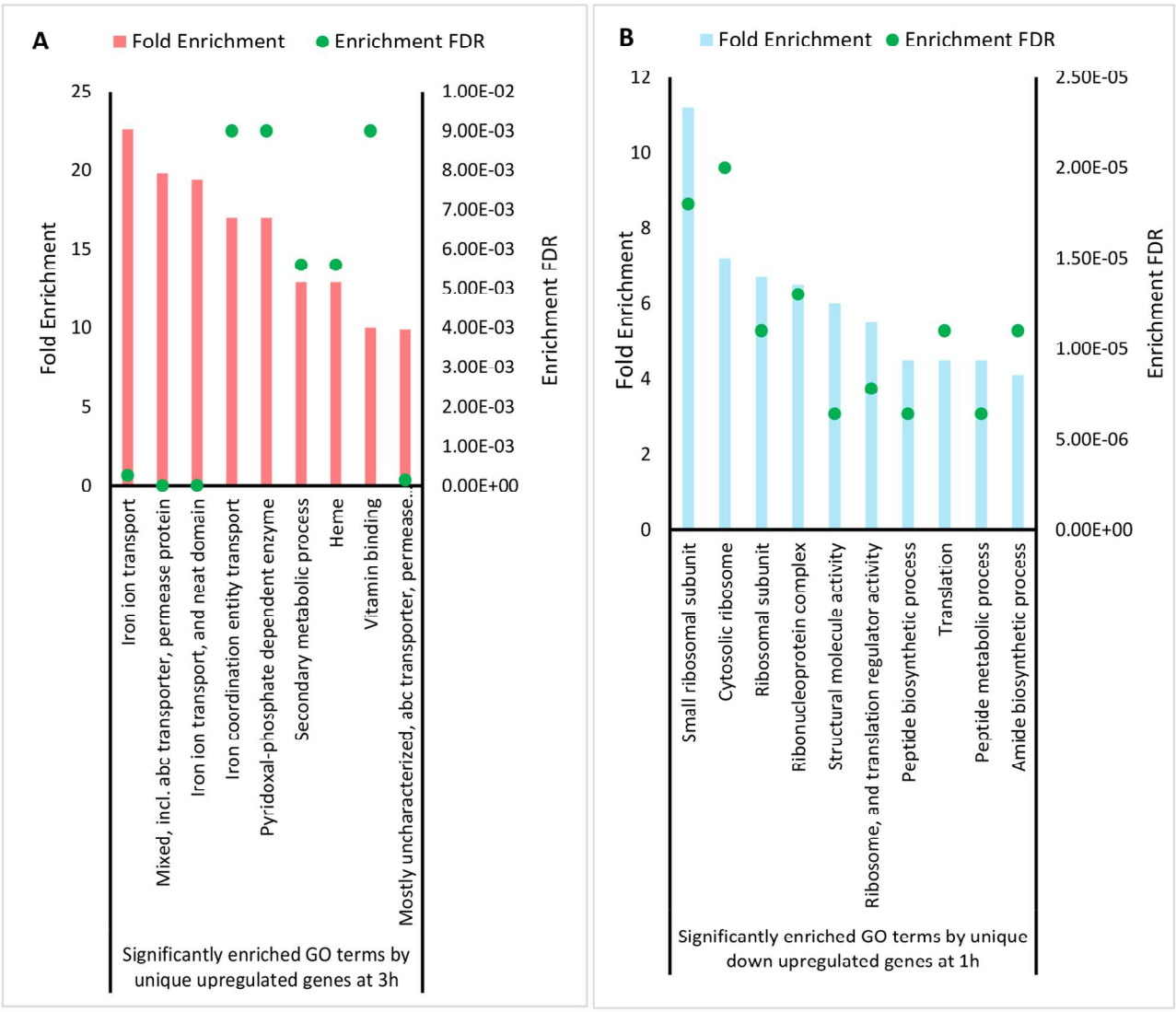

Fig. 7 Different significantly enriched top 10 GO terms analyzed from unique differentially expressed genes at 1 and 3 h of exposure with tonsillar cells. Horizontal line represents the different significant GO terms identified by GO analysis, left vertical represents the fold enrichment value, and right vertical line with enrichment false discovery rate (FDR). **A)** The uniquely upregulated genes at 3 h involved in top 9 GO terms. All red bars indicate the significant GO terms. No significantly enriched GO terms were identified from unique down regulated genes at 3 h in the GO analysis with FDR < 0.05. **B)** The uniquely downregulated at 1 h involved in top 10 GO terms. All blue bars indicate the significant GO terms. No significantly enriched GO terms were identified from unique up regulated genes at 1 h in the GO analysis with FDR < 0.05

The upregulated genes responsible for iron acquisition and transport namely *isdACEFHI* and *sbn*ABCDE were only expressed at 3 h exposure to host cells. Some of the genes such as *dps, sle1* and *icaA* were also uniquely upregulated after 3 h of exposure to tonsillar cells. After 1 h exposure, the genes *metE, sdrD* and *msrA2* were upregulated while *emp, chp* and *sbnH* were downregulated (Fig. 8B).

In summary, some of the DEGs were present both after 1 h and 3 h exposure to host, which indicates a more constant need in making proteins that help in e.g., methionine biosynthesis and destruction of hydrogen peroxide (katA). Similarly, some of the unique DEGs at 1 h were found to be involved in cell division and methionine synthesis, whereas at 3 h of exposure, *S. aureus* mostly expressed genes having a role in iron acquisition, iron hemostasis including cell attachment and stress response. These results demonstrate *S. aureus* ability to adapt to the changing environment that might occur over time.

RT-qPCR confirmation

To verify the DEGs obtained from the RNA-seq datasets of *S. aureus* during exposure to tonsillar cells, reverse transcription qPCR experiments were performed for six genes (*ilvC, metI, metE, icaA, emp*, and *rpsT*). For all the six genes tested, the RT-qPCR data showed a general

Table 1 Top biological processes enriched by uniquely upregulated genes at 3 h identified from GO analysis. Genes were significantly involved (FDR < 0.05) in the respective pathways detected by GO enrichment analysis

Fold Enrichment	GO terms	Genes
36	Iron import into cell	*isdI, isdF*
27	Iron coordination entity transport	*isdC, isdE, isdF*
24	Iron ion transport	*isdI, isdC, isdE, isdF*
24	Cellular metal ion homeostasis	*isdI, isdF*
24	Heme transport	*isdC, isdE*
24	Establishment of localization in cell	*isdI, isdF*
24	Iron ion homeostasis	*isdI, isdF*
18	Cell and biological adhesion	*sdrC, icaA*
18	Cellular cation homeostasis	*isdI, isdF*
8	Transition metal ion transport	*isdI, isdC, isdE, isdF*
6	Alpha-amino acid biosynthetic process	*sbnA, argH, argG, trpF, trpB*

correlation with RNA-seq data in log2fold changes, where three of them were significant (Table 2). Overall, the correlation between RNA-seq and RT-qPCR is good, validating the RNA-seq data.

Discussion

In this study, we used the human tonsillar cell line (HTEpiC) to identify possible *S. aureus* determinants involved during *S. aureus* throat colonization. The suitability of using HTEpiC as an in vitro model for investigating the key determinants in *S. aureus* exposure to human tonsillar cells has not been previously verified, making our study the first to investigate this interaction. We observed that after 1 or 3 h of *S. aureus* presence, the host cells remained viable, as indicated by the negligible levels of LDH released by the host cells. This finding suggests that the HTEpiC cell line is suitable for studying the interaction between *S. aureus* and human tonsillar cells without compromising the viability of the host cells.

Numerous transcriptomics studies have investigated the transcriptomics profiling of *S. aureus* during

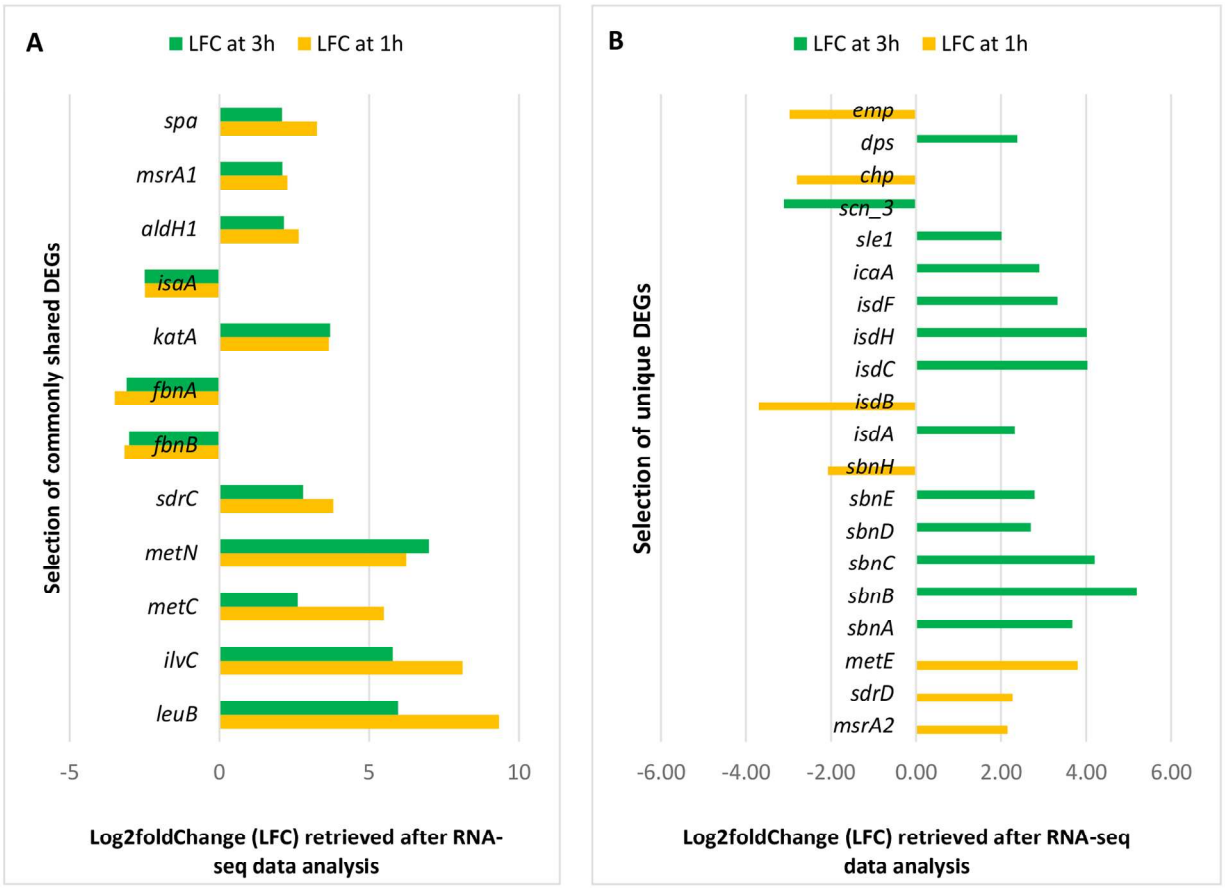

Fig. 8 Selected common and unique DEGs in *S. aureus* exposed to tonsillar cells at 1 and 3 h identified from RNA-seq. Log2fold change retrieved after RNA-seq analysis. **(A)** DEGs detected both at 1 and 3 h after exposure to host cells. **(B)** DEGs uniquely expressed either at 1 or 3 h after exposure to host cells

Table 2 RNA-seq data validation by RT-qPCR using six selected genes (*ilvC*, *metI*, *metE*, *icaA*, *emp*, and *rpsT*). RNA-seq values represent log_2 fold change of transcripts inferred by bioinformatics prediction. RT-qPCR values represent the mean log_2 fold change in transcripts. The value represents the log2 of the relative fold change between control (*S. aureus* only) and test (*S. aureus* with host). For both the analysis, log_2 fold change and adjusted p-value is presented. The p-value of less than 0.05 was significant

	Log2fold change		Adjusted P value	
Gene Symbol	**RNA seq**	**RT qPCR**	**RNA-seq**	**qRT-PCR**
ilvc	8.18	35	0.0000	0.0280
metI	2.95	2	0.0000	0.3351
metE	3.8	5	0.0000	0.1472
icaA	2.95	6	0.0009	0.0043
emp	-2.97	-1	0.0004	0.3831
rpst	-2.23	0.7	0.0000	0.0050

colonization of the nasal and vaginal regions, as well as during infections of the lung and skin [19–22]. However, there is limited knowledge about *S. aureus* transcriptome in throat colonization. To the best of our knowledge, the present study is the first to investigate the unique determinants expressed by *S. aureus* when exposed to tonsillar cells for 1 or 3 h. We found that some transcripts were commonly expressed at both time points, while others were expressed uniquely at 1 or 3 h exposure to host cells. Specifically, at 3 h, we observed differentially expressed genes such as *sbnABCDE, isdACHF, dps, sle1, icaA*, and *scn_3*. Conversely, at the 1 h time point, we observed differentially expressed genes such as *metE, sdrD, msrA2, emp, chp, isdB* and *sbnH*. Notably, some genes such as *fnbA*, *fnbB*, and *isaA* were commonly downregulated at both time points. These findings indicate the transcriptional response of *S. aureus* during interaction with tonsillar cells, suggesting that these genes may play an important role in *S. aureus* throat colonization.

S. aureus exhibited almost 1 log10 difference in growth in presence of host cells compared to growth in absence of host cells. This observation suggests that the bacteria may be receiving additional nutrients from host, e.g., following host cell lysis, as indicated by the slight increase in LDH released by the infected host cells compared to the uninfected cells. The *sle1* gene (also known as *aaa*) is one of the important peptidoglycan hydrolases associated with cell separation in *S. aureus* [23, 24]. Interestingly, *sle1* was upregulated after 3 h exposure to host cells, perhaps as a consequence of the bacterial growth in presence of host cells.

S. aureus adhesion to host cells is a prerequisite for colonization and is therefore considered a major risk factor for subsequent development of staphylococcal infection [25]. A total of 35 *S. aureus* adhesins have been examined previously [6, 26, 27]. *S. aureus* surface proteins, including clumping factor B (ClfB), iron-regulated surface protein A (IsdA), serine-aspartate repeat-containing protein (Sdr)C, SdrD and surface protein G (SasG), as well as wall teichoic acid, have been identified to promote *S.aureus* adherence to nasal epithelial cells and is involved during nasal colonization [26, 28–31]. Notably, our transcriptomics data show nine significantly differentially expressed genes (*isdA*, *isdB*, *isdH*, *sdrC*, *sdrD*, *fnbA*, *fnbB*, *isaA* and *spa*) encoding surface-bound proteins. This is consistent with observations reported in previous studies indicating the differential expression of *sdrC, sdrD, isdA* and *IsdB* [32–34] in *S. aureus* in the presence of host.

Comparison of *in-vivo* and *in-vitro* gene expression profiles across different human niches has shown that *S. aureus* colonization of the anterior nares is strongly controlled by adhesins and iron availability [7, 35]. In this study, we found that most of the *S. aureus* iron regulated surface determinants (*isd*) genes were highly upregulated after 3 h of *S. aureus* exposure to tonsillar cell. This finding was consistent with a similar study performed during *S. aureus* vaginal colonization [21]. The Isd system modulates the acquisition of heme enabling the bacterial to extract nutrients such as iron from its environment [36]. Furthermore, we found that several other genes important in iron homeostasis such as *sbnA, sbnB, sbnC, sbnD, sbnE* and *sbnH* were also expressed. These genes are responsible for encoding proteins for the biosynthesis of staphyloferrin B (*sbn*ABCDEH) and its transport system (SirABC) [37].

S. aureus employs various mechanisms to evade host immune defences for its survival within the host [25, 38]. In our study, we found that *scn_3* (encoding Staphylococcal complement inhibitor) and *chp* (encoding Chemotaxis inhibitory protein), involved in countering the first line of host defence mechanisms were downregulated when *S. aureus* was exposed to host cells. This is contradictory to an earlier finding [7], which could potentially be attributed to the absence of neutrophils in our experimental setup. On the other hand, catalase (*katA*)was upregulated consistent with reports from a previous study [7]. Catalase play a crucial role in protecting cells against the toxic effects of hydrogen peroxide, and it is required for survival, persistence, and nasal colonization of *S. aureus* [39]. Similarly, the Dps family protein, which protects DNA under starvation conditions was also upregulated only after 3 h. This suggests that the expression of stress

response genes may play a major role in the survival of *S. aureus* during throat colonization. The differential expression of these genes further indicates the dynamic adaptation of *S. aureus* to the host environment, particularly in response to the host immune defenses and nutrient availability.

The DEGs analysis of *S. aureus* cocultured with tonsillar cells, also revealed that various amino acid biosynthesis operons were upregulated. Notably, methionine synthase (*metE*) was reported to be upregulated only during 1 h of exposure, while *metC* was strongly upregulated at 1 h compared to 3 h. Previously, cystathionine-v-synthase (*metI*) has been reported to be strongly expressed during *S. aureus* colonization [30, 37, 40]. Upregulation of other methionine biosynthesis genes like cystathionine-b-lyase (*metC*), *metE, metH*, and *metI* including two L-methionine ABC-transport systems (*metN* and *metN2*) have also been reported [7]. These upregulated methionine biosynthesis genes represent a potential target for new antimicrobial strategies [41, 42] for combating *S. aureus* infection.

In our study, *fnbA* and *fnbB* were downregulated after both 1 and 3 h of exposure to tonsillar cells. This contrasts with previous studies indicating the role of FnbA and FnbB in promoting bacterial adhesion, biofilm formation and infections [43, 44]. Our findings suggest that these surface proteins [45] might be less relevant for *S. aureus* in presence of tonsillar cells in our experimental conditions. Transcription of *fnbA* and *fnbB* is downregulated in the post exponential phase of *S. aureus* growth [46]. This raises the possibility that the bacterial growth phase at the selected time points in this study may have influenced the expression pattern of *fnbA* and *fnbB*.

The common DEGs are important when *S. aureus* are exposed to a tonsillar cell line at both the time points, and the unique expression pattern might represent the importance of those genes either at 1 or 3 h. Overall, the transcriptome of *S. aureus* at 3 h showed increased upregulation of genes associated with iron acquisition while this was not observed at 1 h. This may suggest that over time *S. aureus* experiences a reduced level of available iron and increased competition for the ion [47, 48]. While *icaA* was upregulated at 3 h, potentially enhancing bacterial adhesion, the expression of *icaD*, a gene encoding IcaD that functions together with IcaA, was found to be commonly shared but with higher levels at 3 h compared to 1 h (lfc 5 at 1 h and lfc 6.5 at 3 h). Similarly, *sdrC* was noted as a commonly upregulated gene during the meeting of *S. aureus* with the tonsillar cell line. The level of expression of *sdrC* at 3 h (lfc 3.8) was higher than at 1 h (lfc 2.7), which may suggest increased SdrC-mediated adherence to tonsillar cells over time.

One of the limitations in our study, is that only one *S. aureus* strain and one type of mammalian cell line were included in the RNA-seq experiment. Another limitation is low mapping efficacy obtained in the 1 h test samples. To recover higher concentration of RNA required for RNA-seq, the in vitro experiment could have been performed in bigger cell culture dishes.

In conclusion, we have shown the suitability of using HTEpiC as an in vitro model for investigating key determinants in *S. aureus* involved in throat colonization. Our results reveal that some genes are commonly expressed, while others are uniquely expressed either at 1 or 3 h, indicating adaptation to the environment in presence of the tonsillar cell line. The up-regulated genes might be targets for intervention to prevent *S. aureus* throat colonization or infection in the future. Investigating the impact of other bacterial strain(s) present in the throat microbiome, as well as transcriptomic analysis of host cells using an ex-vivo model, could provide further insights into how *S. aureus* survival factors are influenced during co-colonization of the throat niche.

Methods

Experimental design

An overview of the experimental setup in this study is illustrated in Fig. 9. A Staphylococcus aureus throat isolate was cultured with or without a tonsillar cell line prior to RNA-seq to find differentially expressed genes (DEGs).

Human tonsil epithelial cells

Human Tonsil Epithelial Cells (HTEpiC) were purchased from Sciencell, United States (Cat #2560) and was isolated from a 10-year-old male. The tonsillar cells were cultured using Tonsil Epithelial Cell Medium (TEpiCM, Sciencell, Cat #2561) supplemented with 1% Tonsil Epithelial Cell Growth Supplement (TEpiCGS, Sciencell, Cat #2572) and penicillin/streptomycin solution (P/S, Sciencell, Cat #0503) (hereafter referred to as complete medium) at 37 °C in a 5% CO_2 incubator. Prior to the culturing of HTEpiC, the T-75 tissue culture flask was coated with 2 µg/cm^2 poly-L-lysin (PLL) (Sciencell, Cat #0403) and incubated at 37 °C for 2 h or overnight. Morphology, growth, and multiplication of HTEpiC were checked regularly. The complete medium was changed every third day until the cells reached 70% confluency and thereafter every second day until 90% confluent culture was observed (Additional file 6, Figure S2). At approximately 90% confluency, subculturing was initiated with trypsinization of cells using 0.25% trypsin/EDTA solution (T/E, Cat #0183) and handled according to the manufacturer´s instructions (Sciencell Research Laboratories, California).

Bacterial strains and growth conditions

A *Staphylococcus aureus* strain TR145 (spa-type t045, clonal complex 15) isolated from the throat in a healthy,

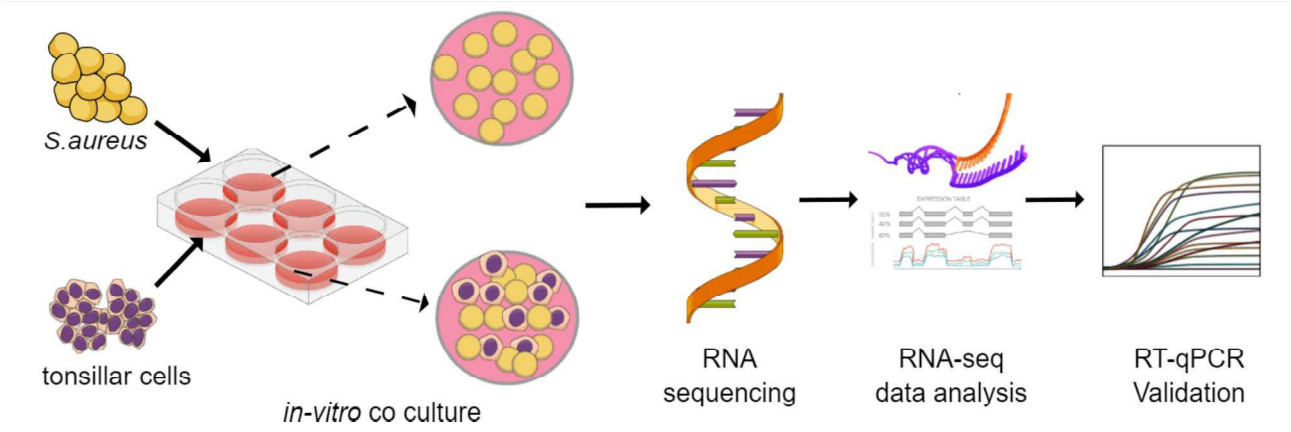

Fig. 9 Overview of the experimental setup in this study

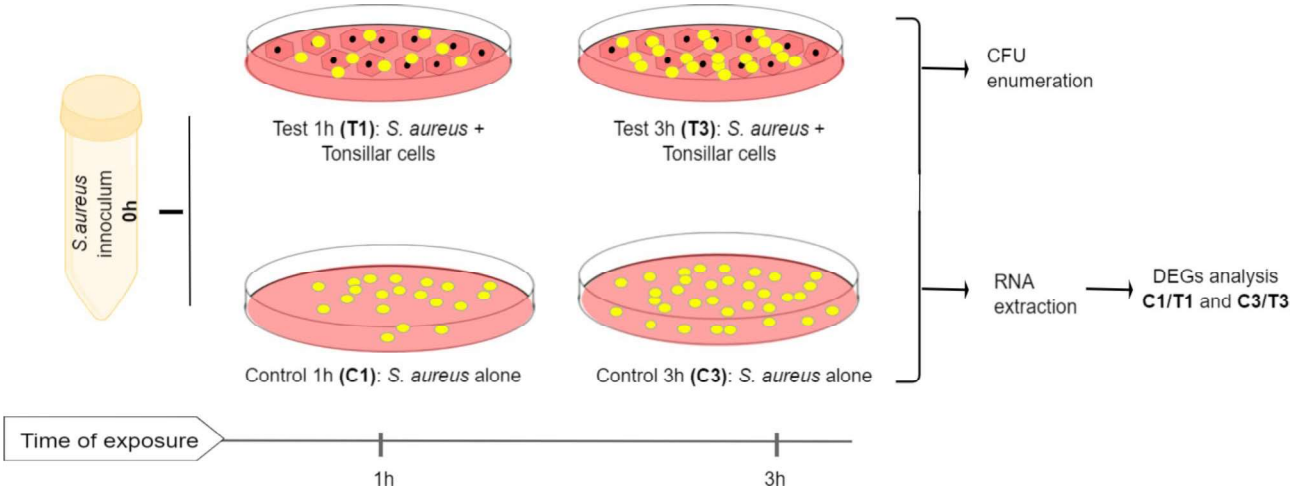

Fig. 10 Schematic representation of the in vitro co-culturing of *S. aureus* with or without tonsillar cells. *S. aureus* (inoculum) was added to PLL-coated wells containing host media in absence (controls) or presence of monolayer of host cells (tests) at MOI 5 and incubated for 1 or 3 h. Three independent experiments were run in triplicates. The adhered bacteria were collected and plated for either CFU enumeration or RNA extraction. The RNA samples were further processed for RNA-seq followed by DEGs analysis (C1/T1 and C3/T3)

adult individual in the Tromsø 6 study [49, 50] was used in this study. A single colony of *S. aureus* TR145 was transferred to 10 ml of Tryptic Soy Broth (TSB) and the culture was incubated overnight with shaking at 220 revolutions per minute (rpm) at 37 °C. One ml of overnight *S. aureus* culture was inoculated into 9 ml fresh TSB and incubated at 37 °C with shaking at 220 rpm for 1–2 h to reach the logarithmic growth phase ($0D_{600}$ 0.8–1.2). Freshly prepared logarithmic growth culture was subsequently harvested by centrifugation at 5000 rpm for 10 min at room temperature. The bacterial pellet was washed twice with autoclaved phosphate-buffered saline (PBS) and dissolved in 1 ml PBS.

The bacterial culture was adjusted to OD=0.4 (corresponding to approximately 1×10^8 CFU/ml) and used as inoculum to infect the host cells. To confirm the colony forming units (CFU), serial dilution of the inoculum was done followed by plate enumeration. The plating was done in triplicate onto TSA plates and left to incubate for 24 h at 37 °C. The bacterial colonies were then counted, and the average CFU/ml was calculated.

In vitro culturing of *S. aureus* with/without tonsillar cell line

HTEpiC was cultured until passage four and seeded at a density of ~4×10^5 viable cells per well in six well plates for adhesion assay or ~7×10^4 viable cells per well in 24 well plates coated with PLL. The HTEpiC was grown until confluence, washed with Dulbecco's Phosphate-Buffered Saline (DPBS, Sciencell, Cat #SC0303) and added TEpiCM. *S. aureus* TR145 at OD_{600nm} of 0.4 in complete medium (without antibiotics) was added to HTEpiC monolayer or to empty wells in a number corresponding to Multiplicity of Infection (MOI)=5 and incubated for 1 and 3 h (Fig. 10, to capture the initial stages of *S. aureus*-host cell interaction [51, 52]. Images of host

cells in absence or in presence of *S. aureus* for 1 and 3 h are shown in Additional file 6, Figure S3.

After 1 and 3 h post-infection, the media was aspirated, and the host cells were washed twice with fresh media/DPBS to remove unbound bacteria. The host cells were then trypsinized and lysed with Triton-X. The released bacteria were then collected from three technical replicates and pooled together. At both time points, bacteria seeded into PLL coated plates without host cells (Fig. 10, control 1 h and control 3 h) were also collected using scraping technique followed by visual inspection of the wells by microscopy to ensure that most of bacteria were recovered from the well.

An aliquot of the bacterial suspensions was serially diluted and plated on TSA agars for CFU determination. The remaining bacteria were centrifuged immediately at 5000 rpm at room temperature for 10 min. The bacterial pellets were resuspended in 100 µl of RNAprotect® Bacterial Reagent (Qiagen, Cat #76,506) followed by 5 s vortexing and incubation for 5 min at RT. After the final centrifugation (5000 rpm, 10 min, room temperature), bacterial pellets were preserved at -80 °C until total RNA isolation.

Cytotoxicity assay

Host cell lactate dehydrogenase (LDH) released into supernatants after 1 and 3 h of post-infection was quantified using CytoTox96®Non-Radioactive Cytotoxicity Assay (Promega, G1781), according to the manufacturer's instructions. Positive control (HTEpiC infected with *S. aureus*) included in the cytotoxicity assay represented 100% cell death after adding 2 µl of lysis solution (9% W/V Triton-X-100) per 100 µl volume. Both background control (only complete medium) and negative control (non-infected HTEpiC) were included for each condition at both time points. For the quantification of the sample, colorimetric measurement of LDH release was measured at 490 nm using a standard 96-well plate reader, analyzed using SoftMax Pro Software. Sample readings (from three technical replicates) were divided by the positive control for cell lysis to result in a percentage of total cell death for each sample.

Table 3 Twelve RNA samples were processed for NGS library preparation and RNA-seq

Replicate number	Sample ID	Group
1st Biological replicates	*S. aureus* only _1h	C1h_Rep1
	S. aureus only _3h	C3h_Rep1
	S. aureus + tonsillar cells_1h	T1h_Rep1
	S. aureus + tonsillar cells_3h	T3h_Rep1
2nd Biological replicates	*S. aureus* only _1h	C1h_Rep2*
	S. aureus only _3h	C3h_Rep2
	S. aureus + tonsillar cells_1h	T1h_Rep2
	S. aureus + tonsillar cells_3h	T3h_Rep2
3rd Biological replicates	*S. aureus* only _1h	C1h_Rep3
	S. aureus only _3h	C3h_Rep3
	S. aureus + tonsillar cells_1h	T1h_Rep3
	S. aureus + tonsillar cells_3h	T3h_Rep3

Note **This control sample had the lowest RNA concentration and did not reveal the presence of RNA after library preparation and was therefore not processed for RNA-seq library preparation*

Bacterial lysis and RNA preparation

Bacterial pellets in RNAprotect® at -80 °C were thawed and suspended in 100 µl of TE buffer (10 mM Tris Cl, 1 mM EDTA, pH 8; Sigma-Aldrich) containing the lysozyme (0.5 µl, 0.1 mg/ml; Sigma-Aldrich) and lysostaphin (0.5 µl, 10 mg/ml; Sigma-Aldrich) and incubated at 37 °C for 10 min. The bacterial suspensions were transferred to a 0.5 ml Safe-Lock centrifuge tube containing acid-washed glass beads (0.1 mm diameter, Cat.No. 11,079,101, BioSpec product) and disrupted using *Precellys® Evolution* homogenizer (Precellys Evolution, bertin technologies) at 4500 rpm, 40 s x 2 cycle, 4 min pause on ice.

After homogenization, total RNA was isolated, following the recommendations of the manufacturer (Qiagen RNeasy Mini Kit, Cat.No. 74,104). RNA was eluted with 40 µl of nuclease-free water (Ambion; Darmstadt, Germany) and the eluate was used to re-eluate (30–35 µl) to achieve higher RNA concentration. DNase treatment was performed using Heat and Run kit (ArcticZymes, Norway) according to the manufacturer's instructions. RNA quantity and integrity were measured by Nanodrop1000 spectrophotometer (Thermo Scientific; Waltham, MA, USA or Biolab), and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively.

NGS library construction and RNA-sequencing

Total RNA extracted from three replicates of *S. aureus* TR145 grown in absence of host cells collected at time points of 1 and 3 h (*S. aureus* only, control samples (C)) and three replicates of *S. aureus* TR145 after 1 and 3 h exposure to host cells (*S. aureus* + tonsillar cells, test samples (T)) were selected for RNA-seq library preparation (Table 3).

Depletion of rRNA was performed with the RiboCop depletion kit (Lexogen, cat no: 127 (RiboCop rRNA depletion kit for Gram Positive Bacteria (G+)), according to the manufacturer's protocol. In total, 12 RNA samples (Table 3) were processed for library construction using Lexogen's CORALL™ Total RNA-Seq Kit with RiboCop (Cat.No.96; EU, CH, USA).

All samples were run with 16 PCR cycles for the final library amplification step. One of the samples had too low concentration (~ 1.5 ng/µl) after fragmentation and did not proceed for sequencing. Otherwise, all steps were according to the manufacturer's protocol. The samples were sequenced on an Illumina 550 platform, with dual

indexes, and paired end mode. The final sequencing concentration was 1.8 pM.

RNA-seq data analysis

Total RNA reads were generated from 11 samples in two runs of RNA-seq (Table 3). The illumina paired end data were then mapped against *S. aureus* TR145 reference genome to retrieve bacterial reads that were uniquely mapped. The remaining most reads representing eukaryotic RNA were eliminated bioinformatically. Only bacterial reads were further processed for differentially expressed genes (DEGs) analysis. The detailed RNA-seq bioinformatics pipeline is illustrated in Fig. 11.

Each library was pre-processed for quality check using FASTQC/0.11.9-Java-11 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Filtering (removable of adaptor dimer reads) and trimming (removable of low-quality bases) was performed by Trimmomatic/0.39-Java-11 (http://www.usadellab.org/cms/?page=trimmomatic). Only those sequences with quality score Q>20 and a minimum of 55 nucleotide sequence length were retained in the dataset. The final quality check was performed in the trimmed file. *S. aureus* strain TR145 (SAMEA112465883) was previously whole genome sequenced and kindly provided to us (M Røkeberg Olsen, unpublished results). The genome from *S. aureus* TR145 was used as a reference genome for the mapping performed using Bowtie2/2.4.4-GCC-10.3.0 (https://bowtie-bio.sourceforge.net/bowtie2/index.shtml).

Reads mapped to reference (*S. aureus* TR145) gene was identified using HTSeq counting tool (https://htseq.readthedocs.io/en/release_0.11.1/count.html). After aligning reads to a reference and generating count files, it was further analyzed by DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html) to explore any DEGs present in the sample. The DE was further curated to give only those genes which show p- value adjusted (padj) along the indication of gene name. Threshold for padj was adjusted to less than 0.05 (padj<0.05) and for log2fold change greater than 2 (lfc ≥ |2|). Any gene that followed these two thresholds was a good starting point for identifying significant genes. The DEGs were visualized from plots such as PCA and Venn diagram in R using ggplot2. Further, these DEGs were analyzed using ShinyGO 0.76.2 (http://bioinformatics.sdstate.edu/go/), a graphical tool for gene ontology (GO) enrichment analysis. DEGs being involved in different GO terms such as molecular function, biological process, and cellular components were identified. The GO terms with false discovery rate (FDR) less than 0.05 were considered significantly enriched.

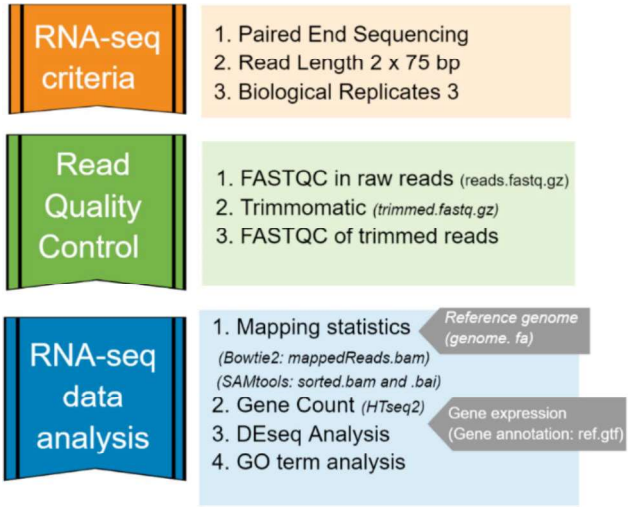

Fig. 11 RNA-seq workflow analysis used in this study

Table 4 Primer sequence and expected amplicon size of the six selected genes used for qRT-PCR in this study

Gene	Primer Sequence (5′-3′)	Amplicon size (bp)	Efficiency E (%)	Correlation coefficient (R^2)
groEL	GCACCAGTTCGTCAAATTGC (L) CACTCGTTTGTAGCAGCGTT (R)	110	115	1.00
ilvC	AAACGGACGCTTTACAAGGC (L) GTCAAAAGAACGACCTGGGC (R)	131	121	0.97
metI	ACATGGTATTGCATCATTCGCT (L) ATGTGCCGCCGTATAAATCG (R)	116	120	0.94
metE	CGAAAGCGTGCGTACTTCAA (L) TCTCGGCTTTGTGGGAATGA (R)	120	118	0.90
icaA	CGACGTTGGCTACTGGGATA (L) TGCTTCCAAAGACCTCCCAA (R)	150	122	0.94
emp	CGCGTGAATGTAACAACAAACA (L) CTTGTAGTGGGTTTGCGTAGT (R)	138	114	0.98
rpsT	CTGAAGCACGCAACATTTCAC (L) ACTTTGAGCAGCTTTGTCTACT (R)	140	119	0.92

Validation by qRT-PCR

Validation of RNA-seq data was performed by qRT-PCR. Six genes with different expression profiles were selected based on fold change, p-value, and functions. For instance, gene with highest fold change and lowest p-value (*ilvC*), gene with medium fold change but commonly expressed (*metI*), uniquely expressed genes involved in cellular process (*metI, emp*, *icaA*), molecular function (*rspt*), and both in the molecular function and biological process (*metE*) (Table 4). The mRNA transcripts of the selected DEGs were quantified using the LightCycler® 96 Instrument Software, Version 1.1.1

- Service Pack SP1 according to the manufacturer's instructions (Table 4).

RNA was reverse transcribed into cDNA using the High-capacity cDNA Reverse Transcription kits (Applied Biosystems, Forster City, CA, USA, Cat #4,368,814) according to the manufacturer's protocol. Two microliter of generated cDNA / negative RT control was used as a template for the generation of amplicons from gene of interest. Each qRT-PCR reaction was performed in a final volume of 25 µl. The final concentration of 100 nM of each primer pair was added to 12.5 µl of SYBR® Green PCR Master Mix (1X, Takyon™ qPCR MasterMixes for SYBR® assays containing Low ROX passive reference, Eurogentec, Fremont, CA, USA, Cat #UF-LSMT-B0701). The PCR reactions were generated in a thermal cycler: 95 °C for 15 s; 40 cycles of 60 °C for 60 s, 95 °C for 15 s, and a final extension of 60 °C for 15 s. RT negative control, and PCR Negative control samples with sterile water were also included. All qRT-PCR experiments were performed using three biological replicates and three technical replicates. The Quantification cycle values (Cq) were determined, and relative fold differences were calculated by Delta-Delta Cq method using *groEL* as the reference gene. The expression profiles of the six genes analyzed relative to the *groEL* was compared with the values of Deseq2 analysis obtained from the RNA-Seq data.

Abbreviations

aldh1	Aldehyde dehydrogenase 1
Clf	Clumping factor
cDNA	Complementary Deoxyribonucleic acid
CFU	Colony forming units
chp	Calcineurin B homologous protein
cna	Collagen adhesion
cq	Quantification cycle
DEGs	Differentially expressed genes
dps	DNA protection during starvation protein
DPBS	Dulbecco's Phosphate-Buffered Saline
eap	Extracellular adherence protein
ebh	Extracellular matrix-binding protein ebh
ebps	Elastin-binding protein EbpS
ecm	Extracellular matrix
emp	Extracellular matrix protein-binding protein
FnBPs	Fibronectin binding proteins
HaCaT	Human Keratinocyte Cell Culture
HTEpiC	Human tonsil epithelial cells
icaA	Intracellular adhesion A
ilvC	Ketol-acid reductoisomerase
GO	Gene ontology
isaA	Immunodominant staphylococcal antigen A
isd	Iron-regulated surface determinants
katA	Catalase
leuC	Leucine biosynthesis
LDH	Lactate dehydrogenase
met	Methionine biosynthesis
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
MOI	Multiplicity of infection
NEAT	NEAr Transporter domain
$0D_{600}$	Optical density
PCA	Principal component analysis
PBS	Phosphate-buffered saline
P/S	Penicillin/ streptomycin solution
PLL	Poly-L-lysin
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
rpsT	Encoding ribosomal protein S20
RNA-seq	RNA-sequencing
rpm	Revolutions per minute
sas	Staphylococcus aureus surface protein
Scn_3	Staphylococcal complement inhibitor 3
sbn	Staphyloferrin B biosynthesis protein
sdrC	Serine-aspartate repeat-containing protein C
sle1	Cell separation gene of *S. aureus*
spa	Staphylococcal protein A

sraP	Serine-rich adhesion for platelets
SNM	Synthetic nasal medium
TEpiCM	Tonsil Epithelial Cell Medium
TEpiCGS, Tonsil Epithelial Cell Growth Supplement; T/E	Trypsin/EDTA solution
TSB	Tryptic soy broth

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-023-02919-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Authors' contributions
A.M.H. and M.J. supervised, and conceptualized the project, and provided resources. S.B., C.A., M.J. and A.M.H. designed the study. S.B. and K.J. performed the in vitro experiments. C.A. supervised laboratory work. S.B. analyzed the data. S.B., C.A., M.J. and A.M.H. interpreted the data. S.B. prepared the first version of the manuscript. All authors reviewed the manuscript, gave inputs, and approved the final manuscript.

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Data Availability
Raw RNA-seq reads and processed files generated in this study can be found in the European Nucleotide Archive (ENA) repository with the GEO accession number GSE226317 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE226317) under the project number PRJNA939634. The FASTQ files of reference genome (*S. aureus* TR145 strain) and annotation files used in this study is deposited in ENA with BioSample accession SAMEA112465883 (https://www.ebi.ac.uk/ena/browser/view/PRJEB59355) under the project number PRJEB59355. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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Publisher's Note

Table S1: Samples and RNA concentrations used in this study.

Replicate number	Sample ID	Group	RNA concentration (ng/µl) by Nanodrop	RNA sequencing performed
1st Biological replicates	*S. aureus* only _1h	C1h_Rep1	10.8	Yes
	S. aureus only _3h	C3h_Rep1	80.6	Yes
	S. aureus + tonsillar cells_1h	T1h_Rep1	45.8	Yes
	S. aureus + tonsillar cells_3h	T3h_Rep1	876	Yes
2nd Biological replicates	*S. aureus* only _1h	C1h_Rep2*	1.5	No*
	S. aureus only _3h	C3h_Rep2	31.6	Yes
	S. aureus + tonsillar cells_1h	T1h_Rep2	127.8	Yes
	S. aureus + tonsillar cells_3h	T3h_Rep2	404	Yes
3rd Biological replicates	*S. aureus* only _1h	C1h_Rep3	7	Yes
	S. aureus only _3h	C3h_Rep3	15.3	Yes
	S. aureus + tonsillar cells_1h	T1h_Rep3	23.2	Yes
	S. aureus + tonsillar cells_3h	T3h_Rep3	480	Yes

**This control sample had the lowest RNA concentration, did not reveal the presence of RNA after library preparation, and was therefore not processed for RNA sequencing.*

Table S2: Summary of RNA-seq raw reads, and mapping efficacy. *X represents the raw reads generated per library. Y represents the reads retrieved after filtering and removing low-quality bases and adaptor contaminations.*

Group	RNA-Seq reads before (X) and after (Y) quality filtering and trimming		Mapping efficacy* (%)
	X	Y	
C1h_Rep1	40,210,520	40,210,170	93
C3h_Rep1	36,355,999	33,882,141	89
T1h_Rep1	34,216,742	34,216,316	< 20
T3h_Rep1	41,855,924	39,522,800	63
C3h_Rep2	53,642,437	53,566,920	94
T1h_Rep2	49,011,990	49,011,329	<20
T3h_Rep2	40,504,599	33,350,138	55
C1h_Rep3	53,136,173	53,122,689	<20
C3h_Rep3	41,671,571	38,692,332	88
T1h_Rep3	45,491,307	45,416,993	<20
T3h_Rep3	41,044,431	41,043,179	75

*The reference genome used in this study is S. aureus TR145. The mapping efficiency percentage represents the uniquely mapped reads against the S. aureus genome. All the other unmapped reads, especially in samples treated with host cells contained RNA reads from eukaryotic cells, which were eliminated bioinformatically.

Table S3. Four hundred and thirty differentially expressed genes (DEGs) identified in S. aureus after 1h of exposure with tonsillar cells identified by RNA-seq. *Out of 430 only 310 genes were pre-annotated. Out of 310 DEGs, 105 were identified as uniquely expressed genes only at 1h (gene name marked in bold) and the remaining 205 as commonly shared DEGs between two exposure time points.*

transcript_id	log2FoldChange	pvalue	padj	gene name
t145_t084_12690_gene	9.55	4.30E-19	1.40E-17	NA
t145_t084_04400_gene	9.33	8.30E-20	2.90E-18	*leuB*
t145_t084_04390_gene	8.66	3.10E-26	1.90E-24	*leuC*
t145_t084_04410_gene	8.43	1.10E-29	8.90E-28	*leuA_1*
t145_t084_04430_gene	8.42	1.90E-18	5.60E-17	*ilvH*
t145_t084_04420_gene	8.18	1.10E-35	1.50E-33	*ilvC*
t145_t084_12680_gene	7.97	2.50E-16	5.70E-15	NA
t145_t084_23200_gene	7.45	2.70E-38	4.00E-36	*salL*
t145_t084_19190_gene	7.45	4.00E-39	7.30E-37	*metP_2*
t145_t084_23210_gene	7.32	3.90E-47	9.20E-45	NA
t145_t084_11060_gene	7.31	4.60E-09	4.00E-08	*thrC*
t145_t084_11070_gene	7.24	3.20E-31	3.20E-29	*nadX*
t145_t084_03490_gene	7.2	6.30E-14	1.00E-12	***czrA***
t145_t084_07330_gene	6.92	1.10E-55	3.80E-53	NA
t145_t084_10440_gene	6.89	1.20E-28	8.20E-27	*dapA*
t145_t084_11050_gene	6.85	8.60E-22	3.40E-20	*thrB_1*
t145_t084_21540_gene	6.82	3.20E-11	3.90E-10	NA
t145_t084_10420_gene	6.69	1.60E-26	9.70E-25	*dapH_1*
t145_t084_23220_gene	6.59	3.80E-77	4.60E-74	*ykoD_2*
t145_t084_10450_gene	6.54	8.40E-29	6.10E-27	asd
t145_t084_10430_gene	6.5	2.60E-30	2.30E-28	*dapB*
t145_t084_24480_gene	6.47	1.00E-19	3.50E-18	NA
t145_t084_04440_gene	6.29	3.50E-119	8.20E-116	*ilvB*
t145_t084_23240_gene	6.28	4.00E-37	5.50E-35	NA
t145_t084_04380_gene	6.27	9.00E-15	1.60E-13	*leuD*
t145_t084_19200_gene	6.24	2.20E-54	6.60E-52	*metN*
t145_t084_10410_gene	6.09	4.00E-23	1.90E-21	*scmP_2*
t145_t084_21270_gene	6.08	5.30E-22	2.10E-20	*carA_2*
t145_t084_04630_gene	6.04	2.70E-14	4.50E-13	NA
t145_t084_11080_gene	5.95	2.50E-17	6.70E-16	*yclM*
t145_t084_19180_gene	5.84	5.10E-58	2.00E-55	*metQ_2*
t145_t084_10460_gene	5.84	9.70E-24	4.60E-22	*lysC*
t145_t084_21280_gene	5.81	4.10E-19	1.30E-17	*lcfB*
t145_t084_04450_gene	5.8	4.10E-67	2.40E-64	*ilvD*
t145_t084_24340_gene	5.8	7.80E-35	9.30E-33	NA
t145_t084_07320_gene	5.57	3.00E-22	1.20E-20	*serA*
t145_t084_14530_gene	5.55	4.00E-22	1.60E-20	*oppF_2*
t145_t084_19970_gene	5.49	5.10E-15	9.60E-14	*metC*
t145_t084_10400_gene	5.42	1.00E-21	4.00E-20	*alr1_2*
t145_t084_23230_gene	5.41	4.30E-51	1.10E-48	*ykoC*
t145_t084_01780_gene	5.2	3.80E-74	3.00E-71	*panS*
t145_t084_04370_gene	5.19	4.10E-15	8.00E-14	*ilvA*
t145_t084_14520_gene	5.11	1.80E-33	1.80E-31	*dppE_2*
t145_t084_23390_gene	5.06	1.10E-03	3.60E-03	*icaD*
t145_t084_21550_gene	4.91	2.40E-10	2.50E-09	NA
t145_t084_19980_gene	4.8	4.10E-15	8.00E-14	*yitJ*
t145_t084_03480_gene	4.72	2.60E-11	3.10E-10	***czcD_1***
t145_t084_19810_gene	4.65	6.40E-08	4.70E-07	NA
t145_t084_10390_gene	4.64	1.40E-30	1.30E-28	*lysA*
t145_t084_22840_gene	4.64	2.00E-20	7.40E-19	*hutH*
t145_t084_17830_gene	4.55	4.80E-10	4.70E-09	NA
t145_t084_14540_gene	4.53	2.50E-16	5.60E-15	*oppD_2*
t145_t084_20120_gene	4.53	5.00E-07	3.30E-06	*efeM*
t145_t084_21170_gene	4.36	4.40E-03	1.20E-02	***fruA_2***
t145_t084_20130_gene	4.29	6.00E-09	5.10E-08	*ydaF*
t145_t084_04550_gene	4.28	3.30E-11	3.90E-10	*nrgA*
t145_t084_24040_gene	4.28	1.20E-27	8.50E-26	*yhdG*
t145_t084_14560_gene	4.24	1.30E-35	1.60E-33	*oppB_2*
t145_t084_21530_gene	4.2	7.10E-19	2.30E-17	NA

t145_t084_14140_gene	4.18	1.90E-16	4.40E-15	NA
t145_t084_14290_gene	4.16	4.90E-31	4.60E-29	*acp*
t145_t084_15470_gene	4.12	6.50E-08	4.80E-07	*argO*
t145_t084_24450_gene	4.12	8.50E-08	6.10E-07	*ldhD_1*
t145_t084_15320_gene	4.1	3.80E-24	1.80E-22	*metQ_1*
t145_t084_24670_gene	4.03	2.70E-17	7.30E-16	NA
t145_t084_14550_gene	4	3.00E-62	1.40E-59	*oppC_2*
t145_t084_02130_gene	4	1.90E-29	1.40E-27	*ydbM*
t145_t084_19960_gene	4	1.70E-09	1.50E-08	*metI*
t145_t084_23350_gene	3.93	1.20E-11	1.50E-10	*lipA_2*
t145_t084_20240_gene	3.91	2.90E-03	8.70E-03	NA
t145_t084_22820_gene	3.9	8.80E-34	9.60E-32	NA
t145_t084_21290_gene	3.89	5.00E-03	1.40E-02	*caiA*
t145_t084_19990_gene	3.8	1.00E-12	1.40E-11	***metE***
t145_t084_18000_gene	3.8	5.30E-25	2.90E-23	*sdrC*
t145_t084_11540_gene	3.8	4.00E-03	1.10E-02	NA
t145_t084_24680_gene	3.77	7.20E-09	6.10E-08	NA
t145_t084_21830_gene	3.77	1.50E-06	9.00E-06	*nrtD*
t145_t084_16260_gene	3.73	6.80E-47	1.50E-44	*hisC_2*
t145_t084_10360_gene	3.7	8.90E-13	1.20E-11	NA
t145_t084_23250_gene	3.66	9.00E-16	1.90E-14	NA
t145_t084_02360_gene	3.65	1.00E-04	4.30E-04	NA
t145_t084_22270_gene	3.65	4.70E-08	3.60E-07	*butA*
t145_t084_11010_gene	3.65	8.30E-30	6.80E-28	*katA*
t145_t084_07190_gene	3.59	2.90E-20	1.10E-18	*aroA_1*
t145_t084_15330_gene	3.57	5.20E-12	6.60E-11	*metP_1*
t145_t084_19100_gene	3.56	2.20E-27	1.40E-25	*gltA*
t145_t084_02890_gene	3.55	1.40E-06	8.30E-06	*adhR*
t145_t084_23600_gene	3.55	1.60E-22	6.80E-21	NA
t145_t084_01710_gene	3.51	1.90E-29	1.40E-27	*yghA*
t145_t084_07220_gene	3.44	1.50E-02	3.50E-02	*acuA*
t145_t084_11340_gene	3.42	4.60E-09	4.00E-08	*glnR*
t145_t084_14490_gene	3.42	4.50E-05	2.00E-04	*oppD_1*
t145_t084_01800_gene	3.42	5.90E-13	8.50E-12	NA
t145_t084_14080_gene	3.41	4.10E-06	2.20E-05	NA
t145_t084_01790_gene	3.39	2.80E-41	5.60E-39	NA
t145_t084_20070_gene	3.38	2.00E-15	4.00E-14	NA
t145_t084_14090_gene	3.38	1.40E-05	7.10E-05	NA
t145_t084_14100_gene	3.34	1.90E-13	2.90E-12	NA
t145_t084_00290_gene	3.34	4.80E-14	7.80E-13	*abgT*
t145_t084_20230_gene	3.27	4.70E-03	1.30E-02	*ulaC*
t145_t084_22420_gene	3.26	2.40E-10	2.50E-09	*spa*
t145_t084_06900_gene	3.26	1.20E-09	1.10E-08	***ribBA***
t145_t084_05550_gene	3.2	9.70E-14	1.50E-12	***bcp***
t145_t084_03610_gene	3.19	4.80E-17	1.20E-15	*abgB*
t145_t084_22800_gene	3.18	2.90E-14	4.90E-13	*metXA*
t145_t084_06450_gene	3.18	2.30E-03	7.00E-03	NA
t145_t084_21490_gene	3.15	1.50E-06	8.90E-06	NA
t145_t084_11180_gene	3.14	2.10E-03	6.70E-03	NA
t145_t084_15340_gene	3.13	4.50E-10	4.40E-09	*metN2*
t145_t084_21520_gene	3.1	8.20E-13	1.20E-11	*ggt*
t145_t084_24110_gene	3.1	1.40E-07	9.90E-07	***cocE***
t145_t084_24310_gene	3.04	2.20E-15	4.40E-14	NA
t145_t084_21180_gene	3.03	8.70E-04	3.00E-03	NA
t145_t084_14500_gene	3.02	5.00E-04	1.80E-03	*oppC_1*
t145_t084_20370_gene	3.02	4.40E-20	1.50E-18	*ybbH_2*
t145_t084_19600_gene	3.01	1.20E-04	5.00E-04	NA
t145_t084_22810_gene	3.01	1.40E-08	1.10E-07	NA
t145_t084_19110_gene	2.99	9.00E-07	5.70E-06	***hdfR_2***
t145_t084_14870_gene	2.99	3.70E-20	1.30E-18	NA
t145_t084_18070_gene	2.98	9.40E-05	4.10E-04	NA
t145_t084_11000_gene	2.96	9.80E-06	5.00E-05	***rpmG2_2***
t145_t084_21850_gene	2.95	1.20E-02	2.80E-02	NA
t145_t084_06890_gene	2.95	3.60E-07	2.30E-06	***ribE***
t145_t084_09320_gene	2.93	3.30E-10	3.30E-09	NA

t145_t084_21840_gene	2.93	3.10E-10	3.10E-09	NA
t145_t084_19730_gene	2.92	2.50E-13	3.80E-12	***nfrA***
t145_t084_02470_gene	2.92	2.20E-05	1.10E-04	NA
t145_t084_15980_gene	2.92	5.30E-11	6.10E-10	*hpf*
t145_t084_01420_gene	2.89	2.70E-09	2.40E-08	NA
t145_t084_20110_gene	2.88	8.60E-14	1.40E-12	*efeN*
t145_t084_06690_gene	2.87	3.30E-11	3.90E-10	***yvgN_1***
t145_t084_21810_gene	2.86	3.20E-04	1.20E-03	*ssuC*
t145_t084_21800_gene	2.86	5.50E-03	1.50E-02	NA
t145_t084_20220_gene	2.86	1.10E-04	4.80E-04	NA
t145_t084_07450_gene	2.85	6.20E-04	2.20E-03	NA
t145_t084_00650_gene	2.85	7.50E-15	1.40E-13	NA
t145_t084_23300_gene	2.84	3.30E-03	9.50E-03	***hisB***
t145_t084_04890_gene	2.83	1.20E-06	7.20E-06	NA
t145_t084_00260_gene	2.82	1.80E-05	8.80E-05	NA
t145_t084_14480_gene	2.82	6.20E-03	1.70E-02	***oppF_1***
t145_t084_24320_gene	2.81	5.30E-16	1.10E-14	NA
t145_t084_06880_gene	2.8	4.20E-08	3.30E-07	***ribD***
t145_t084_23280_gene	2.79	1.60E-07	1.10E-06	*hisD*
t145_t084_01850_gene	2.79	8.10E-03	2.10E-02	NA
t145_t084_11040_gene	2.79	1.70E-14	2.90E-13	NA
t145_t084_19400_gene	2.78	2.10E-04	8.50E-04	*lpl2_4*
t145_t084_04730_gene	2.78	3.00E-07	2.00E-06	***dapE***
t145_t084_18110_gene	2.77	1.80E-10	2.00E-09	***hchA***
t145_t084_24690_gene	2.77	2.30E-25	1.30E-23	*lipR*
t145_t084_06150_gene	2.76	5.10E-14	8.30E-13	*traP*
t145_t084_23270_gene	2.73	3.70E-04	1.40E-03	*hisG*
t145_t084_16920_gene	2.72	2.10E-05	9.90E-05	***wecD***
t145_t084_23860_gene	2.72	9.60E-13	1.30E-11	*bsaA_2*
t145_t084_10950_gene	2.72	4.80E-03	1.30E-02	NA
t145_t084_18060_gene	2.72	5.20E-15	9.80E-14	***ppaX***
t145_t084_10490_gene	2.71	2.00E-02	4.50E-02	***pstS***
t145_t084_18080_gene	2.7	1.20E-25	7.30E-24	*ilvE*
t145_t084_06090_gene	2.69	1.50E-12	2.10E-11	***prsA***
t145_t084_00270_gene	2.68	4.50E-06	2.40E-05	NA
t145_t084_23790_gene	2.66	8.00E-05	3.50E-04	NA
t145_t084_04970_gene	2.65	1.50E-16	3.60E-15	*aldH1*
t145_t084_14370_gene	2.65	6.60E-11	7.50E-10	NA
t145_t084_01410_gene	2.64	6.90E-04	2.40E-03	***hrtA_1***
t145_t084_11330_gene	2.63	1.70E-07	1.20E-06	*glnA*
t145_t084_24440_gene	2.62	2.10E-13	3.20E-12	*bacF*
t145_t084_21680_gene	2.62	9.20E-07	5.80E-06	***yecD***
t145_t084_14380_gene	2.61	3.00E-07	2.00E-06	***yjbK***
t145_t084_21870_gene	2.61	8.10E-04	2.80E-03	***aldA***
t145_t084_24520_gene	2.6	7.20E-08	5.30E-07	*ydfJ*
t145_t084_10740_gene	2.59	1.90E-08	1.50E-07	NA
t145_t084_03620_gene	2.56	1.60E-12	2.10E-11	NA
t145_t084_00870_gene	2.55	8.20E-16	1.70E-14	NA
t145_t084_14390_gene	2.53	3.80E-13	5.50E-12	***yjbI***
t145_t084_20080_gene	2.53	1.90E-15	3.80E-14	*tatAy*
t145_t084_01220_gene	2.52	9.70E-05	4.10E-04	NA
t145_t084_23990_gene	2.51	8.00E-25	4.20E-23	*acsA_2*
t145_t084_16280_gene	2.5	9.70E-15	1.70E-13	***opuBA***
t145_t084_05560_gene	2.5	5.40E-13	7.80E-12	***ghrB_1***
t145_t084_19090_gene	2.49	8.50E-10	8.10E-09	*gltB*
t145_t084_19370_gene	2.49	1.60E-02	3.80E-02	***lpl2_2***
t145_t084_24490_gene	2.48	8.30E-11	9.50E-10	NA
t145_t084_20440_gene	2.47	1.40E-08	1.10E-07	NA
t145_t084_17130_gene	2.47	1.10E-05	5.80E-05	NA
t145_t084_15930_gene	2.46	1.10E-05	5.60E-05	NA
t145_t084_04540_gene	2.45	5.10E-04	1.90E-03	*yeeD*
t145_t084_02900_gene	2.44	6.40E-10	6.20E-09	NA
t145_t084_13870_gene	2.43	7.90E-03	2.10E-02	NA
t145_t084_02180_gene	2.42	6.70E-04	2.40E-03	***fdhD***
t145_t084_00890_gene	2.41	5.60E-08	4.20E-07	***tcyC_1***

t145_t084_07940_gene	2.41	9.40E-08	6.80E-07	NA
t145_t084_06460_gene	2.4	1.40E-06	8.30E-06	NA
t145_t084_23970_gene	2.4	6.30E-04	2.20E-03	NA
t145_t084_23310_gene	2.38	3.60E-03	1.00E-02	***hisH***
t145_t084_07470_gene	2.37	3.00E-10	3.10E-09	NA
t145_t084_23260_gene	2.36	2.70E-07	1.80E-06	*hisZ*
t145_t084_04930_gene	2.36	4.20E-15	8.20E-14	NA
t145_t084_21100_gene	2.35	1.20E-22	5.10E-21	*tarJ'*
t145_t084_06630_gene	2.35	1.40E-04	5.80E-04	NA
t145_t084_24920_gene	2.34	5.10E-04	1.90E-03	NA
t145_t084_14430_gene	2.34	8.40E-18	2.40E-16	***mecA***
t145_t084_08200_gene	2.33	2.40E-06	1.40E-05	***fgd_1***
t145_t084_13350_gene	2.33	1.20E-10	1.40E-09	NA
t145_t084_21670_gene	2.32	3.80E-08	2.90E-07	***ipdC***
t145_t084_19380_gene	2.31	9.20E-03	2.40E-02	NA
t145_t084_01310_gene	2.31	3.00E-06	1.60E-05	***paiA***
t145_t084_14360_gene	2.29	3.30E-10	3.30E-09	***yjbM***
t145_t084_23320_gene	2.28	9.90E-03	2.50E-02	***hisA***
t145_t084_11020_gene	2.28	3.10E-09	2.80E-08	*lysP_2*
t145_t084_20090_gene	2.28	1.90E-05	9.10E-05	*tatC2*
t145_t084_10670_gene	2.27	4.80E-03	1.30E-02	***trpC***
t145_t084_17990_gene	2.27	1.40E-03	4.40E-03	***sdrD***
t145_t084_10760_gene	2.27	2.50E-09	2.20E-08	*msrA1*
t145_t084_24470_gene	2.27	2.30E-12	3.00E-11	***copA***
t145_t084_11370_gene	2.25	2.50E-07	1.70E-06	***bsaA_1***
t145_t084_06770_gene	2.25	1.20E-13	1.80E-12	***tal***
t145_t084_18440_gene	2.25	2.90E-08	2.30E-07	***gabR***
t145_t084_16550_gene	2.21	1.30E-02	3.20E-02	NA
t145_t084_21820_gene	2.2	7.70E-04	2.70E-03	*cmpC*
t145_t084_16230_gene	2.2	6.40E-09	5.40E-08	*dtpT*
t145_t084_21150_gene	2.19	1.50E-02	3.50E-02	***gatC_2***
t145_t084_09360_gene	2.19	2.10E-09	1.90E-08	***zwf***
t145_t084_01700_gene	2.18	1.10E-05	5.40E-05	*scmP_1*
t145_t084_18390_gene	2.18	1.70E-03	5.50E-03	***mcsA***
t145_t084_16250_gene	2.18	7.30E-13	1.00E-11	NA
t145_t084_15940_gene	2.17	2.90E-05	1.40E-04	***yfbR***
t145_t084_23290_gene	2.17	1.30E-05	6.40E-05	*hisC_3*
t145_t084_15410_gene	2.16	1.20E-06	7.20E-06	***ydbP***
t145_t084_04640_gene	2.16	3.10E-12	4.00E-11	***yafV***
t145_t084_19610_gene	2.16	2.50E-03	7.60E-03	NA
t145_t084_01470_gene	2.15	1.70E-09	1.50E-08	*aaeA*
t145_t084_10160_gene	2.15	2.80E-24	1.40E-22	***msrA2***
t145_t084_21420_gene	2.14	3.40E-06	1.80E-05	NA
t145_t084_09640_gene	2.14	1.30E-05	6.30E-05	***rpsA***
t145_t084_24650_gene	2.13	3.80E-06	2.00E-05	***ydaP***
t145_t084_00840_gene	2.12	5.10E-07	3.30E-06	***gpmA_1***
t145_t084_18130_gene	2.12	2.30E-11	2.80E-10	*scmP_3*
t145_t084_14570_gene	2.12	1.80E-02	4.20E-02	NA
t145_t084_21110_gene	2.11	1.30E-18	3.80E-17	*tarI2*
t145_t084_19590_gene	2.11	2.20E-04	8.70E-04	*qorB*
t145_t084_16670_gene	2.11	1.30E-04	5.50E-04	NA
t145_t084_07590_gene	2.11	1.30E-06	7.70E-06	***citZ***
t145_t084_07310_gene	2.11	1.10E-10	1.20E-09	*gph_1*
t145_t084_24500_gene	2.11	4.30E-05	2.00E-04	*rocA*
t145_t084_24870_gene	2.11	2.30E-03	7.00E-03	***mhqD***
t145_t084_19640_gene	2.1	1.30E-03	4.20E-03	NA
t145_t084_19390_gene	2.1	2.00E-02	4.60E-02	***lpl2_3***
t145_t084_23450_gene	2.09	4.60E-04	1.70E-03	NA
t145_t084_20400_gene	2.06	1.60E-03	5.00E-03	*sglT*
t145_t084_06520_gene	2.06	3.80E-04	1.40E-03	NA
t145_t084_00880_gene	2.05	3.80E-03	1.10E-02	***tcyB***
t145_t084_21090_gene	2.05	1.10E-14	2.00E-13	***tarK***
t145_t084_07350_gene	2.05	4.10E-06	2.20E-05	*glpQ_1*
t145_t084_23620_gene	2.04	8.10E-13	1.10E-11	NA

t145_t084_11410_gene	2.04	5.10E-07	3.30E-06	***glpD***
t145_t084_07230_gene	2.03	6.30E-04	2.30E-03	*acsA_1*
t145_t084_01480_gene	2.03	1.30E-06	7.70E-06	*mdtD*
t145_t084_17170_gene	2.03	2.40E-03	7.40E-03	***mntA***
t145_t084_02870_gene	2.02	7.40E-04	2.60E-03	NA
t145_t084_12760_gene	2.02	1.40E-02	3.30E-02	***argF***
t145_t084_22600_gene	2.01	1.40E-02	3.30E-02	NA
t145_t084_10190_gene	2	6.60E-04	2.30E-03	NA
t145_t084_12890_gene	-2	1.20E-03	4.00E-03	NA
t145_t084_18680_gene	-2	4.00E-17	1.00E-15	*hpt*
t145_t084_12590_gene	-2.01	5.10E-15	9.60E-14	*murD*
t145_t084_13450_gene	-2.02	1.70E-08	1.40E-07	*pdhD*
t145_t084_07900_gene	-2.03	7.70E-08	5.60E-07	***fpgS***
t145_t084_11810_gene	-2.04	9.70E-17	2.40E-15	*uppS*
t145_t084_16380_gene	-2.04	1.50E-05	7.20E-05	***queD***
t145_t084_08150_gene	-2.05	1.60E-16	3.80E-15	NA
t145_t084_22400_gene	-2.05	1.20E-02	3.00E-02	***yfhA***
t145_t084_13960_gene	-2.06	1.60E-08	1.30E-07	*menH*
t145_t084_01980_gene	-2.06	1.00E-05	5.10E-05	*odh*
t145_t084_22300_gene	-2.07	2.20E-05	1.10E-04	***sbnH***
t145_t084_11620_gene	-2.08	8.60E-15	1.50E-13	NA
t145_t084_12030_gene	-2.09	5.70E-06	2.90E-05	*rpsP*
t145_t084_11830_gene	-2.11	1.50E-08	1.20E-07	*pyrH*
t145_t084_11680_gene	-2.11	9.20E-05	4.00E-04	***rpsO***
t145_t084_18200_gene	-2.12	2.40E-09	2.10E-08	*rpoB*
t145_t084_16950_gene	-2.13	2.20E-07	1.50E-06	NA
t145_t084_12490_gene	-2.13	6.20E-12	7.90E-11	***ileS***
t145_t084_21980_gene	-2.14	1.80E-02	4.20E-02	NA
t145_t084_19550_gene	-2.14	8.50E-03	2.20E-02	***ssl4_2***
t145_t084_06360_gene	-2.17	3.90E-05	1.80E-04	NA
t145_t084_18140_gene	-2.17	1.30E-06	7.70E-06	***tuf***
t145_t084_17780_gene	-2.18	2.90E-06	1.60E-05	NA
t145_t084_13040_gene	-2.18	2.00E-09	1.80E-08	*pheT*
t145_t084_08460_gene	-2.19	3.00E-14	5.00E-13	***aroE***
t145_t084_17790_gene	-2.2	1.80E-10	1.90E-09	NA
t145_t084_16850_gene	-2.21	6.20E-05	2.80E-04	NA
t145_t084_02650_gene	-2.21	3.20E-07	2.10E-06	***rplR***
t145_t084_12460_gene	-2.22	8.50E-05	3.70E-04	*lspA*
t145_t084_08320_gene	-2.22	3.80E-16	8.40E-15	NA
t145_t084_02330_gene	-2.23	1.20E-04	5.00E-04	*cntE_2*
t145_t084_08410_gene	-2.24	4.30E-05	1.90E-04	***entA***
t145_t084_13050_gene	-2.25	2.50E-06	1.40E-05	*pheS*
t145_t084_01130_gene	-2.27	1.80E-02	4.30E-02	NA
t145_t084_02820_gene	-2.3	3.50E-08	2.70E-07	***rpsI***
t145_t084_00400_gene	-2.3	8.60E-05	3.70E-04	***cntE_1***
t145_t084_02660_gene	-2.31	3.00E-06	1.60E-05	***rpsE***
t145_t084_18190_gene	-2.32	5.40E-08	4.10E-07	*rpoC*
t145_t084_02640_gene	-2.33	1.30E-05	6.30E-05	***rplF***
t145_t084_03780_gene	-2.33	1.40E-07	1.00E-06	***ywlC***
t145_t084_18180_gene	-2.35	1.60E-12	2.10E-11	*rplGB*
t145_t084_11610_gene	-2.35	1.80E-27	1.20E-25	*phaB*
t145_t084_22610_gene	-2.35	3.30E-07	2.20E-06	*dus*
t145_t084_15580_gene	-2.35	5.60E-04	2.00E-03	NA
t145_t084_16390_gene	-2.36	2.60E-12	3.50E-11	***queE_2***
t145_t084_18160_gene	-2.36	1.40E-09	1.30E-08	***rpsG***
t145_t084_02810_gene	-2.37	3.50E-09	3.10E-08	*rplM*
t145_t084_10320_gene	-2.4	1.70E-07	1.20E-06	*brnQ_1*
t145_t084_09960_gene	-2.41	3.40E-06	1.90E-05	NA
t145_t084_06030_gene	-2.41	2.90E-17	7.70E-16	NA
t145_t084_00390_gene	-2.41	3.20E-05	1.50E-04	***cntF***
t145_t084_17690_gene	-2.43	1.10E-06	6.90E-06	NA
t145_t084_01990_gene	-2.44	4.20E-09	3.60E-08	***mleN_2***
t145_t084_03670_gene	-2.45	2.30E-10	2.40E-09	*pyrG*
t145_t084_17510_gene	-2.45	1.20E-04	5.10E-04	NA

t145_t084_17700_gene	-2.46	4.20E-16	9.20E-15	NA
t145_t084_13540_gene	-2.46	1.00E-05	5.10E-05	*ythB*
t145_t084_02630_gene	-2.47	6.30E-08	4.70E-07	*rpsH*
t145_t084_11800_gene	-2.47	7.50E-13	1.10E-11	NA
t145_t084_11760_gene	-2.47	1.40E-17	3.80E-16	*rimP*
t145_t084_22310_gene	-2.48	1.70E-05	8.20E-05	***garL***
t145_t084_24350_gene	-2.49	9.80E-10	9.30E-09	*isaA*
t145_t084_11110_gene	-2.5	1.20E-04	4.90E-04	*nucH*
t145_t084_03740_gene	-2.5	7.90E-08	5.80E-07	*rpmE2*
t145_t084_13430_gene	-2.51	2.60E-03	7.90E-03	***puuR***
t145_t084_18150_gene	-2.53	1.60E-10	1.70E-09	***fusA***
t145_t084_12600_gene	-2.56	4.60E-16	9.90E-15	*mraY*
t145_t084_07410_gene	-2.56	1.50E-05	7.30E-05	*yfcA*
t145_t084_01550_gene	-2.58	1.20E-18	3.80E-17	*gltS*
t145_t084_18770_gene	-2.59	1.50E-10	1.60E-09	*rplY*
t145_t084_09950_gene	-2.61	3.80E-17	9.80E-16	*rlmL*
t145_t084_03770_gene	-2.65	1.50E-11	1.90E-10	*prmC*
t145_t084_02580_gene	-2.65	6.00E-08	4.50E-07	*rpsQ*
t145_t084_12360_gene	-2.65	1.30E-10	1.40E-09	NA
t145_t084_12450_gene	-2.66	4.80E-20	1.70E-18	***rluD_2***
t145_t084_03750_gene	-2.67	3.00E-14	4.90E-13	*tdk*
t145_t084_11740_gene	-2.69	7.70E-15	1.40E-13	NA
t145_t084_18170_gene	-2.71	5.50E-16	1.20E-14	***rpsL***
t145_t084_11750_gene	-2.71	2.00E-25	1.20E-23	*nusA*
t145_t084_13160_gene	-2.71	2.70E-12	3.60E-11	NA
t145_t084_13550_gene	-2.73	9.60E-06	4.90E-05	*ythA*
t145_t084_18250_gene	-2.73	7.10E-15	1.30E-13	*rplK*
t145_t084_08130_gene	-2.74	6.30E-08	4.70E-07	*hisS*
t145_t084_02510_gene	-2.74	1.10E-12	1.50E-11	*rplW*
t145_t084_07840_gene	-2.75	9.10E-09	7.60E-08	NA
t145_t084_07970_gene	-2.76	1.00E-06	6.40E-06	*rplU*
t145_t084_14040_gene	-2.78	3.20E-15	6.30E-14	*catD*
t145_t084_02600_gene	-2.79	6.50E-12	8.30E-11	*rplX*
t145_t084_00380_gene	-2.81	3.60E-06	2.00E-05	***cntD***
t145_t084_04770_gene	-2.83	6.80E-05	3.00E-04	***chp***
t145_t084_01230_gene	-2.83	2.40E-05	1.10E-04	*treP_1*
t145_t084_00370_gene	-2.84	5.00E-04	1.80E-03	***cntC***
t145_t084_02520_gene	-2.85	2.20E-09	2.00E-08	*rplB*
t145_t084_02620_gene	-2.86	1.50E-10	1.60E-09	*rpsZ*
t145_t084_10870_gene	-2.86	1.60E-10	1.70E-09	*opuD_2*
t145_t084_20990_gene	-2.87	1.20E-06	7.50E-06	***lrgB***
t145_t084_02610_gene	-2.88	2.80E-11	3.30E-10	*rplE*
t145_t084_11850_gene	-2.91	2.20E-19	7.20E-18	*rpsB*
t145_t084_13320_gene	-2.92	2.10E-19	7.00E-18	*typA*
t145_t084_02590_gene	-2.95	2.80E-11	3.30E-10	*rplN*
t145_t084_10980_gene	-2.95	5.90E-10	5.70E-09	*guaC*
t145_t084_02530_gene	-2.96	5.10E-12	6.50E-11	*rpsS*
t145_t084_11720_gene	-2.97	5.30E-39	9.10E-37	*infB*
t145_t084_15590_gene	-2.97	1.00E-04	4.40E-04	***emp***
t145_t084_13420_gene	-2.97	3.80E-07	2.50E-06	***potA***
t145_t084_07980_gene	-2.98	3.80E-07	2.50E-06	NA
t145_t084_03760_gene	-3	9.90E-23	4.50E-21	*prfA*
t145_t084_11730_gene	-3.02	5.00E-18	1.50E-16	*rplGA*
t145_t084_14070_gene	-3.02	5.20E-04	1.90E-03	NA
t145_t084_07920_gene	-3.04	2.10E-05	1.00E-04	NA
t145_t084_13410_gene	-3.05	9.50E-09	7.90E-08	***potB***
t145_t084_16510_gene	-3.05	2.20E-06	1.20E-05	***ydjF***
t145_t084_22750_gene	-3.06	5.20E-15	9.80E-14	*purA*
t145_t084_16370_gene	-3.06	8.00E-21	3.00E-19	*queC*
t145_t084_07990_gene	-3.06	1.40E-06	8.30E-06	***rpmA***
t145_t084_08140_gene	-3.07	1.60E-13	2.40E-12	*aspS*
t145_t084_18240_gene	-3.15	2.80E-13	4.10E-12	*rplA*
t145_t084_00030_gene	-3.16	3.70E-10	3.70E-09	*fnbB*
t145_t084_02550_gene	-3.19	6.20E-14	9.90E-13	*rpsC*
t145_t084_07360_gene	-3.21	3.10E-21	1.20E-19	*rpsD*

t145_t084_02490_gene	-3.21	3.90E-12	5.10E-11	*rplC*
t145_t084_02480_gene	-3.27	8.50E-11	9.60E-10	*rpsJ*
t145_t084_12000_gene	-3.28	4.80E-14	7.80E-13	*rplS*
t145_t084_02570_gene	-3.29	9.20E-14	1.40E-12	*rpmC*
t145_t084_02540_gene	-3.31	4.40E-16	9.70E-15	*rplV*
t145_t084_16140_gene	-3.37	1.30E-05	6.50E-05	***yclQ***
t145_t084_19880_gene	-3.49	9.30E-12	1.20E-10	***rpsR***
t145_t084_03160_gene	-3.49	1.10E-38	1.70E-36	NA
t145_t084_00020_gene	-3.5	1.40E-07	1.00E-06	*fnbA*
t145_t084_19900_gene	-3.51	6.90E-15	1.30E-13	*rpsF*
t145_t084_02500_gene	-3.53	3.20E-13	4.70E-12	*rplD*
t145_t084_19890_gene	-3.56	3.90E-13	5.70E-12	*ssbA_2*
t145_t084_04790_gene	-3.58	1.60E-04	6.60E-04	*hlb_2*
t145_t084_07740_gene	-3.6	9.00E-08	6.50E-07	*rpmI*
t145_t084_13390_gene	-3.62	1.60E-12	2.10E-11	*potD*
t145_t084_12440_gene	-3.62	1.40E-17	4.00E-16	*pyrR*
t145_t084_00820_gene	-3.63	2.80E-10	2.80E-09	*sbi*
t145_t084_14650_gene	-3.64	1.10E-02	2.70E-02	***leuA_2***
t145_t084_21660_gene	-3.65	1.10E-34	1.20E-32	*ptsG_3*
t145_t084_02560_gene	-3.69	4.60E-16	9.90E-15	*rplP*
t145_t084_13140_gene	-3.7	5.90E-09	5.00E-08	***isdB***
t145_t084_18230_gene	-3.72	1.10E-16	2.70E-15	*rplJ*
t145_t084_07750_gene	-3.73	2.70E-11	3.20E-10	*rplT*
t145_t084_01020_gene	-3.73	8.50E-03	2.20E-02	*nasE*
t145_t084_07730_gene	-3.76	3.80E-17	9.90E-16	*infC*
t145_t084_04120_gene	-3.76	7.70E-17	1.90E-15	*cshA*
t145_t084_13400_gene	-3.81	4.00E-10	4.00E-09	*ydcV*
t145_t084_18220_gene	-3.89	5.30E-17	1.30E-15	*rplL*
t145_t084_09970_gene	-3.92	2.10E-14	3.50E-13	NA
t145_t084_12020_gene	-3.96	1.80E-17	4.90E-16	*rimM*
t145_t084_04760_gene	-3.99	1.30E-24	6.50E-23	***hlb_1***
t145_t084_12010_gene	-4	1.20E-13	1.80E-12	*trmD*
t145_t084_21410_gene	-4.06	1.50E-14	2.60E-13	NA
t145_t084_15300_gene	-4.15	2.30E-16	5.20E-15	NA
t145_t084_17230_gene	-4.23	2.70E-05	1.30E-04	***mrpC***
t145_t084_12380_gene	-4.27	1.00E-17	2.90E-16	*pyrF*
t145_t084_15600_gene	-4.32	1.10E-06	6.60E-06	NA
t145_t084_12390_gene	-4.37	2.80E-24	1.40E-22	*carB*
t145_t084_12370_gene	-4.37	5.80E-30	5.00E-28	*pyrE*
t145_t084_12400_gene	-4.55	1.80E-22	7.70E-21	*carA_1*
t145_t084_21000_gene	-4.59	1.30E-13	1.90E-12	*lrgA*
t145_t084_12410_gene	-4.75	5.80E-18	1.70E-16	*pyrC*
t145_t084_21320_gene	-4.95	7.90E-17	2.00E-15	NA
t145_t084_02000_gene	-5.06	1.40E-25	7.90E-24	*ssaA2*
t145_t084_12420_gene	-5.21	1.30E-18	3.80E-17	*pyrB*
t145_t084_12430_gene	-5.21	8.30E-23	3.80E-21	*pyrP*
t145_t084_19450_gene	-5.6	7.40E-19	2.30E-17	NA
t145_t084_19460_gene	-5.75	8.70E-12	1.10E-10	*ssl5_1*

NOTE:

NA gene not annotated

genes in bold uniquely expressed genes at 1h of exposure

Table S4. Three hundred and forty-eight differentially expressed genes (DEGs) were identified in S. aureus after 3h of exposure with tonsillar cells identified by RNA-seq. Out of 348 of which only 258 genes were pre-annotated. Out of 258 genes, 53 being identified as uniquely expressed genes at 3h (gene name maked in bold) and remaining 205 as commonly shared DEGs between both the exposure time points.

transcript_id	log2FoldChange	pvalue	padj	gene name
t145_t084_12680_gene	7.57	3.90E-23	1.20E-21	NA
t145_t084_19190_gene	7.25	1.00E-71	4.00E-69	*metP_2*
t145_t084_19200_gene	6.99	1.00E-104	8.20E-102	*metN*
t145_t084_23200_gene	6.84	9.10E-47	1.30E-44	*salL*
t145_t084_12690_gene	6.81	5.00E-16	8.40E-15	NA
t145_t084_10460_gene	6.74	3.80E-39	3.70E-37	*lysC*
t145_t084_23210_gene	6.68	9.20E-66	2.80E-63	NA
t145_t084_21550_gene	6.53	1.70E-23	5.40E-22	NA
t145_t084_23390_gene	6.53	5.90E-06	2.90E-05	*icaD*
t145_t084_19180_gene	6.52	1.60E-99	9.50E-97	*metQ_2*
t145_t084_10450_gene	6.44	1.60E-35	1.30E-33	*asd*
t145_t084_21540_gene	6.4	2.00E-15	3.00E-14	NA
t145_t084_07330_gene	6.29	5.30E-61	1.30E-58	NA
t145_t084_10440_gene	6.09	3.30E-29	1.60E-27	*dapA*
t145_t084_21270_gene	6.02	1.30E-29	6.70E-28	*carA_2*
t145_t084_23220_gene	6	4.70E-88	2.20E-85	*ykoD_2*
t145_t084_04400_gene	5.96	7.40E-15	1.10E-13	*leuB*
t145_t084_10430_gene	5.88	1.10E-32	7.60E-31	*dapB*
t145_t084_04430_gene	5.87	1.40E-36	1.10E-34	*ilvH*
t145_t084_07320_gene	5.85	2.40E-30	1.40E-28	*serA*
t145_t084_11080_gene	5.82	3.10E-21	7.50E-20	*yclM*
t145_t084_10420_gene	5.81	4.00E-26	1.60E-24	*dapH_1*
t145_t084_04420_gene	5.78	2.10E-29	1.10E-27	*ilvC*
t145_t084_04410_gene	5.71	8.90E-22	2.30E-20	*leuA_1*
t145_t084_21530_gene	5.48	3.70E-42	4.00E-40	NA
t145_t084_11070_gene	5.34	3.20E-22	8.50E-21	*nadX*
t145_t084_24340_gene	5.31	5.40E-37	4.60E-35	NA
t145_t084_01780_gene	5.2	1.60E-106	1.90E-103	*panS*
t145_t084_22360_gene	5.19	1.90E-17	3.50E-16	***sbnB***
t145_t084_14140_gene	5.19	1.40E-30	8.70E-29	NA
t145_t084_11060_gene	5.15	3.50E-06	1.80E-05	*thrC*
t145_t084_04390_gene	5.14	6.00E-17	1.10E-15	*leuC*
t145_t084_10410_gene	5.07	6.10E-21	1.50E-19	*scmP_2*
t145_t084_21830_gene	5.04	7.50E-15	1.10E-13	*nrtD*
t145_t084_21280_gene	4.99	1.90E-24	6.40E-23	*lcfB*
t145_t084_23230_gene	4.99	2.30E-61	6.10E-59	*ykoC*
t145_t084_21820_gene	4.93	9.10E-19	1.80E-17	*cmpC*
t145_t084_10400_gene	4.91	7.70E-23	2.30E-21	*alr1_2*
t145_t084_24040_gene	4.9	1.90E-44	2.30E-42	*yhdG*
t145_t084_14290_gene	4.84	2.70E-52	5.00E-50	*acp*
t145_t084_14510_gene	4.8	2.50E-08	1.80E-07	***oppB_1***
t145_t084_22810_gene	4.79	2.50E-24	8.30E-23	NA
t145_t084_21810_gene	4.78	2.20E-13	2.90E-12	*ssuC*
t145_t084_23240_gene	4.75	1.50E-31	9.80E-30	NA
t145_t084_15320_gene	4.73	1.10E-39	1.10E-37	*metQ_1*
t145_t084_04440_gene	4.65	4.10E-113	9.70E-110	*ilvB*
t145_t084_04380_gene	4.61	6.20E-13	7.60E-12	*leuD*
t145_t084_11050_gene	4.54	5.20E-13	6.40E-12	*thrB_1*
t145_t084_21800_gene	4.52	3.10E-07	1.90E-06	NA
t145_t084_04450_gene	4.36	1.30E-57	2.80E-55	*ilvD*
t145_t084_22820_gene	4.32	4.60E-55	9.20E-53	NA
t145_t084_21520_gene	4.27	9.50E-30	5.10E-28	*ggt*
t145_t084_04370_gene	4.22	1.40E-13	1.80E-12	*ilvA*
t145_t084_22350_gene	4.2	9.10E-33	6.60E-31	*sbnC*
t145_t084_15330_gene	4.17	9.10E-20	1.90E-18	*metP_1*

t145_t084_14520_gene	4.08	3.50E-27	1.50E-25	*dppE_2*
t145_t084_10390_gene	4.05	1.20E-29	6.30E-28	*lysA*
t145_t084_13120_gene	4.03	7.80E-22	2.00E-20	***isdC***
t145_t084_07250_gene	4.02	2.50E-19	5.10E-18	***isdH***
t145_t084_21840_gene	3.97	1.90E-22	5.30E-21	NA
t145_t084_22840_gene	3.9	1.50E-19	3.10E-18	*hutH*
t145_t084_13110_gene	3.88	2.80E-10	2.50E-09	NA
t145_t084_14530_gene	3.87	3.20E-14	4.50E-13	*oppF_2*
t145_t084_15340_gene	3.78	2.60E-17	4.80E-16	*metN2*
t145_t084_23270_gene	3.77	7.10E-09	5.30E-08	*hisG*
t145_t084_23350_gene	3.76	3.80E-13	4.80E-12	*lipA_2*
t145_t084_11010_gene	3.7	5.60E-38	5.10E-36	*katA*
t145_t084_22370_gene	3.68	4.10E-24	1.40E-22	***sbnA***
t145_t084_04550_gene	3.63	2.20E-14	3.10E-13	*nrgA*
t145_t084_13100_gene	3.62	4.20E-11	4.10E-10	***isdE***
t145_t084_14550_gene	3.61	5.30E-66	1.80E-63	*oppC_2*
t145_t084_23860_gene	3.6	5.30E-28	2.40E-26	*bsaA_2*
t145_t084_14560_gene	3.59	1.00E-32	7.10E-31	*oppB_2*
t145_t084_14100_gene	3.55	2.50E-20	5.60E-19	NA
t145_t084_20240_gene	3.48	8.40E-04	2.70E-03	NA
t145_t084_01710_gene	3.47	1.70E-40	1.80E-38	*yghA*
t145_t084_01700_gene	3.43	6.10E-15	9.20E-14	*scmP_1*
t145_t084_15470_gene	3.42	5.30E-10	4.50E-09	*argO*
t145_t084_14540_gene	3.42	3.50E-12	4.00E-11	*oppD_2*
t145_t084_07190_gene	3.42	8.10E-23	2.40E-21	*aroA_1*
t145_t084_04630_gene	3.37	1.40E-06	7.80E-06	NA
t145_t084_23600_gene	3.37	1.90E-25	7.20E-24	NA
t145_t084_13090_gene	3.33	3.90E-09	3.00E-08	*isdF*
t145_t084_21490_gene	3.32	1.20E-08	8.60E-08	NA
t145_t084_23250_gene	3.3	2.70E-16	4.60E-15	NA
t145_t084_06460_gene	3.27	1.50E-13	1.90E-12	NA
t145_t084_13070_gene	3.23	1.90E-06	1.00E-05	***isdG_1***
t145_t084_01790_gene	3.22	1.60E-49	2.60E-47	NA
t145_t084_20220_gene	3.19	1.20E-06	6.50E-06	NA
t145_t084_06450_gene	3.19	4.70E-04	1.60E-03	NA
t145_t084_01800_gene	3.19	2.70E-15	4.10E-14	NA
t145_t084_22800_gene	3.17	1.00E-17	2.00E-16	*metXA*
t145_t084_19100_gene	3.17	3.10E-28	1.40E-26	*gltA*
t145_t084_16260_gene	3.15	1.40E-43	1.60E-41	*hisC_2*
t145_t084_21400_gene	3.14	1.70E-07	1.10E-06	***uhpT***
t145_t084_24320_gene	3.1	1.30E-25	5.00E-24	NA
t145_t084_24440_gene	3.07	1.10E-22	3.10E-21	*bacF*
t145_t084_15980_gene	3.03	2.10E-14	3.10E-13	*hpf*
t145_t084_00290_gene	3.03	7.70E-15	1.10E-13	*abgT*
t145_t084_07220_gene	3.02	1.40E-02	3.30E-02	*acuA*
t145_t084_23260_gene	3.02	2.10E-14	3.00E-13	*hisZ*
t145_t084_03620_gene	3	1.10E-20	2.60E-19	NA
t145_t084_20130_gene	2.98	1.30E-06	7.40E-06	*ydaF*
t145_t084_20120_gene	2.96	1.50E-04	5.90E-04	*efeM*
t145_t084_21560_gene	2.96	2.90E-12	3.30E-11	***gsiA***
t145_t084_23400_gene	2.95	2.70E-04	9.80E-04	***icaA***
t145_t084_19960_gene	2.95	6.60E-08	4.40E-07	*metI*
t145_t084_03610_gene	2.93	2.20E-18	4.30E-17	*abgB*
t145_t084_23280_gene	2.92	2.10E-10	1.90E-09	*hisD*
t145_t084_24450_gene	2.88	2.10E-05	9.70E-05	*ldhD_1*
t145_t084_02130_gene	2.87	3.30E-22	8.80E-21	*ydbM*
t145_t084_24520_gene	2.87	8.90E-12	9.60E-11	*ydfJ*
t145_t084_24310_gene	2.86	1.80E-18	3.50E-17	NA
t145_t084_24480_gene	2.85	4.20E-06	2.20E-05	NA
t145_t084_16230_gene	2.83	5.70E-17	1.00E-15	*dtpT*
t145_t084_13080_gene	2.82	9.50E-08	6.20E-07	***srtB***
t145_t084_24670_gene	2.81	1.60E-11	1.60E-10	NA

t145_t084_20230_gene	2.8	4.70E-03	1.30E-02	*ulaC*
t145_t084_22330_gene	2.79	1.40E-06	7.60E-06	***sbnE***
t145_t084_18000_gene	2.78	2.60E-17	4.80E-16	***sdrC***
t145_t084_01420_gene	2.76	1.00E-10	9.80E-10	NA
t145_t084_22340_gene	2.7	1.80E-03	5.50E-03	***sbnD***
t145_t084_21290_gene	2.7	1.20E-02	2.90E-02	*caiA*
t145_t084_07310_gene	2.69	1.20E-20	2.80E-19	*gph_1*
t145_t084_20070_gene	2.68	1.10E-12	1.30E-11	NA
t145_t084_19970_gene	2.61	6.30E-07	3.60E-06	*metC*
t145_t084_09320_gene	2.59	4.10E-10	3.50E-09	NA
t145_t084_24680_gene	2.58	6.70E-06	3.30E-05	NA
t145_t084_19810_gene	2.55	7.40E-04	2.40E-03	NA
t145_t084_19720_gene	2.53	4.10E-06	2.10E-05	***tcyP***
t145_t084_24030_gene	2.52	2.50E-07	1.50E-06	NA
t145_t084_20250_gene	2.48	1.10E-04	4.40E-04	***ulaA***
t145_t084_22270_gene	2.48	3.20E-05	1.40E-04	*butA*
t145_t084_23990_gene	2.47	4.40E-30	2.60E-28	*acsA_2*
t145_t084_02360_gene	2.46	2.30E-03	6.80E-03	NA
t145_t084_06920_gene	2.45	2.60E-04	9.30E-04	***putB***
t145_t084_04540_gene	2.45	6.10E-06	3.00E-05	*yeeD*
t145_t084_11040_gene	2.44	4.50E-14	6.30E-13	NA
t145_t084_21890_gene	2.44	8.30E-09	6.20E-08	***isdI***
t145_t084_14490_gene	2.44	2.90E-04	1.00E-03	*oppD_1*
t145_t084_02890_gene	2.43	9.40E-05	3.80E-04	*adhR*
t145_t084_06470_gene	2.43	1.20E-05	5.60E-05	NA
t145_t084_11330_gene	2.41	8.60E-08	5.70E-07	*glnA*
t145_t084_24500_gene	2.4	1.80E-07	1.10E-06	*rocA*
t145_t084_00710_gene	2.4	4.00E-09	3.10E-08	***irtA***
t145_t084_10650_gene	2.4	1.00E-12	1.20E-11	***trpB***
t145_t084_19090_gene	2.39	2.40E-11	2.50E-10	*gltB*
t145_t084_03100_gene	2.39	8.90E-08	5.80E-07	NA
t145_t084_03550_gene	2.38	1.10E-17	2.10E-16	***dps***
t145_t084_10360_gene	2.38	2.40E-07	1.50E-06	NA
t145_t084_20110_gene	2.38	4.90E-13	6.20E-12	*efeN*
t145_t084_14500_gene	2.35	7.10E-04	2.30E-03	*oppC_1*
t145_t084_11340_gene	2.34	6.90E-06	3.40E-05	*glnR*
t145_t084_04530_gene	2.34	1.10E-10	1.00E-09	NA
t145_t084_21110_gene	2.33	8.70E-28	3.80E-26	*tarI2*
t145_t084_13130_gene	2.32	6.90E-05	2.80E-04	***isdA***
t145_t084_19400_gene	2.31	2.00E-04	7.60E-04	*lpl2_4*
t145_t084_20370_gene	2.31	5.70E-16	9.50E-15	*ybbH_2*
t145_t084_23290_gene	2.31	4.00E-08	2.70E-07	*hisC_3*
t145_t084_10660_gene	2.3	3.70E-11	3.60E-10	***trpF***
t145_t084_07350_gene	2.28	2.30E-09	1.80E-08	*glpQ_1*
t145_t084_06150_gene	2.28	3.00E-12	3.40E-11	*traP*
t145_t084_14830_gene	2.28	2.40E-04	8.70E-04	***argH***
t145_t084_14090_gene	2.27	7.10E-05	2.90E-04	NA
t145_t084_14820_gene	2.26	1.30E-04	5.10E-04	***argG***
t145_t084_00640_gene	2.25	2.00E-11	2.00E-10	***cpdA_1***
t145_t084_20080_gene	2.24	9.20E-16	1.50E-14	*tatAy*
t145_t084_11020_gene	2.23	8.10E-11	7.80E-10	*lysP_2*
t145_t084_13910_gene	2.21	1.00E-10	9.70E-10	***dapX***
t145_t084_01480_gene	2.21	3.30E-09	2.60E-08	*mdtD*
t145_t084_21850_gene	2.2	5.30E-03	1.40E-02	NA
t145_t084_14080_gene	2.19	4.00E-04	1.40E-03	NA
t145_t084_02900_gene	2.18	3.80E-10	3.30E-09	NA
t145_t084_00650_gene	2.18	1.90E-11	1.90E-10	NA
t145_t084_18080_gene	2.17	2.80E-21	6.80E-20	*ilvE*
t145_t084_19980_gene	2.17	3.70E-05	1.60E-04	*yitJ*
t145_t084_24690_gene	2.16	4.50E-20	9.70E-19	*lipR*
t145_t084_24850_gene	2.16	4.00E-07	2.40E-06	***mhqR***

t145_t084_04970_gene	2.15	3.90E-14	5.40E-13	*aldH1*
t145_t084_14870_gene	2.15	6.90E-14	9.40E-13	NA
t145_t084_20400_gene	2.12	2.10E-04	7.80E-04	*sglT*
t145_t084_01470_gene	2.12	1.70E-11	1.80E-10	*aaeA*
t145_t084_23150_gene	2.11	1.30E-06	7.40E-06	NA
t145_t084_00870_gene	2.09	9.60E-14	1.30E-12	NA
t145_t084_07210_gene	2.09	2.40E-03	7.10E-03	***acuC***
t145_t084_22420_gene	2.09	5.70E-06	2.90E-05	*spa*
t145_t084_00570_gene	2.08	1.30E-05	6.40E-05	***norB_1***
t145_t084_07450_gene	2.08	5.30E-03	1.40E-02	NA
t145_t084_10760_gene	2.08	3.30E-10	2.90E-09	*msrA1*
t145_t084_21100_gene	2.07	2.80E-22	7.60E-21	*tarJ'*
t145_t084_20090_gene	2.05	1.50E-05	6.80E-05	*tatC2*
t145_t084_03110_gene	2.03	3.30E-07	2.00E-06	NA
t145_t084_18130_gene	2.02	3.30E-13	4.20E-12	*scmP_3*
t145_t084_07230_gene	2.02	1.30E-04	5.00E-04	*acsA_1*
t145_t084_19170_gene	2.01	4.80E-05	2.00E-04	***sle1***
t145_t084_07860_gene	-2.02	1.00E-11	1.10E-10	***hemL1***
t145_t084_01230_gene	-2.03	5.80E-04	2.00E-03	*treP_1*
t145_t084_16730_gene	-2.03	6.20E-07	3.60E-06	NA
t145_t084_24940_gene	-2.03	1.60E-02	3.60E-02	NA
t145_t084_02610_gene	-2.04	1.20E-07	7.80E-07	*rplE*
t145_t084_15380_gene	-2.04	1.80E-02	4.00E-02	NA
t145_t084_11810_gene	-2.05	1.50E-21	3.60E-20	*uppS*
t145_t084_07970_gene	-2.05	5.10E-05	2.20E-04	*rplU*
t145_t084_18250_gene	-2.05	6.20E-11	6.00E-10	*rplK*
t145_t084_13450_gene	-2.06	1.30E-10	1.20E-09	*pdhD*
t145_t084_00970_gene	-2.07	1.00E-02	2.50E-02	NA
t145_t084_07800_gene	-2.07	5.50E-07	3.20E-06	***engB***
t145_t084_12030_gene	-2.07	4.30E-07	2.60E-06	*rpsP*
t145_t084_02620_gene	-2.07	1.10E-07	7.20E-07	*rpsZ*
t145_t084_01980_gene	-2.08	5.80E-07	3.30E-06	*odh*
t145_t084_02600_gene	-2.08	8.00E-09	6.00E-08	*rplX*
t145_t084_13400_gene	-2.09	9.10E-05	3.70E-04	*ydcV*
t145_t084_08300_gene	-2.1	8.40E-15	1.20E-13	***trmR***
t145_t084_02590_gene	-2.11	9.30E-08	6.10E-07	*rplN*
t145_t084_19240_gene	-2.11	1.30E-05	6.10E-05	NA
t145_t084_00820_gene	-2.11	3.90E-05	1.70E-04	*sbi*
t145_t084_18680_gene	-2.12	4.70E-25	1.70E-23	*hpt*
t145_t084_16110_gene	-2.12	3.30E-07	2.00E-06	NA
t145_t084_02520_gene	-2.13	5.40E-07	3.20E-06	*rplB*
t145_t084_18240_gene	-2.13	3.10E-08	2.20E-07	*rplA*
t145_t084_01030_gene	-2.15	3.30E-03	9.30E-03	***nasF***
t145_t084_10970_gene	-2.16	2.00E-06	1.10E-05	NA
t145_t084_12590_gene	-2.16	1.20E-21	3.00E-20	*murD*
t145_t084_08810_gene	-2.17	4.20E-10	3.60E-09	***trmK***
t145_t084_07820_gene	-2.18	5.90E-07	3.40E-06	NA
t145_t084_04680_gene	-2.22	6.70E-05	2.80E-04	***groS***
t145_t084_11800_gene	-2.22	2.80E-13	3.60E-12	NA
t145_t084_10320_gene	-2.22	4.20E-08	2.90E-07	*brnQ_1*
t145_t084_24360_gene	-2.22	5.50E-05	2.30E-04	NA
t145_t084_08560_gene	-2.23	8.90E-09	6.60E-08	*rpsT*
t145_t084_18180_gene	-2.23	4.50E-14	6.20E-13	*rplGB*
t145_t084_16370_gene	-2.23	2.40E-20	5.40E-19	*queC*
t145_t084_12890_gene	-2.24	5.10E-05	2.20E-04	NA
t145_t084_02340_gene	-2.26	1.00E-02	2.50E-02	NA
t145_t084_22990_gene	-2.27	8.80E-03	2.20E-02	NA
t145_t084_13980_gene	-2.27	1.20E-10	1.10E-09	***pchA***
t145_t084_02330_gene	-2.27	3.00E-06	1.60E-05	*cntE_2*
t145_t084_12000_gene	-2.28	4.20E-09	3.30E-08	*rplS*
t145_t084_02580_gene	-2.3	1.30E-07	8.20E-07	*rpsQ*
t145_t084_15120_gene	-2.32	1.00E-04	4.00E-04	NA

t145_t084_07840_gene	-2.32	1.90E-08	1.40E-07	NA
t145_t084_03740_gene	-2.32	2.40E-08	1.70E-07	*rpmE2*
t145_t084_01550_gene	-2.34	2.80E-20	6.20E-19	*gltS*
t145_t084_02570_gene	-2.34	1.80E-09	1.40E-08	*rpmC*
t145_t084_07140_gene	-2.34	1.40E-10	1.30E-09	NA
t145_t084_22610_gene	-2.36	6.00E-09	4.60E-08	*dus*
t145_t084_19890_gene	-2.37	6.30E-08	4.20E-07	*ssbA_2*
t145_t084_17690_gene	-2.38	4.10E-08	2.80E-07	NA
t145_t084_02500_gene	-2.39	3.20E-08	2.20E-07	*rplD*
t145_t084_17720_gene	-2.4	1.10E-10	1.10E-09	***galK***
t145_t084_07830_gene	-2.4	4.30E-17	7.90E-16	***hemC***
t145_t084_08320_gene	-2.42	1.00E-23	3.40E-22	NA
t145_t084_11830_gene	-2.45	1.30E-13	1.70E-12	*pyrH*
t145_t084_12460_gene	-2.45	1.10E-06	6.20E-06	*lspA*
t145_t084_03670_gene	-2.46	9.50E-13	1.10E-11	*pyrG*
t145_t084_02510_gene	-2.47	5.20E-13	6.40E-12	*rplW*
t145_t084_06030_gene	-2.47	9.00E-23	2.60E-21	NA
t145_t084_11820_gene	-2.48	9.70E-30	5.10E-28	***frr***
t145_t084_19900_gene	-2.48	6.70E-10	5.70E-09	*rpsF*
t145_t084_18220_gene	-2.48	2.20E-09	1.70E-08	*rplL*
t145_t084_07410_gene	-2.49	1.80E-06	9.80E-06	*yfcA*
t145_t084_02810_gene	-2.5	3.40E-12	3.80E-11	*rplM*
t145_t084_07150_gene	-2.5	6.00E-24	2.00E-22	NA
t145_t084_24350_gene	-2.5	6.10E-12	6.70E-11	*isaA*
t145_t084_13050_gene	-2.51	3.70E-09	2.90E-08	*pheS*
t145_t084_07130_gene	-2.52	6.60E-16	1.10E-14	NA
t145_t084_02530_gene	-2.52	4.10E-11	4.00E-10	*rpsS*
t145_t084_13040_gene	-2.53	6.80E-15	1.00E-13	*pheT*
t145_t084_02550_gene	-2.53	2.60E-11	2.60E-10	*rpsC*
t145_t084_04790_gene	-2.53	3.90E-04	1.40E-03	*hlb_2*
t145_t084_21740_gene	-2.54	4.20E-04	1.40E-03	***yagU***
t145_t084_01020_gene	-2.56	1.90E-02	4.10E-02	*nasE*
t145_t084_18200_gene	-2.57	6.50E-16	1.10E-14	*rpoB*
t145_t084_02560_gene	-2.58	1.70E-10	1.60E-09	*rplP*
t145_t084_02540_gene	-2.59	9.10E-13	1.10E-11	*rplV*
t145_t084_17710_gene	-2.59	8.60E-21	2.00E-19	***thrB_2***
t145_t084_21220_gene	-2.61	1.10E-03	3.40E-03	***hmp***
t145_t084_13160_gene	-2.61	5.40E-14	7.40E-13	NA
t145_t084_13390_gene	-2.61	6.80E-09	5.10E-08	*potD*
t145_t084_11750_gene	-2.61	8.70E-30	4.80E-28	*nusA*
t145_t084_12600_gene	-2.64	7.40E-22	2.00E-20	*mraY*
t145_t084_15300_gene	-2.64	1.70E-10	1.60E-09	NA
t145_t084_18770_gene	-2.65	2.00E-13	2.60E-12	*rplY*
t145_t084_08140_gene	-2.66	6.40E-13	7.80E-12	*aspS*
t145_t084_18190_gene	-2.67	2.50E-12	2.90E-11	*rpoC*
t145_t084_13320_gene	-2.67	2.40E-20	5.40E-19	*typA*
t145_t084_09960_gene	-2.7	2.70E-09	2.20E-08	NA
t145_t084_21230_gene	-2.7	1.60E-02	3.70E-02	NA
t145_t084_11760_gene	-2.7	2.20E-26	9.10E-25	*rimP*
t145_t084_18230_gene	-2.71	1.40E-11	1.40E-10	*rplJ*
t145_t084_21000_gene	-2.71	5.00E-07	2.90E-06	*lrgA*
t145_t084_11610_gene	-2.74	3.60E-51	6.10E-49	*phaB*
t145_t084_13960_gene	-2.74	5.40E-18	1.10E-16	*menH*
t145_t084_09950_gene	-2.75	8.00E-24	2.60E-22	*rlmL*
t145_t084_13550_gene	-2.75	4.60E-07	2.70E-06	*ythA*
t145_t084_03750_gene	-2.77	3.30E-19	6.80E-18	*tdk*
t145_t084_13540_gene	-2.77	1.70E-08	1.20E-07	*ythB*
t145_t084_08130_gene	-2.79	5.30E-10	4.50E-09	*hisS*
t145_t084_11720_gene	-2.8	9.80E-44	1.20E-41	*infB*
t145_t084_03770_gene	-2.8	8.20E-16	1.30E-14	*prmC*
t145_t084_09970_gene	-2.8	1.10E-12	1.30E-11	NA
t145_t084_02490_gene	-2.81	1.10E-11	1.20E-10	*rplC*

t145_t084_03760_gene	-2.82	1.40E-25	5.30E-24	*prfA*
t145_t084_24810_gene	-2.86	1.70E-04	6.60E-04	***ldhD_2***
t145_t084_11630_gene	-2.86	5.00E-29	2.30E-27	NA
t145_t084_09720_gene	-2.87	2.10E-03	6.20E-03	NA
t145_t084_02000_gene	-2.87	1.70E-11	1.80E-10	*ssaA2*
t145_t084_11740_gene	-2.88	5.70E-23	1.70E-21	NA
t145_t084_12360_gene	-2.93	2.60E-16	4.50E-15	NA
t145_t084_07360_gene	-2.94	2.50E-22	6.90E-21	*rpsD*
t145_t084_07750_gene	-2.95	3.40E-09	2.70E-08	*rplT*
t145_t084_13970_gene	-2.96	6.60E-28	2.90E-26	***menD***
t145_t084_11620_gene	-3	1.30E-37	1.10E-35	*NA*
t145_t084_00030_gene	-3.01	2.50E-11	2.50E-10	*fnbB*
t145_t084_10870_gene	-3.04	2.60E-14	3.60E-13	*opuD_2*
t145_t084_17700_gene	-3.05	1.70E-30	1.10E-28	NA
t145_t084_11730_gene	-3.08	4.30E-25	1.50E-23	*rplGA*
t145_t084_07740_gene	-3.1	2.50E-07	1.50E-06	*rpmI*
t145_t084_11110_gene	-3.1	4.10E-08	2.80E-07	*nucH*
t145_t084_02480_gene	-3.1	5.20E-12	5.70E-11	*rpsJ*
t145_t084_21330_gene	-3.11	9.20E-16	1.50E-14	***scn_3***
t145_t084_10980_gene	-3.14	7.50E-15	1.10E-13	*guaC*
t145_t084_07730_gene	-3.16	2.00E-15	3.00E-14	*infC*
t145_t084_03160_gene	-3.21	1.40E-47	2.10E-45	NA
t145_t084_11850_gene	-3.28	7.10E-30	4.00E-28	*rpsB*
t145_t084_22750_gene	-3.37	1.80E-22	5.00E-21	*purA*
t145_t084_24160_gene	-3.45	7.90E-09	5.90E-08	***pyrD***
t145_t084_04120_gene	-3.45	1.10E-17	2.10E-16	*cshA*
t145_t084_00020_gene	-3.72	3.50E-10	3.10E-09	*fnbA*
t145_t084_21660_gene	-3.77	2.10E-46	2.70E-44	*ptsG_3*
t145_t084_15600_gene	-3.93	6.70E-07	3.80E-06	NA
t145_t084_12370_gene	-4.02	9.80E-34	7.30E-32	*pyrE*
t145_t084_12380_gene	-4.03	6.00E-20	1.30E-18	*pyrF*
t145_t084_12440_gene	-4.07	1.40E-27	6.00E-26	*pyrR*
t145_t084_21350_gene	-4.08	3.70E-08	2.60E-07	***pflA***
t145_t084_12020_gene	-4.15	3.80E-25	1.40E-23	*rimM*
t145_t084_12010_gene	-4.21	8.70E-19	1.70E-17	*trmD*
t145_t084_12390_gene	-4.28	3.90E-29	1.90E-27	*carB*
t145_t084_01140_gene	-4.4	7.30E-07	4.20E-06	***narT***
t145_t084_21410_gene	-4.67	1.70E-23	5.40E-22	NA
t145_t084_12420_gene	-4.72	2.30E-20	5.40E-19	*pyrB*
t145_t084_10010_gene	-4.82	1.70E-08	1.20E-07	***tdcB***
t145_t084_12400_gene	-4.94	5.70E-34	4.40E-32	*carA_1*
t145_t084_12410_gene	-5.11	6.10E-26	2.40E-24	*pyrC*
t145_t084_12430_gene	-5.23	1.00E-30	6.40E-29	*pyrP*
t145_t084_21320_gene	-5.25	2.30E-23	7.00E-22	NA
t145_t084_19450_gene	-5.25	8.00E-21	1.90E-19	NA
t145_t084_19460_gene	-5.29	1.30E-12	1.50E-11	*ssl5_1*

NOTE:

NA gene not annotated

genes in bold uniquely expressed genes at 3h of exposure

Table S5. Total genes in S. aureus after 1h and 3h of exposure with tonsillar cells identified from DESeq analysis. One thousand five hundred and forty-four genes (1544) are listed below sorted according to the Log2FoldChange (LFC) value. Deseq2 analysis result file at 1h represents the comparison between S. aureus alone (C1) versus S. aureus infected to the host cell (T1). Similarly, the deseq2 analysis result file at 3h represents the comparison between S. aureus alone (C3) and S. aureus infected to the host cell (T3).

DEseq2 analysis result file at 1h (C1 vs T1)			DEseq2 analysis result file at 3h (C3 vs T3)		
log2FoldChange	**padj**	**gene_name**	**log2FoldChange**	**padj**	**gene_name**
9.33	2.88E-18	*leuB*	7.25	4.02E-69	*metP_2*
8.66	1.90E-24	*leuC*	6.99	8.15E-102	*metN*
8.43	8.90E-28	*leuA_1*	6.84	1.27E-44	*salL*
8.42	5.58E-17	*ilvH*	6.74	3.65E-37	*lysC*
8.18	1.48E-33	*ilvC*	6.53	2.94E-05	*icaD*
7.45	4.04E-36	*salL*	6.52	9.50E-97	*metQ_2*
7.45	7.29E-37	*metP_2*	6.44	1.30E-33	*asd*
7.31	3.99E-08	*thrC*	6.09	1.60E-27	*dapA*
7.24	3.22E-29	*nadX*	6.02	6.68E-28	*carA_2*
7.20	1.00E-12	*czrA*	6.00	2.22E-85	*ykoD_2*
6.89	8.17E-27	*dapA*	5.96	1.10E-13	*leuB*
6.85	3.43E-20	*thrB_1*	5.88	7.56E-31	*dapB*
6.69	9.74E-25	*dapH_1*	5.87	1.12E-34	*ilvH*
6.59	4.56E-74	*ykoD_2*	5.85	1.43E-28	*serA*
6.54	6.06E-27	*asd*	5.82	7.51E-20	*yclM*
6.50	2.30E-28	*dapB*	5.81	1.58E-24	*dapH_1*
6.29	8.23E-116	*ilvB*	5.78	1.05E-27	*ilvC*
6.27	1.60E-13	*leuD*	5.71	2.27E-20	*leuA_1*
6.24	6.57E-52	*metN*	5.34	8.55E-21	*nadX*
6.09	1.86E-21	*scmP_2*	5.20	1.94E-103	*panS*
6.08	2.15E-20	*carA_2*	5.19	3.46E-16	*sbnB*
5.95	6.67E-16	*yclM*	5.15	1.81E-05	*thrC*
5.84	2.02E-55	*metQ_2*	5.14	1.07E-15	*leuC*
5.84	4.64E-22	*lysC*	5.07	1.47E-19	*scmP_2*
5.81	1.33E-17	*lcfB*	5.04	1.10E-13	*nrtD*
5.80	2.44E-64	*ilvD*	4.99	6.41E-23	*lcfB*
5.57	1.24E-20	*serA*	4.99	6.09E-59	*ykoC*
5.55	1.64E-20	*oppF_2*	4.93	1.82E-17	*cmpC*
5.49	9.64E-14	*metC*	4.91	2.26E-21	*alr1_2*
5.42	4.00E-20	*alr1_2*	4.90	2.34E-42	*yhdG*
5.41	1.15E-48	*ykoC*	4.84	4.96E-50	*acp*
5.20	3.01E-71	*panS*	4.80	1.76E-07	*oppB_1*
5.19	7.98E-14	*ilvA*	4.78	2.86E-12	*ssuC*
5.11	1.81E-31	*dppE_2*	4.73	1.11E-37	*metQ_1*
5.06	3.65E-03	*icaD*	4.65	9.68E-110	*ilvB*
4.80	7.98E-14	*yitJ*	4.61	7.57E-12	*leuD*
4.72	3.10E-10	*czcD_1*	4.54	6.40E-12	*thrB_1*
4.64	1.27E-28	*lysA*	4.36	2.79E-55	*ilvD*
4.64	7.42E-19	*hutH*	4.27	5.12E-28	*ggt*
4.53	5.62E-15	*oppD_2*	4.22	1.82E-12	*ilvA*
4.53	3.26E-06	*efeM*	4.20	6.56E-31	*sbnC*
4.36	1.25E-02	*fruA_2*	4.17	1.93E-18	*metP_1*
4.29	5.14E-08	*ydaF*	4.08	1.46E-25	*dppE_2*
4.28	3.89E-10	*nrgA*	4.05	6.31E-28	*lysA*
4.28	8.47E-26	*yhdG*	4.03	2.01E-20	*isdC*
4.24	1.58E-33	*oppB_2*	4.02	5.12E-18	*isdH*
4.16	4.64E-29	*acp*	3.90	3.12E-18	*hutH*

4.12	4.78E-07	*argO*	3.87	4.55E-13	*oppF_2*
4.12	6.14E-07	*ldhD_1*	3.78	4.84E-16	*metN2*
4.10	1.83E-22	*metQ_1*	3.77	5.35E-08	*hisG*
4.00	1.43E-59	*oppC_2*	3.76	4.80E-12	*lipA_2*
4.00	1.41E-27	*ydbM*	3.70	5.12E-36	*katA*
4.00	1.55E-08	*metI*	3.68	1.38E-22	*sbnA*
3.93	1.45E-10	*lipA_2*	3.63	3.07E-13	*nrgA*
3.89	1.38E-02	*caiA*	3.62	4.15E-10	*isdE*
3.80	1.43E-11	*metE*	3.61	1.82E-63	*oppC_2*
3.80	2.90E-23	*sdrC*	3.60	2.40E-26	*bsaA_2*
3.77	8.98E-06	*nrtD*	3.59	7.09E-31	*oppB_2*
3.73	1.47E-44	*hisC_2*	3.47	1.81E-38	*yghA*
3.65	3.63E-07	*butA*	3.43	9.24E-14	*scmP_1*
3.65	6.85E-28	*katA*	3.42	4.51E-09	*argO*
3.59	1.05E-18	*aroA_1*	3.42	3.95E-11	*oppD_2*
3.57	6.58E-11	*metP_1*	3.42	2.37E-21	*aroA_1*
3.56	1.40E-25	*gltA*	3.33	3.04E-08	*isdF*
3.55	8.31E-06	*adhR*	3.23	1.03E-05	*isdG_1*
3.51	1.41E-27	*yghA*	3.17	2.00E-16	*metXA*
3.44	3.54E-02	*acuA*	3.17	1.41E-26	*gltA*
3.42	3.95E-08	*glnR*	3.15	1.57E-41	*hisC_2*
3.42	2.04E-04	*oppD_1*	3.14	1.05E-06	*uhpT*
3.34	7.77E-13	*abgT*	3.07	3.05E-21	*bacF*
3.27	1.32E-02	*ulaC*	3.03	3.05E-13	*hpf*
3.26	2.48E-09	*spa*	3.03	1.13E-13	*abgT*
3.26	1.09E-08	*ribBA*	3.02	3.31E-02	*acuA*
3.20	1.51E-12	*bcp*	3.02	2.96E-13	*hisZ*
3.19	1.21E-15	*abgB*	2.98	7.44E-06	*ydaF*
3.18	4.93E-13	*metXA*	2.96	5.85E-04	*efeM*
3.13	4.40E-09	*metN2*	2.96	3.27E-11	*gsiA*
3.10	1.15E-11	*ggt*	2.95	9.85E-04	*icaA*
3.10	9.87E-07	*cocE*	2.95	4.37E-07	*metI*
3.02	1.83E-03	*oppC_1*	2.93	4.28E-17	*abgB*
3.02	1.55E-18	*ybbH_2*	2.92	1.87E-09	*hisD*
2.99	5.66E-06	*hdfR_2*	2.88	9.73E-05	*ldhD_1*
2.96	4.98E-05	*rpmG2_2*	2.87	8.80E-21	*ydbM*
2.95	2.35E-06	*ribE*	2.87	9.57E-11	*ydfJ*
2.92	3.76E-12	*nfrA*	2.83	1.03E-15	*dtpT*
2.92	6.09E-10	*hpf*	2.82	6.18E-07	*srtB*
2.88	1.36E-12	*efeN*	2.80	1.27E-02	*ulaC*
2.87	3.89E-10	*yvgN_1*	2.79	7.57E-06	*sbnE*
2.86	1.22E-03	*ssuC*	2.78	4.84E-16	*sdrC*
2.84	9.52E-03	*hisB*	2.70	5.47E-03	*sbnD*
2.82	1.67E-02	*oppF_1*	2.70	2.87E-02	*caiA*
2.80	3.26E-07	*ribD*	2.69	2.77E-19	*gph_1*
2.79	1.12E-06	*hisD*	2.61	3.60E-06	*metC*
2.78	8.45E-04	*lpl2_4*	2.53	2.13E-05	*tcyP*
2.78	1.99E-06	*dapE*	2.48	4.40E-04	*ulaA*
2.77	1.96E-09	*hchA*	2.48	1.40E-04	*butA*
2.77	1.29E-23	*lipR*	2.47	2.57E-28	*acsA_2*
2.76	8.32E-13	*traP*	2.45	9.30E-04	*putB*
2.75	9.39E-02	*icaB_1*	2.45	3.05E-05	*yeeD*
2.73	1.39E-03	*hisG*	2.44	6.17E-08	*isdI*
2.72	9.88E-05	*wecD*	2.44	1.03E-03	*oppD_1*
2.72	1.33E-11	*bsaA_2*	2.43	3.76E-04	*adhR*
2.72	9.75E-14	*ppaX*	2.41	5.67E-07	*glnA*
2.71	4.51E-02	*pstS*	2.40	1.09E-06	*rocA*
2.70	7.30E-24	*ilvE*	2.40	3.13E-08	*irtA*
2.69	2.08E-11	*prsA*	2.40	1.20E-11	*trpB*
2.65	3.63E-15	*aldH1*	2.39	2.45E-10	*gltB*
2.64	2.45E-03	*hrtA_1*	2.38	2.10E-16	*dps*
2.63	1.17E-06	*glnA*	2.38	6.16E-12	*efeN*
2.62	3.16E-12	*bacF*	2.35	2.34E-03	*oppC_1*
2.62	5.76E-06	*yecD*	2.34	3.41E-05	*glnR*
2.61	1.98E-06	*yjbK*	2.33	3.76E-26	*tarI2*
2.61	2.80E-03	*aldA*	2.32	2.85E-04	*isdA*

2.60	5.30E-07	*ydfJ*	2.31	7.59E-04	*lpl2_4*
2.53	5.54E-12	*yjbI*	2.31	9.49E-15	*ybbH_2*
2.53	3.84E-14	*tatAy*	2.31	2.72E-07	*hisC_3*
2.51	4.21E-23	*acsA_2*	2.30	3.63E-10	*trpF*
2.50	1.71E-13	*opuBA*	2.28	1.82E-08	*glpQ_1*
2.50	7.85E-12	*ghrB_1*	2.28	3.43E-11	*traP*
2.49	8.10E-09	*gltB*	2.28	8.68E-04	*argH*
2.49	3.77E-02	*lpl2_2*	2.26	5.12E-04	*argG*
2.45	1.88E-03	*yeeD*	2.25	2.00E-10	*cpdA_1*
2.42	2.38E-03	*fdhD*	2.24	1.48E-14	*tatAy*
2.41	4.25E-07	*tcyC_1*	2.23	7.78E-10	*lysP_2*
2.38	1.04E-02	*hisH*	2.21	9.67E-10	*dapX*
2.36	1.81E-06	*hisZ*	2.21	2.63E-08	*mdtD*
2.35	5.12E-21	*tarJ'*	2.17	6.83E-20	*ilvE*
2.34	2.42E-16	*mecA*	2.17	1.61E-04	*yitJ*
2.33	1.35E-05	*fgd_1*	2.16	9.67E-19	*lipR*
2.32	2.92E-07	*ipdC*	2.16	2.38E-06	*mhqR*
2.31	1.64E-05	*paiA*	2.15	5.38E-13	*aldH1*
2.29	3.33E-09	*yjbM*	2.12	7.84E-04	*sglT*
2.29	1.54E-01	*gatB_2*	2.12	1.78E-10	*aaeA*
2.28	2.52E-02	*hisA*	2.09	7.10E-03	*acuC*
2.28	2.78E-08	*lysP_2*	2.09	2.86E-05	*spa*
2.28	9.14E-05	*tatC2*	2.08	6.42E-05	*norB_1*
2.27	1.35E-02	*trpC*	2.08	2.87E-09	*msrA1*
2.27	4.45E-03	*sdrD*	2.07	7.61E-21	*tarJ'*
2.27	2.24E-08	*msrA1*	2.05	6.85E-05	*tatC2*
2.27	3.00E-11	*copA*	2.02	4.17E-12	*scmP_3*
2.25	1.70E-06	*bsaA_1*	2.02	4.99E-04	*acsA_1*
2.25	1.82E-12	*tal*	2.01	2.05E-04	*sle1*
2.25	2.28E-07	*gabR*	1.98	5.53E-03	*hutU*
2.23	1.07E-01	*icaB_2*	1.98	1.16E-04	*metE*
2.20	2.69E-03	*cmpC*	1.98	1.69E-07	*ybaN*
2.20	5.40E-08	*dtpT*	1.98	4.24E-10	*proC_2*
2.19	3.53E-02	*gatC_2*	1.97	5.71E-11	*copA*
2.19	1.93E-08	*zwf*	1.96	9.89E-07	*bcp*
2.18	5.38E-05	*scmP_1*	1.95	2.33E-07	*nfrA*
2.18	5.53E-03	*mcsA*	1.94	3.25E-06	*hchA*
2.17	1.38E-04	*yfbR*	1.93	1.16E-11	*tal*
2.17	6.45E-05	*hisC_3*	1.91	1.68E-09	*pcp*
2.16	7.18E-06	*ydbP*	1.91	9.16E-11	*fbiB*
2.16	4.02E-11	*yafV*	1.90	2.73E-02	*hisB*
2.15	1.53E-08	*aaeA*	1.90	1.50E-02	*trpC*
2.15	1.37E-22	*msrA2*	1.88	4.76E-09	*yjbI*
2.14	2.42E-01	*pstA*	1.87	9.01E-03	*tcyB*
2.14	6.33E-05	*rpsA*	1.86	1.42E-05	*tcyC_1*
2.13	2.01E-05	*ydaP*	1.86	9.73E-09	*ghrB_1*
2.13	4.54E-01	*ywqD_1*	1.85	8.46E-09	*ppaX*
2.12	3.27E-06	*gpmA_1*	1.84	1.51E-13	*perR*
2.12	2.83E-10	*scmP_3*	1.83	2.42E-04	*purF*
2.11	3.82E-17	*tarI2*	1.83	3.18E-08	*ylmA*
2.11	8.68E-04	*qorB*	1.82	1.54E-01	*icaB_1*
2.11	9.18E-02	*xerD_3*	1.80	1.23E-02	*sdrD*
2.11	7.66E-06	*citZ*	1.79	5.79E-07	*czcD_2*
2.11	1.24E-09	*gph_1*	1.78	1.33E-03	*cocE*
2.11	1.97E-04	*rocA*	1.78	1.56E-04	*paiA*
2.11	6.99E-03	*mhqD*	1.77	1.53E-04	*bcrA_2*
2.10	4.59E-02	*lpl2_3*	1.76	4.31E-04	*dapE*
2.06	4.96E-03	*sglT*	1.75	1.09E-08	*yxeI*
2.05	1.09E-02	*tcyB*	1.74	6.17E-03	*wecD*
2.05	1.97E-13	*tarK*	1.74	1.28E-16	*ahpC*
2.05	2.19E-05	*glpQ_1*	1.69	3.50E-11	*mecA*
2.04	3.27E-06	*glpD*	1.68	2.51E-03	*qorB*
2.03	2.27E-03	*acsA_1*	1.65	1.23E-02	*mccB*
2.03	7.65E-06	*mdtD*	1.64	5.40E-11	*tarK*
2.03	7.45E-03	*mntA*	1.63	1.99E-06	*yjbM*
2.02	6.10E-02	*pstB3*	1.63	3.89E-09	*kdpD*

2.02	3.33E-02	*argF*	1.62	2.20E-02	*czcD_1*
2.00	1.18E-08	*ebpS*	1.62	8.96E-06	*prsA*
2.00	4.23E-06	*trpB*	1.61	7.65E-07	*ahpF*
1.99	7.33E-02	*icaA*	1.61	9.92E-04	*dhaK*
1.98	1.63E-03	*ribH*	1.60	1.80E-05	*crr*
1.97	8.03E-02	*malL*	1.59	1.71E-04	*citZ*
1.97	3.47E-02	*msrAB*	1.59	4.47E-04	*rarD*
1.94	1.19E-06	*mshD_2*	1.59	1.76E-02	*iolG*
1.93	1.78E-01	*lacA*	1.59	2.57E-01	*fruA_2*
1.91	4.46E-04	*ssl7_3*	1.59	6.68E-03	*purN*
1.91	1.96E-02	*malG*	1.58	6.84E-06	*zwf*
1.90	1.30E-08	*kdpD*	1.58	4.36E-04	*dhaL*
1.88	2.44E-07	*msrB*	1.58	8.82E-05	*mccA*
1.86	1.24E-03	*rocD2_1*	1.58	3.45E-04	*mntH*
1.85	3.83E-02	*csbB*	1.57	9.96E-06	*yvyI*
1.84	6.51E-02	*wbgU*	1.57	2.02E-02	*nanA*
1.84	5.30E-07	*yccX*	1.57	4.84E-04	*sbnF*
1.84	1.11E-05	*crr*	1.56	4.51E-02	*hisH*
1.82	1.74E-04	*icd*	1.56	1.77E-04	*brnQ_3*
1.81	7.67E-06	*pepT_1*	1.56	7.90E-03	*gfo*
1.81	1.47E-03	*purM*	1.56	5.38E-02	*argF*
1.80	3.28E-02	*ulaA*	1.55	7.17E-05	*glpD*
1.80	1.58E-05	*dapX*	1.54	1.01E-10	*manP*
1.80	3.77E-04	*recX*	1.54	1.07E-03	*purM*
1.80	1.18E-04	*brnQ_3*	1.53	3.88E-04	*ogt*
1.79	6.33E-02	*ssl4_3*	1.53	3.41E-05	*atl_3*
1.79	1.29E-02	*ftnA*	1.53	5.82E-02	*aldA*
1.79	1.70E-04	*trpF*	1.52	1.11E-02	*hdfR_2*
1.78	8.39E-04	*bcrA_2*	1.51	1.81E-03	*rpsA*
1.78	9.94E-06	*rbsD*	1.51	1.45E-09	*ywpJ_1*
1.77	7.11E-03	*fbp*	1.51	2.09E-03	*trpD2*
1.77	2.38E-04	*yiiM*	1.51	4.69E-04	*icd*
1.77	2.59E-05	*cpdA_1*	1.51	2.14E-08	*fepC*
1.76	1.15E-02	*mhqA_3*	1.51	2.13E-03	*yjbK*
1.76	8.06E-02	*lacE*	1.51	1.58E-01	*xerD_3*
1.76	6.61E-03	*mntB_1*	1.50	9.86E-11	*ccpA*
1.75	2.32E-06	*pcp*	1.49	1.63E-01	*murQ*
1.74	1.41E-09	*pitA*	1.48	1.81E-04	*accC*
1.74	4.38E-07	*murI*	1.48	2.29E-04	*orr*
1.73	2.20E-02	*gpmA_2*	1.48	2.06E-07	*yafV*
1.71	1.31E-04	*vraR*	1.48	4.21E-14	*msrA2*
1.71	7.08E-06	*luxS*	1.46	2.09E-04	*purL*
1.71	1.72E-01	*phnD2*	1.45	1.15E-02	*hfq*
1.71	5.32E-06	*yicL*	1.45	6.62E-04	*bsaA_1*
1.70	2.47E-02	*ybaK*	1.45	7.58E-06	*msrB*
1.70	1.75E-01	*ssl7_1*	1.45	7.37E-05	*gabR*
1.70	1.94E-03	*gsiA*	1.45	1.67E-02	*yrrK*
1.69	1.16E-01	*icaC*	1.45	2.53E-06	*opuBA*
1.68	2.28E-03	*sarX*	1.45	1.64E-05	*pxpA*
1.68	5.56E-03	*norB_1*	1.44	1.11E-01	*czrA*
1.67	1.60E-03	*nagB_2*	1.44	5.42E-03	*yfbR*
1.67	2.91E-02	*mntB_2*	1.44	1.26E-03	*yveA*
1.67	1.98E-04	*acnA*	1.42	1.35E-02	*bglK*
1.67	7.51E-03	*cobB*	1.40	7.04E-09	*pitA*
1.66	6.04E-09	*ywpJ_1*	1.40	6.55E-05	*agrB*
1.66	1.01E-04	*kdpE*	1.38	1.34E-01	*hisA*
1.65	1.99E-05	*pnoA*	1.38	5.95E-05	*kdpE*
1.64	1.68E-01	*lukDv_1*	1.37	1.49E-04	*farB_1*
1.62	5.33E-10	*ccpA*	1.37	6.55E-03	*agrA*
1.62	1.78E-02	*gfo*	1.37	2.09E-01	*icaB_2*
1.62	4.72E-02	*msmX*	1.36	1.44E-07	*tagU_2*
1.60	5.31E-02	*entS*	1.35	3.38E-05	*nhaK_1*
1.58	4.39E-03	*yxdL*	1.35	4.49E-15	*fda*
1.57	8.46E-02	*RBKS*	1.34	1.36E-04	*rbsK*
1.56	1.57E-02	*copZ*	1.34	1.57E-03	*yiiM*
1.56	2.02E-03	*mccA*	1.33	1.55E-03	*yvgN_1*

1.53	1.84E-05	*mgt*	1.32	1.16E-04	*panE*
1.52	8.35E-02	*hutU*	1.32	2.20E-02	*tcyC_2*
1.52	9.16E-02	*ctsR*	1.31	1.69E-01	*hutI*
1.50	1.82E-06	*nrdE*	1.31	9.96E-06	*fur*
1.49	4.46E-05	*polA*	1.31	1.21E-04	*luxS*
1.48	9.88E-03	*dhaK*	1.30	6.31E-03	*msrC*
1.48	2.63E-02	*yehR*	1.29	2.24E-03	*trpA*
1.48	1.74E-01	*argR_2*	1.28	3.26E-04	*rbsD*
1.46	2.85E-03	*sbnA*	1.28	5.39E-02	*ltaE*
1.45	1.60E-01	*lukDv_2*	1.27	1.22E-02	*sarZ*
1.45	2.28E-02	*sle1*	1.26	6.92E-03	*nhoA*
1.45	4.36E-04	*farB_1*	1.26	9.52E-07	*aroC*
1.44	2.95E-03	*hutG*	1.26	1.67E-14	*nrdR*
1.44	7.20E-02	*iolG*	1.25	1.05E-01	*fhuD_1*
1.44	5.97E-01	*esaB*	1.25	2.10E-02	*nfuA*
1.44	3.83E-03	*ogt*	1.25	1.52E-04	*odhB*
1.43	2.45E-01	*ssl3*	1.25	1.81E-01	*lnrL_2*
1.43	6.70E-03	*rarD*	1.24	4.77E-04	*miaA*
1.43	4.30E-05	*lcdH*	1.24	1.17E-04	*ebpS*
1.43	6.74E-07	*perR*	1.23	2.44E-02	*purE*
1.43	1.79E-01	*ampR*	1.23	7.83E-02	*mntB_2*
1.42	3.27E-03	*trxA*	1.23	1.12E-03	*yjlD*
1.42	1.53E-05	*dnaN*	1.22	1.35E-01	*hrtA_1*
1.41	7.92E-05	*oxyR*	1.21	2.27E-04	*yccX*
1.40	1.41E-02	*sarZ*	1.21	1.57E-04	*mgt*
1.40	1.84E-05	*ypdF*	1.21	1.72E-01	*lpl2_3*
1.40	2.47E-10	*pepF1_2*	1.20	6.08E-05	*purD*
1.40	7.73E-02	*nanA*	1.20	9.27E-04	*pepT_1*
1.39	1.77E-04	*yxeI*	1.19	2.07E-04	*sodA*
1.38	1.14E-02	*nhoA*	1.19	3.77E-03	*yclN*
1.38	1.73E-01	*purQ*	1.19	2.01E-01	*gatC_2*
1.37	1.38E-02	*nrdI*	1.18	2.91E-03	*nadK*
1.37	1.10E-05	*glcT*	1.18	2.82E-01	*oppF_1*
1.37	2.53E-04	*nhaK_1*	1.18	1.07E-03	*mshD_2*
1.35	9.44E-03	*purH*	1.17	7.49E-02	*gatD_2*
1.34	1.56E-01	*ureA*	1.17	4.92E-04	*acr1*
1.34	2.15E-01	*gnu*	1.17	1.60E-02	*yxdL*
1.34	2.58E-01	*oppB_1*	1.16	1.81E-04	*murI*
1.32	2.38E-01	*ybbH_3*	1.16	5.91E-05	*dnaA*
1.32	1.86E-02	*mhqR*	1.16	1.50E-02	*mgsR*
1.32	5.58E-06	*yfmJ*	1.15	2.31E-01	*lukDv_1*
1.31	9.37E-03	*proC_1*	1.14	1.07E-01	*rpmG2_2*
1.31	1.19E-03	*rbsK*	1.14	6.73E-03	*pxpC_1*
1.30	1.18E-02	*vraS*	1.13	1.09E-02	*proC_1*
1.30	8.97E-02	*hdfR_1*	1.13	2.55E-06	*ddl*
1.30	1.21E-02	*ytmI*	1.13	1.17E-02	*ydbP*
1.29	2.45E-01	*hutI*	1.12	1.35E-03	*arcC1*
1.29	6.67E-04	*yvgN_2*	1.12	2.68E-03	*yajO*
1.28	8.54E-03	*tadA_1*	1.12	2.92E-02	*trpE*
1.27	4.11E-02	*nfuA*	1.11	1.08E-01	*yclO*
1.26	1.53E-01	*phoB*	1.11	2.72E-04	*lcdH*
1.26	6.23E-02	*cycB*	1.11	5.19E-05	*glcT*
1.26	9.63E-04	*mhqA_2*	1.11	2.11E-03	*tag*
1.26	4.55E-03	*pxpA*	1.11	6.31E-02	*rclA*
1.25	6.03E-03	*hit*	1.11	6.43E-02	*mntB_1*
1.25	1.86E-01	*glcU_2*	1.10	3.70E-02	*rocD2_1*
1.25	3.24E-04	*dps*	1.10	5.54E-02	*aur*
1.24	2.82E-05	*yugI*	1.10	8.74E-02	*feuB*
1.24	8.24E-06	*pycA*	1.10	1.19E-04	*bacC*
1.24	2.69E-02	*nudF*	1.09	1.95E-01	*cntL*
1.24	3.63E-06	*nrdF*	1.09	1.42E-06	*bmrA*
1.23	9.41E-04	*ytpA*	1.09	9.04E-04	*yvgN_2*
1.23	2.63E-02	*irtA*	1.08	1.98E-01	*lukDv_2*
1.22	1.71E-04	*dnaA*	1.08	1.71E-02	*purH*
1.22	3.07E-01	*ssl5_2*	1.07	3.63E-02	*trpG_1*
1.21	1.12E-01	*nixA*	1.06	1.03E-03	*aroB*

1.21	3.40E-01	*glnQ*	1.06	2.77E-01	*ybbH_3*
1.21	2.95E-05	*yfkN_1*	1.06	2.88E-03	*dat*
1.20	6.77E-03	*czcD_2*	1.06	5.27E-02	*pxpB_1*
1.20	1.46E-01	*essG_1*	1.05	1.45E-01	*mntA*
1.20	4.00E-03	*tag*	1.05	1.23E-03	*dtd*
1.20	3.81E-03	*miaA*	1.05	6.99E-03	*cycA_2*
1.20	5.66E-05	*tagU_2*	1.04	1.71E-02	*trxA*
1.19	8.79E-03	*nadK*	1.03	2.14E-03	*cysK*
1.19	2.25E-01	*bceA_2*	1.03	2.26E-01	*glcU_2*
1.18	1.80E-03	*cysK*	1.01	4.81E-07	*spxA*
1.16	7.06E-03	*atl_3*	1.01	1.51E-03	*mhqA_2*
1.16	3.54E-02	*dhaL*	1.01	2.34E-03	*folD*
1.16	4.79E-01	*dapH_3*	1.00	3.40E-01	*malL*
1.16	3.50E-03	*panE*	1.00	1.54E-02	*acnA*
1.16	2.08E-01	*putB*	1.00	7.67E-03	*hly*
1.15	3.37E-02	*mgsR*	1.00	9.93E-04	*ptpA*
1.15	7.18E-03	*yajO*	1.00	4.73E-02	*nrdI*
1.15	2.63E-03	*odhB*	1.00	1.59E-02	*gluD*
1.14	1.95E-01	*mcsB*	1.00	3.26E-02	*ydaP*
1.13	1.77E-02	*purL*	1.00	6.94E-05	*pycA*
1.13	3.87E-02	*yveA*	0.99	3.98E-07	*cggR*
1.13	2.62E-01	*essG_6*	0.99	2.24E-03	*sauU*
1.12	1.87E-04	*aroC*	0.99	3.03E-03	*polA*
1.12	3.86E-01	*murQ*	0.99	2.85E-01	*dhaM*
1.12	3.15E-01	*crtQ*	0.98	1.81E-01	*accB_1*
1.12	4.22E-02	*catE_2*	0.98	1.62E-02	*hit*
1.12	2.08E-07	*rutB*	0.97	4.90E-01	*gatB_2*
1.12	3.84E-01	*lukEv*	0.96	3.61E-03	*speA*
1.11	2.20E-02	*gcvH*	0.96	5.32E-04	*panB*
1.11	1.07E-01	*rclA*	0.96	1.02E-01	*garL*
1.11	4.83E-02	*msrC*	0.95	2.45E-01	*asp2*
1.10	1.56E-03	*ptpA*	0.95	2.79E-04	*recG*
1.10	3.21E-01	*purS*	0.95	6.92E-07	*rutB*
1.10	8.16E-03	*ebh_1*	0.95	4.19E-02	*vraS*
1.10	1.34E-02	*arcC1*	0.94	2.26E-01	*fdhD*
1.09	1.18E-01	*bglK*	0.94	4.53E-02	*recX*
1.09	3.19E-01	*comC*	0.94	2.88E-01	*purQ*
1.09	2.10E-02	*gluD*	0.93	1.18E-03	*nrdE*
1.08	1.47E-01	*tcyP*	0.93	1.96E-01	*malG*
1.08	1.22E-01	*hfq*	0.93	3.45E-02	*hssR*
1.08	7.77E-03	*cysJ*	0.93	3.25E-02	*tadA_1*
1.08	4.54E-01	*essG_7*	0.92	1.99E-02	*sspB*
1.07	5.29E-04	*flp*	0.92	2.78E-01	*csbB*
1.07	8.09E-02	*trpG_1*	0.92	6.27E-02	*feoB*
1.06	8.37E-03	*cynR*	0.92	2.32E-03	*dnaN*
1.06	8.54E-03	*ylmA*	0.91	1.08E-01	*cobB*
1.06	7.01E-02	*yfhP*	0.91	7.28E-03	*yicL*
1.05	8.08E-03	*hssS*	0.91	3.47E-02	*ppaC*
1.05	1.46E-01	*yrrK*	0.91	1.92E-01	*ytrA*
1.04	9.40E-02	*mta*	0.91	3.58E-02	*fadN*
1.04	2.24E-01	*argH*	0.91	5.66E-03	*oxyR*
1.04	1.36E-01	*nhaK_2*	0.90	6.03E-02	*catE_2*
1.04	4.35E-03	*graR_1*	0.90	3.72E-04	*recA*
1.03	3.95E-03	*ysdC_2*	0.90	1.13E-03	*smc_2*
1.03	2.91E-01	*acuC*	0.90	2.81E-01	*purS*
1.03	5.70E-03	*lacR*	0.89	7.93E-02	*fgd_1*
1.03	1.56E-02	*yvyI*	0.89	1.38E-02	*odhA*
1.03	6.78E-05	*fhs*	0.89	3.64E-01	*icaC*
1.03	4.44E-03	*dnaD*	0.88	1.58E-01	*fbp*
1.03	2.54E-01	*glpQ_2*	0.88	1.36E-01	*copZ*
1.02	5.73E-03	*pgi*	0.88	1.95E-01	*nixA*
1.02	4.22E-02	*cidB*	0.87	2.08E-01	*isdB*
1.01	1.24E-02	*phoP*	0.87	2.90E-01	*cntK*
1.01	7.87E-04	*recG*	0.87	7.59E-02	*mnhA1*
1.00	1.69E-03	*graR_2*	0.87	1.34E-01	*cycB*
1.00	4.36E-06	*tagH_1*	0.87	9.27E-04	*yfmJ*

1.00	1.24E-01	*btuD_1*	0.87	1.58E-01	*yfiY*
1.00	9.63E-02	*fetA*	0.87	1.33E-02	*fgd_2*
0.99	1.26E-03	*icaR*	0.86	1.88E-02	*mneS*
0.99	3.49E-01	*yhfK*	0.86	2.48E-01	*entS*
0.98	1.59E-02	*acr1*	0.85	1.58E-01	*sspC*
0.98	3.63E-02	*deoC2*	0.85	4.30E-02	*kimA*
0.98	3.17E-01	*asp2*	0.85	1.21E-02	*luxA*
0.97	3.75E-01	*yydJ*	0.84	2.30E-01	*ybaK*
0.97	1.03E-02	*folD*	0.84	2.34E-01	*gpmA_2*
0.97	2.20E-03	*panB*	0.84	5.73E-02	*gcvH*
0.97	3.94E-01	*crtP*	0.83	4.84E-02	*vraR*
0.97	1.26E-05	*cggR*	0.83	6.48E-02	*ktrB_1*
0.97	6.98E-03	*yfnB*	0.83	1.61E-02	*yoeB*
0.97	1.35E-03	*nagA*	0.83	6.30E-02	*aroD*
0.96	8.22E-02	*mnhA1*	0.82	1.23E-02	*mdrP_2*
0.96	1.29E-02	*mdrP_2*	0.82	3.55E-04	*dnaB*
0.96	1.19E-01	*trpD2*	0.81	1.46E-02	*lpp*
0.96	2.99E-02	*yjlD*	0.80	5.21E-04	*murF*
0.96	1.25E-01	*modA*	0.80	2.00E-02	*lexA*
0.95	2.25E-02	*tmk*	0.80	2.85E-03	*flp*
0.94	1.83E-01	*rihB*	0.79	4.66E-02	*pepS*
0.94	2.45E-01	*mccB*	0.79	2.60E-05	*relA*
0.94	9.24E-06	*relA*	0.79	1.25E-02	*cydD*
0.93	1.34E-01	*clpP*	0.79	1.68E-02	*COQ5*
0.93	4.45E-01	*lnrL_2*	0.79	4.92E-03	*salA*
0.93	4.97E-02	*sarS*	0.78	9.76E-03	*ypdF*
0.92	7.77E-03	*kapB*	0.78	1.74E-02	*yefM*
0.92	4.25E-01	*hmp*	0.78	3.19E-01	*ureA*
0.92	7.30E-04	*pepA_2*	0.78	1.23E-02	*pth*
0.92	1.43E-03	*manP*	0.78	4.31E-01	*gnu*
0.92	6.84E-03	*tagU_1*	0.78	1.34E-02	*yfnB*
0.91	5.85E-02	*mnaA_2*	0.78	4.62E-03	*suhB_2*
0.91	2.72E-05	*ecfA2*	0.77	3.33E-02	*pnoA*
0.90	4.25E-01	*dhaM*	0.77	1.41E-01	*slyA_2*
0.89	2.22E-01	*malR*	0.77	2.86E-03	*ecfA1*
0.89	6.98E-02	*opcR*	0.77	4.20E-01	*cntM*
0.89	2.82E-02	*luxA*	0.77	4.91E-01	*phnD2*
0.89	5.31E-02	*treR_1*	0.76	2.64E-01	*feuC*
0.89	4.31E-03	*suhB_2*	0.75	2.46E-02	*ytnP*
0.88	7.85E-04	*bmrA*	0.75	4.22E-02	*sdrE*
0.88	3.94E-03	*mgtE*	0.75	3.31E-02	*upp*
0.88	6.34E-01	*lacF*	0.74	2.77E-03	*nrdF*
0.87	2.54E-02	*yefM*	0.73	2.57E-01	*cycA_1*
0.87	1.32E-01	*fadN*	0.73	2.53E-02	*lacR*
0.86	2.73E-03	*ydjZ*	0.73	8.81E-04	*walR*
0.86	4.41E-01	*kdpA*	0.72	1.93E-02	*kdpB*
0.86	1.68E-01	*trpE*	0.72	1.60E-01	*gntT*
0.85	8.58E-03	*cinA*	0.72	1.71E-01	*btuD_3*
0.84	4.80E-02	*scrB*	0.72	3.40E-01	*essG_1*
0.84	2.24E-01	*rpmB*	0.71	2.46E-01	*yfhH*
0.84	8.39E-04	*ahpC*	0.71	1.12E-02	*glyA*
0.84	4.66E-01	*bceB_2*	0.71	3.45E-01	*RBKS*
0.83	5.17E-02	*garK_1*	0.71	9.59E-02	*mnaA_2*
0.83	2.71E-01	*lytN*	0.70	1.03E-02	*yibN*
0.83	2.46E-02	*rhaR_2*	0.70	5.95E-02	*tmk*
0.82	4.19E-01	*ebh_2*	0.69	4.70E-01	*glnQ*
0.82	2.11E-01	*tpx*	0.69	5.25E-01	*sspA*
0.82	3.83E-03	*ddl*	0.69	5.10E-02	*ald2*
0.82	2.08E-01	*purF*	0.68	3.84E-01	*msmX*
0.81	2.34E-01	*aur*	0.68	7.12E-02	*fumC*
0.81	1.72E-01	*slyA_2*	0.67	3.84E-01	*fosB*
0.81	1.14E-03	*walR*	0.67	2.91E-03	*cls_2*
0.81	8.34E-02	*manR*	0.67	1.22E-02	*yfkN_1*
0.80	2.54E-02	*pth*	0.66	8.31E-02	*hisF*
0.80	1.51E-01	*mntH*	0.66	3.01E-01	*phoU*
0.80	5.39E-03	*der_2*	0.65	2.28E-01	*yfhP*

0.80	4.00E-01	*fosB*	0.65	3.01E-01	*rihB*
0.79	2.04E-02	*hprK*	0.65	1.29E-02	*hdl IVa*
0.79	4.25E-02	*lpp*	0.65	1.32E-01	*cidB*
0.79	9.65E-03	*rqcH*	0.65	3.10E-01	*ureB*
0.78	1.54E-02	*recD*	0.65	6.97E-04	*ecfA2*
0.78	4.51E-01	*essB*	0.64	3.41E-01	*cntA*
0.78	5.79E-02	*cysS*	0.63	6.18E-01	*pstS*
0.78	2.70E-02	*bceB_3*	0.63	9.03E-04	*pdp*
0.78	5.33E-02	*ywaC*	0.63	3.59E-01	*mhqA_3*
0.77	9.22E-02	*spo0J*	0.63	1.80E-02	*panC*
0.77	1.66E-01	*glyQS*	0.62	5.99E-01	*lpl2_2*
0.77	1.41E-01	*ktrB_1*	0.62	2.99E-01	*btuD_1*
0.77	4.96E-01	*secY_2*	0.62	4.86E-02	*korA*
0.76	2.87E-01	*hup*	0.62	5.57E-02	*lytM*
0.76	5.05E-03	*femA_2*	0.61	2.43E-01	*sarX*
0.76	4.80E-02	*sodA*	0.61	5.36E-01	*essG_2*
0.76	1.12E-01	*degA*	0.61	1.90E-01	*ftsW*
0.76	2.25E-01	*cysA*	0.60	2.56E-01	*mta*
0.75	4.79E-01	*splF*	0.60	2.62E-01	*nanE*
0.75	6.13E-02	*upp*	0.59	4.28E-02	*kapB*
0.75	1.63E-01	*aroD*	0.58	9.81E-02	*srtA*
0.74	1.84E-04	*nrdR*	0.58	3.28E-02	*dnaI*
0.74	2.19E-02	*salA*	0.58	1.85E-01	*sarS*
0.74	5.59E-01	*sspA*	0.58	1.17E-01	*phoP*
0.73	4.27E-01	*ureF*	0.58	1.71E-01	*rpoZ*
0.73	1.52E-02	*ecfA1*	0.58	1.27E-01	*ysdC_1*
0.73	2.29E-01	*gntT*	0.58	3.31E-02	*divIC*
0.72	8.97E-02	*odhA*	0.57	1.86E-01	*mleN_1*
0.72	1.36E-02	*trxB*	0.57	3.43E-01	*mltF*
0.71	2.76E-01	*sufT*	0.56	1.48E-01	*garK_1*
0.71	2.08E-02	*aroA_2*	0.56	2.54E-01	*bdbD*
0.70	5.65E-02	*mqo1*	0.56	5.39E-01	*yfiZ_2*
0.70	2.14E-02	*panC*	0.55	3.47E-01	*deoD1*
0.70	4.15E-01	*femA_3*	0.55	4.98E-01	*yfiZ_1*
0.70	8.34E-02	*proC_2*	0.55	1.96E-01	*nudC*
0.69	2.09E-01	*deoC1*	0.54	3.41E-02	*tarB*
0.69	8.75E-02	*bshC*	0.54	4.91E-02	*rqcH*
0.68	5.92E-01	*essG_3*	0.54	1.11E-02	*pepF1_2*
0.68	2.12E-01	*trpA*	0.53	8.80E-02	*tagU_1*
0.68	8.87E-03	*cls_2*	0.53	1.82E-01	*scrB*
0.68	1.99E-02	*tarB*	0.53	5.86E-01	*yfhA*
0.68	1.14E-01	*pepF1_1*	0.52	3.59E-02	*femA_2*
0.68	1.13E-01	*dat*	0.51	1.90E-01	*ebh_1*
0.68	1.40E-01	*tarI1*	0.51	4.93E-02	*der_2*
0.67	6.77E-02	*addB*	0.51	2.43E-01	*deoC2*
0.67	2.34E-01	*trpS*	0.51	6.69E-02	*nagA*
0.67	1.28E-01	*ecsA*	0.51	2.28E-01	*treR_1*
0.66	1.55E-01	*cycA_2*	0.51	6.58E-02	*aroA_2*
0.66	1.40E-02	*murF*	0.51	2.38E-01	*rex*
0.66	9.25E-02	*aroB*	0.51	5.11E-01	*norB_3*
0.66	3.15E-01	*deoD1*	0.51	1.71E-01	*hssS*
0.66	1.48E-01	*cymR*	0.51	4.44E-01	*hup*
0.66	3.53E-01	*aldC_2*	0.50	5.85E-01	*ampR*
0.66	4.49E-01	*mazE*	0.50	3.53E-01	*pepT_2*
0.66	2.41E-01	*crtM*	0.50	6.01E-01	*essG_6*
0.65	4.25E-02	*glyA*	0.49	6.22E-01	*pstB3*
0.65	2.49E-02	*pncB2*	0.49	2.30E-01	*yifK*
0.65	2.90E-01	*moaD*	0.49	1.41E-01	*graR_1*
0.65	6.21E-01	*tenA*	0.49	2.31E-01	*putP*
0.65	2.07E-01	*ppaC*	0.49	2.46E-01	*degA*
0.65	4.12E-02	*yibN*	0.49	4.57E-01	*lysP_1*
0.64	4.47E-01	*gatD_2*	0.48	4.32E-01	*tpx*
0.64	1.31E-02	*yitU_2*	0.48	2.30E-01	*ecsA*
0.64	6.00E-01	*bioK*	0.47	3.03E-01	*opcR*
0.64	4.95E-02	*menE_1*	0.47	4.86E-01	*ahpD*
0.63	2.03E-02	*folP*	0.47	5.55E-01	*mhqD*

0.63	1.36E-01	*yoeB*	0.47	3.15E-01	*gpmA_1*
0.62	4.75E-01	*argG*	0.47	1.69E-01	*ysdC_2*
0.62	1.61E-02	*xerC*	0.47	3.17E-01	*hutG*
0.62	1.21E-01	*srtA*	0.46	6.75E-02	*pepA_2*
0.62	2.79E-01	*yhfP*	0.45	2.52E-01	*lacG*
0.62	1.59E-01	*agrB*	0.45	2.07E-01	*pnbA*
0.61	5.39E-02	*hdl IVa*	0.45	5.90E-02	*mnaA_1*
0.61	1.37E-01	*speA*	0.45	1.31E-01	*cinA*
0.61	1.31E-01	*ettA*	0.45	2.32E-01	*ywaC*
0.60	2.24E-01	*nudC*	0.45	5.78E-01	*rodA*
0.60	1.83E-01	*oatA_1*	0.44	1.91E-01	*secA1_2*
0.60	1.32E-01	*dtd*	0.44	5.18E-01	*hlgB*
0.60	1.19E-01	*purD*	0.44	4.01E-01	*yfmC_1*
0.60	1.93E-01	*coaE*	0.43	5.57E-01	*hdfR_1*
0.60	3.50E-01	*isdH*	0.43	2.68E-01	*cysJ*
0.60	2.37E-01	*argC*	0.43	3.50E-02	*tagH_1*
0.59	1.30E-01	*mhqA_1*	0.43	6.45E-01	*msrAB*
0.59	4.21E-01	*yfhH*	0.43	1.06E-01	*pnp_2*
0.59	1.91E-01	*sirC*	0.43	3.14E-01	*manR*
0.59	1.18E-01	*fbiB*	0.42	4.18E-01	*glyQS*
0.59	4.73E-01	*essG_5*	0.42	8.53E-01	*ywqE_1*
0.58	1.77E-01	*ysdC_1*	0.42	2.90E-01	*wecC*
0.58	1.91E-01	*fumC*	0.42	1.81E-01	*gtfA_3*
0.58	4.91E-01	*ureB*	0.42	4.12E-01	*mepA*
0.58	6.14E-02	*tagX*	0.41	5.17E-01	*relJ*
0.57	7.43E-02	*mutM*	0.41	8.98E-02	*cpdA_2*
0.57	5.79E-01	*essG_8*	0.41	5.17E-01	*mnhC1*
0.56	4.96E-01	*esxA*	0.41	5.55E-01	*nhaK_2*
0.56	4.69E-02	*ywqN*	0.40	3.85E-01	*walK*
0.56	2.98E-02	*gtfA_2*	0.40	1.05E-01	*sucD*
0.55	2.16E-02	*spxA*	0.40	2.13E-01	*hprK*
0.55	2.41E-01	*scmP_4*	0.40	4.43E-01	*yhfP*
0.55	4.82E-01	*ypjD*	0.40	4.54E-01	*nagB_2*
0.54	4.99E-01	*modB*	0.40	7.30E-02	*gtfA_2*
0.54	7.72E-02	*recA*	0.40	2.58E-01	*rhaR_2*
0.53	5.53E-01	*lpl2_1*	0.39	3.05E-01	*gmk*
0.53	8.12E-02	*coaBC*	0.39	7.26E-01	*psuG*
0.53	3.43E-01	*dapH_2*	0.39	2.34E-01	*sdcS*
0.52	2.53E-01	*putP*	0.39	1.86E-01	*slyA_3*
0.52	4.25E-01	*kdpC*	0.39	4.59E-01	*azoR*
0.52	2.35E-01	*gph_2*	0.38	4.65E-01	*purC*
0.51	4.94E-01	*tcyC_2*	0.38	3.73E-01	*spo0J*
0.51	5.62E-02	*iolS*	0.38	4.40E-01	*hisI*
0.50	1.73E-02	*fda*	0.38	3.70E-01	*panD*
0.50	3.44E-01	*nth_1*	0.37	1.60E-01	*ugtP*
0.50	7.57E-01	*hlgA*	0.37	4.96E-01	*kdpC*
0.50	6.30E-01	*sarT*	0.37	3.31E-01	*bshC*
0.50	4.25E-01	*murG*	0.36	4.86E-01	*slyA_1*
0.49	8.47E-02	*ecfT_1*	0.36	5.55E-01	*yflS*
0.49	2.98E-02	*priA*	0.36	5.84E-02	*hisC_1*
0.49	1.48E-01	*smc_2*	0.36	5.22E-01	*nudF*
0.49	7.00E-02	*ypcP*	0.36	2.96E-01	*addB*
0.49	2.26E-01	*sauU*	0.35	2.57E-01	*comEC*
0.49	3.89E-01	*hssR*	0.35	6.12E-01	*rocD2_2*
0.48	7.37E-02	*cdr*	0.35	2.86E-01	*bceB_3*
0.48	5.54E-01	*selX*	0.35	2.05E-01	*ydjZ*
0.47	5.97E-01	*rodA*	0.35	5.85E-01	*ptsG_2*
0.47	5.63E-01	*cycA_1*	0.35	3.82E-01	*cynR*
0.47	8.68E-02	*cpdA_2*	0.34	2.22E-01	*trxB*
0.47	4.93E-01	*mobB*	0.34	4.38E-01	*uppP*
0.46	3.27E-01	*phrB*	0.34	1.97E-01	*ezrA*
0.46	2.63E-01	*kdpB*	0.34	2.65E-01	*menE_1*
0.45	2.90E-01	*hsdR*	0.33	5.88E-01	*ydaG*
0.45	2.84E-01	*lexA*	0.33	5.97E-01	*yecD*
0.45	5.96E-01	*ahpD*	0.33	1.96E-01	*yfkN_2*
0.45	3.71E-01	*tcaA*	0.33	7.43E-01	*essG_9*

0.44	1.92E-01	*scrK*	0.32	5.65E-01	*sbnH*
0.44	2.97E-01	*ahpF*	0.32	4.74E-01	*sbnI*
0.44	5.48E-02	*pdp*	0.31	3.98E-01	*mhqA_1*
0.44	1.12E-01	*mnaA_1*	0.31	4.86E-01	*bcrA_1*
0.44	3.55E-01	*yifK*	0.31	5.21E-01	*nth_1*
0.44	1.46E-01	*ftsH*	0.31	2.03E-01	*ypcP*
0.43	3.95E-01	*hisF*	0.31	6.72E-01	*epsL*
0.43	5.47E-01	*relJ*	0.31	6.25E-01	*rpe*
0.43	5.56E-02	*swrC*	0.31	6.06E-01	*purK*
0.43	4.50E-01	*dinB*	0.31	4.44E-01	*azo1*
0.43	1.31E-01	*dagK*	0.31	5.39E-01	*ipdC*
0.43	1.77E-01	*rplI*	0.30	6.74E-01	*dppE_1*
0.43	4.41E-01	*tarJ*	0.30	4.30E-01	*ytpA*
0.43	5.06E-01	*rpmG2_1*	0.30	5.68E-01	*ytmI*
0.42	1.89E-01	*pgcA*	0.30	5.75E-01	*serS*
0.42	1.14E-01	*iscS_2*	0.30	3.43E-01	*recD*
0.41	2.36E-01	*ccpN*	0.29	6.72E-01	*yehR*
0.41	4.51E-01	*rnhA*	0.29	2.46E-01	*cdr*
0.41	4.25E-01	*veg*	0.29	1.74E-01	*rsgA*
0.41	2.71E-01	*rhaS_2*	0.29	2.52E-01	*tetA*
0.41	3.91E-01	*mneS*	0.29	4.00E-01	*rhaS_2*
0.41	6.05E-01	*mrnC*	0.29	6.72E-01	*aldC_2*
0.41	2.17E-01	*dnaI*	0.28	2.70E-01	*bshA*
0.40	3.84E-01	*secG*	0.28	6.78E-01	*ureG*
0.40	8.87E-01	*ywqE_1*	0.28	6.42E-01	*ykoE*
0.40	5.51E-01	*rsbU_2*	0.28	8.24E-01	*lukEv*
0.40	1.58E-01	*bepA_1*	0.28	6.79E-01	*ureE*
0.40	5.59E-01	*proP*	0.27	3.81E-01	*ftsZ*
0.39	5.52E-01	*ureD1*	0.27	4.54E-01	*scmP_4*
0.39	4.37E-01	*sasA*	0.27	5.25E-01	*sirC*
0.39	2.74E-01	*fepC*	0.27	7.73E-01	*yclP*
0.38	5.10E-01	*bdbD*	0.27	5.60E-01	*murJ_1*
0.38	6.22E-01	*sau3AIR*	0.27	5.50E-01	*hxlB*
0.38	6.73E-01	*aldC_1*	0.27	3.57E-01	*mfd*
0.38	4.91E-01	*est_2*	0.27	2.62E-01	*atl_2*
0.37	5.64E-01	*isdI*	0.27	3.84E-01	*tagH_2*
0.37	5.02E-01	*gapA1*	0.26	5.69E-01	*crtM*
0.37	7.60E-01	*lpl2_6*	0.26	5.90E-01	*mnhB1*
0.37	4.49E-01	*map_3*	0.26	7.40E-01	*fecD*
0.37	6.67E-01	*ytrA*	0.26	6.08E-01	*gapA1*
0.37	6.20E-01	*cysE*	0.26	4.66E-01	*mscL*
0.37	6.08E-01	*mltF*	0.26	6.15E-01	*sarR*
0.37	4.63E-01	*pepS*	0.25	7.63E-01	*lpl2_1*
0.37	2.22E-01	*ugtP*	0.25	6.28E-01	*dinB*
0.37	3.14E-01	*graS_1*	0.25	4.25E-01	*gdpP*
0.37	1.55E-01	*lgt*	0.25	8.24E-01	*yydJ*
0.36	7.34E-01	*pglF*	0.25	4.98E-01	*radA*
0.36	2.89E-01	*secA1_1*	0.25	3.62E-01	*yedJ*
0.36	2.55E-01	*femB*	0.25	8.25E-01	*argR_2*
0.36	2.89E-01	*tagH_2*	0.25	6.56E-01	*trpS*
0.36	7.28E-01	*zipA*	0.24	3.89E-01	*ftsH*
0.35	4.89E-01	*rex*	0.24	4.46E-01	*glvR*
0.35	5.71E-01	*tarA*	0.24	4.62E-01	*gtfB*
0.35	6.67E-01	*eno*	0.24	5.99E-01	*rlmH*
0.34	2.36E-01	*dnaB*	0.24	4.50E-01	*yugI*
0.34	3.55E-01	*cca*	0.23	7.22E-01	*umuC*
0.34	4.25E-01	*ytnP*	0.22	3.96E-01	*cls_1*
0.34	2.53E-01	*tetA*	0.22	7.86E-01	*cntB*
0.33	5.47E-01	*mnhB1*	0.22	8.56E-01	*lpl2_5*
0.33	4.12E-01	*mnhE1*	0.22	4.12E-01	*dagK*
0.33	5.34E-01	*sbnC*	0.21	5.54E-01	*mraZ*
0.32	6.35E-01	*feoB*	0.20	4.43E-01	*bepA_1*
0.32	4.84E-01	*pnbA*	0.20	5.38E-01	*yqfL*
0.31	5.94E-01	*accC*	0.20	6.68E-01	*xerD_1*
0.31	4.54E-01	*cydD*	0.20	4.70E-01	*fhs*
0.30	3.89E-01	*slyA_3*	0.19	6.24E-01	*pgi*

0.30	6.62E-01	*rpsU*	0.19	5.76E-01	*darA*
0.30	7.29E-01	*yydI*	0.19	5.69E-01	*ccpN*
0.30	6.04E-01	*ftsW*	0.19	6.59E-01	*yheS*
0.29	6.33E-01	*dgk*	0.19	6.24E-01	*aroK*
0.29	8.11E-01	*nasF*	0.19	5.10E-01	*gpsB*
0.29	5.93E-01	*walK*	0.19	8.12E-01	*yydI*
0.29	4.79E-01	*sucC*	0.18	8.13E-01	*ypjD*
0.29	8.22E-01	*opuCD*	0.18	6.52E-01	*rot*
0.29	6.65E-01	*btuD_3*	0.18	6.13E-01	*arlR*
0.28	3.01E-01	*tarF*	0.18	8.65E-01	*kdpA*
0.28	5.83E-01	*hxlB*	0.18	8.74E-01	*crtQ*
0.28	5.59E-01	*yheS*	0.18	8.42E-01	*mazE*
0.27	7.95E-01	*xerD_2*	0.17	5.85E-01	*mgtE*
0.27	5.06E-01	*mscL*	0.17	6.74E-01	*crtN*
0.27	4.30E-01	*metG*	0.17	5.37E-01	*ywqN*
0.26	3.84E-01	*bshA*	0.17	4.77E-01	*ydhF*
0.26	5.10E-01	*korA*	0.17	5.77E-01	*coaBC*
0.25	7.64E-01	*xylB*	0.16	8.84E-01	*splF*
0.25	6.28E-01	*folB*	0.16	8.79E-01	*wbgU*
0.25	4.92E-01	*rihA*	0.16	6.68E-01	*liaR*
0.25	4.88E-01	*gdpP*	0.16	7.30E-01	*oatA_1*
0.24	5.90E-01	*spsB_2*	0.15	5.19E-01	*pdxK*
0.24	6.77E-01	*pspB*	0.15	8.20E-01	*fadA*
0.23	5.10E-01	*nadE*	0.15	5.71E-01	*xerC*
0.23	8.49E-01	*essG_9*	0.15	7.52E-01	*cymR*
0.23	8.28E-01	*zapA*	0.15	8.87E-01	*essG_8*
0.23	5.71E-01	*sdcS*	0.14	8.83E-01	*glpQ_2*
0.23	7.48E-01	*agrA*	0.14	8.25E-01	*murG*
0.22	6.06E-01	*rsmH_2*	0.14	7.37E-01	*mrcA*
0.22	6.68E-01	*tarM*	0.14	6.58E-01	*pgcA*
0.22	6.08E-01	*lytR*	0.13	8.98E-01	*wbpI*
0.22	8.09E-01	*sspC*	0.13	5.88E-01	*lgt*
0.22	5.55E-01	*yitU_1*	0.12	8.88E-01	*femA_3*
0.22	7.14E-01	*araB*	0.12	8.85E-01	*essG_5*
0.22	5.96E-01	*mutS2_2*	0.12	8.97E-01	*xerD_2*
0.22	5.59E-01	*glvR*	0.12	8.63E-01	*cysA*
0.22	6.67E-01	*panD*	0.12	7.13E-01	*metG*
0.22	7.10E-01	*yacL*	0.12	8.42E-01	*yjjP*
0.21	8.68E-01	*essG_2*	0.12	9.01E-01	*mnhD1_1*
0.21	8.71E-01	*argJ*	0.12	7.48E-01	*cca*
0.20	7.41E-01	*yycH*	0.11	7.73E-01	*sucC*
0.20	5.93E-01	*pepA_1*	0.11	8.61E-01	*tagO*
0.20	7.52E-01	*yjjP*	0.11	8.54E-01	*dapH_2*
0.20	6.36E-01	*fur*	0.11	8.84E-01	*ureC*
0.19	5.85E-01	*pta*	0.10	9.26E-01	*yclQ*
0.19	4.75E-01	*ydhF*	0.10	9.45E-01	*comGC*
0.19	9.32E-01	*sarU*	0.10	7.13E-01	*folP*
0.19	8.03E-01	*pheA*	0.10	8.98E-01	*nupC_2*
0.19	7.02E-01	*slmA*	0.10	7.65E-01	*rplI*
0.19	5.75E-01	*yfkN_2*	0.09	8.65E-01	*alaS*
0.18	5.79E-01	*pnp_2*	0.09	9.18E-01	*modB*
0.18	7.68E-01	*pxpC_1*	0.09	8.54E-01	*lipM*
0.18	6.36E-01	*polX*	0.09	9.36E-01	*lacE*
0.18	6.13E-01	*pcrB*	0.09	9.15E-01	*splE*
0.18	7.05E-01	*moaE*	0.08	7.26E-01	*mro*
0.18	4.60E-01	*tarL*	0.08	8.57E-01	*dtd3*
0.18	9.00E-01	*mtlF*	0.08	8.34E-01	*folK*
0.17	5.39E-01	*ftsA*	0.08	8.86E-01	*ywpJ_2*
0.17	8.54E-01	*ribU*	0.08	8.25E-01	*murA1*
0.17	6.63E-01	*mraZ*	0.08	9.08E-01	*ureD1*
0.16	8.41E-01	*essC*	0.08	8.48E-01	*zntB*
0.15	8.45E-01	*mazF*	0.07	8.88E-01	*argC*
0.15	5.71E-01	*rsgA*	0.07	8.84E-01	*recR*
0.15	7.97E-01	*ywpJ_2*	0.07	9.14E-01	*rsmH_1*
0.15	9.15E-01	*ebh_3*	0.07	8.62E-01	*polX*
0.15	5.46E-01	*hisC_1*	0.07	8.65E-01	*graS_1*

0.14	8.09E-01	*femA_1*	0.06	9.24E-01	*tarA*
0.14	8.09E-01	*kimA*	0.06	9.26E-01	*cspLA*
0.14	7.68E-01	*guaB*	0.06	8.62E-01	*pta*
0.14	7.21E-01	*yqfL*	0.06	8.86E-01	*mqo1*
0.14	7.57E-01	*secA1_2*	0.06	9.26E-01	*ssl7_3*
0.14	4.79E-01	*addA*	0.06	8.91E-01	*dnaD*
0.14	7.60E-01	*radA*	0.06	9.44E-01	*sau3AIR*
0.13	8.28E-01	*ybaN*	0.05	8.62E-01	*iolS*
0.13	8.31E-01	*orr*	0.05	9.26E-01	*prfB*
0.13	8.73E-01	*ureC*	0.05	8.71E-01	*yitU_2*
0.12	8.59E-01	*hrtA_2*	0.05	9.44E-01	*bcr_1*
0.12	7.33E-01	*hslV*	0.05	8.69E-01	*iscS_2*
0.12	7.07E-01	*rpiA*	0.05	9.50E-01	*ansA*
0.12	7.49E-01	*mfd*	0.05	9.42E-01	*fabZ*
0.12	8.45E-01	*hslO*	0.05	8.69E-01	*tarF*
0.11	8.53E-01	*spsB_1*	0.05	8.88E-01	*dltA_2*
0.11	7.49E-01	*dltA_2*	0.05	8.97E-01	*tagX*
0.11	6.30E-01	*yodB*	0.05	9.74E-01	*essG_7*
0.10	8.72E-01	*sspB*	0.05	9.51E-01	*mazF*
0.10	8.93E-01	*mnhG1*	0.04	9.50E-01	*fetA*
0.10	8.57E-01	*yutF*	0.04	9.27E-01	*rsmH_2*
0.09	9.09E-01	*mnhC1*	0.04	9.45E-01	*tarI1*
0.09	8.42E-01	*ypdA*	0.03	9.42E-01	*menC*
0.09	7.83E-01	*sucD*	0.03	9.51E-01	*murJ_2*
0.09	8.72E-01	*deoB*	0.03	9.36E-01	*icaR*
0.09	7.94E-01	*ezrA*	0.03	9.69E-01	*lip2*
0.09	8.60E-01	*ald2*	0.03	9.71E-01	*brnQ_2*
0.09	8.20E-01	*recQ_1*	0.02	9.49E-01	*thiN*
0.08	8.85E-01	*rpoZ*	0.02	9.63E-01	*malP*
0.08	9.02E-01	*hisI*	0.02	9.57E-01	*mutM*
0.08	8.75E-01	*bacC*	0.02	9.56E-01	*ecfT_1*
0.08	8.19E-01	*cls_1*	0.02	9.67E-01	*secA1_1*
0.08	9.19E-01	*MroQ*	0.02	9.70E-01	*folA*
0.07	8.15E-01	*pdxK*	0.02	9.67E-01	*bcr_2*
0.07	9.42E-01	*purN*	0.01	9.70E-01	*rhaS_1*
0.07	8.75E-01	*hslU*	0.01	9.69E-01	*pncB2*
0.07	8.87E-01	*arlR*	0.01	9.75E-01	*aes*
0.07	8.98E-01	*gmk*	0.01	9.80E-01	*cysS*
0.06	8.87E-01	*tkt*	0.01	9.77E-01	*scrK*
0.06	9.36E-01	*nanE*	0.01	9.89E-01	*folB*
0.06	9.64E-01	*phnE_2*	0.00	9.92E-01	*hsdR*
0.06	9.17E-01	*acpS*	0.00	9.89E-01	*mntR*
0.05	9.56E-01	*brnQ_2*	-0.01	9.88E-01	*rlmI*
0.05	8.84E-01	*femX*	-0.01	9.77E-01	*fabI*
0.05	8.99E-01	*pgk*	-0.01	9.90E-01	*yhfK*
0.05	9.51E-01	*isdC*	-0.01	9.70E-01	*ydjM*
0.05	9.29E-01	*dtd3*	-0.02	9.83E-01	*lytN*
0.05	9.52E-01	*pepT_2*	-0.02	9.87E-01	*ebh_3*
0.05	9.43E-01	*lacG*	-0.02	9.67E-01	*spsB_2*
0.05	9.43E-01	*moaB*	-0.02	9.46E-01	*ybeY*
0.05	9.06E-01	*spoVG*	-0.02	9.75E-01	*cysE*
0.04	9.67E-01	*purE*	-0.02	9.69E-01	*rsbU_1*
0.04	9.37E-01	*aroK*	-0.02	9.55E-01	*bioB_2*
0.04	9.49E-01	*tarD*	-0.02	9.72E-01	*ssbA_1*
0.04	9.32E-01	*btuF*	-0.03	9.54E-01	*fruA_1*
0.04	9.58E-01	*xpt*	-0.03	9.27E-01	*ftsA*
0.03	9.78E-01	*heI*	-0.03	9.63E-01	*lipL*
0.03	9.49E-01	*def*	-0.04	9.64E-01	*rpmB*
0.03	9.65E-01	*recQ_2*	-0.04	9.19E-01	*recQ_1*
0.03	9.80E-01	*ptsG_1*	-0.04	9.49E-01	*nikE*
0.02	9.56E-01	*birA*	-0.04	9.26E-01	*mgrA*
0.02	9.75E-01	*frdB*	-0.04	9.49E-01	*desR*
0.02	9.86E-01	*hlgB*	-0.04	9.31E-01	*sepF*
0.01	9.85E-01	*recR*	-0.04	9.70E-01	*mtlF*
0.01	9.89E-01	*nirQ*	-0.05	9.73E-01	*dapH_3*
0.01	9.88E-01	*tarS*	-0.05	9.69E-01	*ssl3*

0.00	9.99E-01	*azoR*	-0.05	9.49E-01	*mrnC*
0.00	9.97E-01	*atl_2*	-0.05	9.27E-01	*tagG*
0.00	9.99E-01	*sbnB*	-0.06	9.44E-01	*malR*
0.00	9.99E-01	*ftsZ*	-0.06	9.40E-01	*sufT*
0.00	9.99E-01	*map_2*	-0.06	9.45E-01	*xylB*
0.00	1.00E+00	*grpE*	-0.06	9.14E-01	*spsB_1*
0.00	9.99E-01	*codY*	-0.06	9.39E-01	*selX*
0.00	9.97E-01	*ghrB_2*	-0.07	9.60E-01	*ssl7_1*
0.00	9.99E-01	*graS_2*	-0.07	8.97E-01	*graS_2*
0.00	9.97E-01	*mrcA*	-0.07	9.26E-01	*cspA_2*
0.00	9.95E-01	*mreC*	-0.07	8.57E-01	*ybbH_1*
-0.01	9.95E-01	*yojF*	-0.08	8.33E-01	*rihA*
-0.01	9.95E-01	*ltaE*	-0.08	8.68E-01	*gph_2*
-0.01	9.83E-01	*comEC*	-0.08	8.87E-01	*glcU_1*
-0.01	9.95E-01	*fetB*	-0.09	9.44E-01	*fetB*
-0.01	9.80E-01	*aes*	-0.09	9.07E-01	*crcB_2*
-0.02	9.84E-01	*bioB_1*	-0.09	9.31E-01	*zipA*
-0.02	9.89E-01	*splE*	-0.09	8.71E-01	*rnmV_2*
-0.02	9.83E-01	*rnmV_2*	-0.09	9.34E-01	*wbjC*
-0.02	9.80E-01	*bcrA_1*	-0.10	9.07E-01	*ydjF*
-0.02	9.69E-01	*liaR*	-0.10	7.57E-01	*lysS*
-0.02	9.62E-01	*moaA_1*	-0.10	9.23E-01	*zapA*
-0.02	9.78E-01	*slyA_1*	-0.10	5.86E-01	*yodB*
-0.02	9.69E-01	*azo1*	-0.11	7.52E-01	*graR_2*
-0.02	9.49E-01	*ybeY*	-0.11	8.42E-01	*moaB*
-0.03	9.56E-01	*dnaC*	-0.11	6.24E-01	*IMPDH*
-0.03	9.54E-01	*thiN*	-0.11	7.40E-01	*rsuA*
-0.03	9.60E-01	*uvrC*	-0.11	7.06E-01	*hldD*
-0.03	9.57E-01	*folA*	-0.11	9.10E-01	*pglF*
-0.03	9.74E-01	*lipL*	-0.11	8.79E-01	*modA*
-0.03	9.74E-01	*pxpB_1*	-0.11	9.47E-01	*phnE_1*
-0.04	9.63E-01	*rpe*	-0.11	8.43E-01	*araB*
-0.04	9.68E-01	*mnhD1_1*	-0.11	9.27E-01	*ssl5_2*
-0.05	9.01E-01	*gpsB*	-0.11	8.25E-01	*tarD*
-0.05	9.26E-01	*lytM*	-0.12	8.36E-01	*yacL*
-0.05	9.15E-01	*gtfA_3*	-0.12	7.23E-01	*ytpP*
-0.05	9.24E-01	*guaA*	-0.12	9.48E-01	*pstA*
-0.05	9.20E-01	*ftsL*	-0.12	6.90E-01	*arlS*
-0.05	9.51E-01	*ydaG*	-0.12	7.92E-01	*rhaR_1*
-0.06	9.55E-01	*dppE_1*	-0.13	9.26E-01	*lacA*
-0.06	8.76E-01	*hldD*	-0.13	7.90E-01	*metK*
-0.06	8.30E-01	*hemE*	-0.13	9.06E-01	*ebh_2*
-0.07	9.25E-01	*nikE*	-0.13	8.53E-01	*tcaR*
-0.07	8.68E-01	*bcr_2*	-0.13	8.79E-01	*mobA*
-0.07	8.53E-01	*yacP*	-0.13	7.84E-01	*rnhC*
-0.07	9.36E-01	*ssbA_1*	-0.14	7.24E-01	*fni*
-0.07	9.17E-01	*splA*	-0.14	6.64E-01	*pcrB*
-0.08	9.20E-01	*nth_2*	-0.14	6.10E-01	*mutS_2*
-0.08	8.60E-01	*ybbH_1*	-0.14	5.88E-01	*yggS*
-0.08	9.33E-01	*wbnH*	-0.14	7.76E-01	*ligJ*
-0.08	8.51E-01	*ytpP*	-0.15	6.85E-01	*tkt*
-0.08	9.17E-01	*purK*	-0.15	7.19E-01	*slmA*
-0.09	9.45E-01	*essG_4*	-0.15	6.99E-01	*fadR*
-0.09	8.88E-01	*rnmV_1*	-0.15	9.26E-01	*lacF*
-0.09	8.09E-01	*menF*	-0.16	8.33E-01	*mutS_1*
-0.10	8.49E-01	*rot*	-0.16	6.23E-01	*asnS*
-0.10	8.72E-01	*mleN_1*	-0.16	7.92E-01	*moaD*
-0.10	7.88E-01	*kefB*	-0.16	8.09E-01	*proP*
-0.10	8.52E-01	*ytkD*	-0.16	7.67E-01	*ykoD_1*
-0.10	8.51E-01	*gtaB*	-0.16	7.63E-01	*tarJ*
-0.11	8.49E-01	*malP*	-0.17	5.75E-01	*hslV*
-0.11	8.30E-01	*uvrB*	-0.17	6.28E-01	*pfkA*
-0.11	8.32E-01	*COQ5*	-0.17	7.44E-01	*topA*
-0.11	8.07E-01	*hsdM*	-0.17	4.97E-01	*femX*
-0.11	8.42E-01	*prfB*	-0.17	4.99E-01	*queE_1*
-0.11	7.68E-01	*divIC*	-0.18	6.55E-01	*moaE*

-0.12	8.17E-01	*glcB*	-0.18	6.77E-01	*yutF*
-0.12	7.33E-01	*ruvB_2*	-0.18	4.76E-01	*rpiA*
-0.12	6.95E-01	*yggS*	-0.19	7.29E-01	*ktrB_2*
-0.12	8.22E-01	*rhaR_1*	-0.19	5.37E-01	*mutY*
-0.13	8.27E-01	*sepA*	-0.19	5.72E-01	*treR_2*
-0.13	8.90E-01	*feuB*	-0.19	7.37E-01	*arcB*
-0.13	8.07E-01	*lipM*	-0.20	5.88E-01	*mnhE1*
-0.13	7.94E-01	*pdxT*	-0.20	4.75E-01	*ruvB_2*
-0.13	9.28E-01	*ywqD_2*	-0.20	7.44E-01	*MroQ*
-0.14	7.44E-01	*hemH*	-0.20	7.09E-01	*yycH*
-0.14	6.96E-01	*yhaM*	-0.20	6.39E-01	*pepF1_1*
-0.14	8.98E-01	*essG_10*	-0.20	8.87E-01	*hlgC*
-0.14	8.73E-01	*ureE*	-0.21	6.39E-01	*phrB*
-0.14	7.61E-01	*tadA_2*	-0.21	7.22E-01	*mnhF1*
-0.15	8.42E-01	*arg*	-0.21	4.97E-01	*nrnA*
-0.15	8.51E-01	*umuC*	-0.22	8.09E-01	*ureF*
-0.15	7.13E-01	*pfkA*	-0.22	5.50E-01	*rny*
-0.15	6.31E-01	*mutS_2*	-0.22	6.23E-01	*coaE*
-0.15	7.34E-01	*rlmI*	-0.22	5.80E-01	*cpnA*
-0.15	6.36E-01	*msrR*	-0.22	5.75E-01	*uvrB*
-0.15	7.84E-01	*rlmH*	-0.22	6.04E-01	*arcD*
-0.16	8.34E-01	*cspA_2*	-0.22	4.75E-01	*moaA_1*
-0.16	8.28E-01	*mnhF1*	-0.23	4.42E-01	*mvaA*
-0.16	8.17E-01	*rsbV*	-0.23	8.10E-01	*essG_10*
-0.17	8.39E-01	*ptsG_2*	-0.23	6.68E-01	*sufC*
-0.17	8.07E-01	*desR*	-0.23	7.02E-01	*arg*
-0.17	7.61E-01	*fmtA*	-0.23	8.36E-01	*argJ*
-0.17	5.96E-01	*yedJ*	-0.23	4.36E-01	*femB*
-0.17	6.59E-01	*gtfB*	-0.23	1.39E-01	*addA*
-0.17	6.25E-01	*recN*	-0.23	4.26E-01	*gnd*
-0.18	7.34E-01	*hly*	-0.23	7.97E-01	*mcsA*
-0.18	8.87E-01	*nikA*	-0.23	2.55E-01	*tarL*
-0.19	8.41E-01	*phoU*	-0.23	3.86E-01	*msrR*
-0.20	8.32E-01	*groS*	-0.23	8.29E-01	*crtP*
-0.20	5.52E-01	*yycI*	-0.24	2.66E-01	*hemE*
-0.20	4.96E-01	*whiA*	-0.24	8.14E-01	*crcB_1*
-0.20	6.51E-01	*yqgN*	-0.24	4.31E-01	*nadE*
-0.20	8.76E-01	*asp1*	-0.24	5.49E-01	*guaB*
-0.21	5.70E-01	*hemL2*	-0.25	4.98E-01	*ypdA*
-0.21	4.50E-01	*fabI*	-0.25	5.90E-01	*mrpA*
-0.21	6.63E-01	*tsaE*	-0.25	5.09E-01	*yqgN*
-0.21	5.40E-01	*truA*	-0.25	3.99E-01	*nagB_1*
-0.22	6.06E-01	*menC*	-0.25	3.12E-01	*rnz*
-0.22	5.96E-01	*accD*	-0.25	8.86E-01	*bioD*
-0.22	7.51E-01	*rnc*	-0.25	7.77E-01	*argB*
-0.23	8.21E-01	*uhpT*	-0.25	4.50E-01	*hemH*
-0.23	6.40E-01	*moeA*	-0.26	3.76E-01	*kefB*
-0.23	7.61E-01	*dnaK*	-0.26	5.72E-01	*glpF*
-0.24	7.00E-01	*ssl1*	-0.26	3.89E-01	*treA*
-0.25	6.66E-01	*sarR*	-0.26	4.46E-01	*hsdM*
-0.25	8.09E-01	*argB*	-0.26	3.70E-01	*yhaM*
-0.25	6.94E-01	*ymcA*	-0.27	4.98E-01	*glcB*
-0.26	7.71E-01	*groL*	-0.27	2.41E-01	*nupG*
-0.26	2.87E-01	*pcrA*	-0.27	1.93E-01	*swrC*
-0.26	5.96E-01	*pucK*	-0.27	5.04E-01	*topB*
-0.26	8.45E-01	*clpB*	-0.27	6.22E-01	*recF*
-0.26	5.64E-01	*gltX*	-0.27	5.73E-01	*femA_1*
-0.26	5.68E-01	*murA2*	-0.27	4.51E-01	*fabF*
-0.26	4.43E-01	*arlS*	-0.28	6.42E-01	*rnc*
-0.27	3.55E-01	*lyrA*	-0.28	4.83E-01	*murA2*
-0.27	6.10E-01	*rnhC*	-0.28	5.73E-01	*miaB*
-0.27	6.93E-01	*rbgA*	-0.28	5.03E-01	*greA*
-0.27	8.42E-01	*nikD*	-0.29	6.27E-01	*pckA*
-0.27	6.61E-01	*serS*	-0.29	2.86E-01	*spoVG*
-0.27	7.60E-01	*mobA*	-0.29	5.80E-01	*hhaIM*
-0.27	5.71E-01	*cpnA*	-0.29	6.83E-01	*eno*

-0.27	2.35E-01	*IMPDH*	-0.29	7.91E-01	*asp1*
-0.28	5.13E-01	*mntR*	-0.30	8.13E-01	*essG_3*
-0.28	6.27E-01	*ykoD_1*	-0.30	3.67E-01	*btuF*
-0.28	4.88E-01	*rhaS_1*	-0.30	6.31E-01	*mobB*
-0.29	5.93E-01	*pdxS*	-0.30	3.39E-01	*pepA_1*
-0.30	4.20E-01	*glmM*	-0.30	4.81E-01	*frdB*
-0.30	5.94E-01	*topA*	-0.30	4.70E-01	*secG*
-0.30	6.98E-01	*mtlA*	-0.30	5.86E-01	*nusB*
-0.30	6.25E-01	*tmcAL*	-0.30	3.74E-01	*mqo2*
-0.30	8.80E-01	*comGC*	-0.30	8.36E-01	*capD*
-0.30	7.39E-01	*bioW*	-0.31	2.39E-01	*ghrB_2*
-0.30	5.44E-01	*wecC*	-0.31	5.50E-01	*suhB_1*
-0.30	4.14E-01	*smc_1*	-0.31	5.54E-01	*glpT*
-0.30	2.02E-01	*tpiA*	-0.31	6.78E-01	*lacC_2*
-0.31	5.71E-01	*pxpB_2*	-0.31	3.61E-01	*def*
-0.31	5.47E-01	*pyk*	-0.31	7.63E-01	*comC*
-0.31	2.38E-01	*purB*	-0.32	5.46E-01	*menG*
-0.31	4.79E-01	*sodM*	-0.32	5.78E-01	*rsbV*
-0.31	5.29E-01	*murJ_2*	-0.32	4.01E-01	*ettA*
-0.31	3.60E-01	*trmB*	-0.32	2.74E-01	*argR_1*
-0.32	4.87E-01	*acpP*	-0.32	2.68E-01	*alr1_1*
-0.32	5.64E-01	*splD*	-0.32	3.17E-01	*truB*
-0.32	8.61E-01	*capD*	-0.32	2.81E-01	*yitU_1*
-0.32	4.02E-01	*zntB*	-0.33	5.95E-01	*clpP*
-0.32	6.48E-01	*yflS*	-0.33	1.58E-01	*rsmA*
-0.32	4.27E-01	*rsbW*	-0.33	2.03E-01	*rnj2*
-0.32	5.59E-01	*miaB*	-0.33	2.96E-01	*hslU*
-0.33	5.77E-01	*ktrB_2*	-0.33	4.34E-01	*gtaB*
-0.33	5.13E-01	*uppP*	-0.33	4.51E-01	*sasA*
-0.33	5.55E-01	*rpsN2*	-0.34	5.23E-01	*tmcAL*
-0.33	5.47E-01	*cdaA*	-0.35	4.34E-01	*pyk*
-0.33	5.13E-01	*bceA_1*	-0.35	4.52E-01	*mtlD*
-0.33	3.86E-01	*mgrA*	-0.35	5.68E-01	*pheA*
-0.33	5.21E-01	*frdA*	-0.35	1.45E-01	*lyrA*
-0.34	3.03E-01	*nagB_1*	-0.36	5.39E-01	*ktrA*
-0.34	2.45E-01	*rnj2*	-0.36	2.38E-01	*treP_2*
-0.34	2.55E-01	*plsC*	-0.36	5.75E-01	*ymdB*
-0.34	4.00E-01	*queH*	-0.36	5.01E-01	*ymcA*
-0.34	2.95E-01	*rsuA*	-0.37	1.08E-01	*birA*
-0.34	4.99E-01	*glpF*	-0.37	4.10E-01	*tcaA*
-0.34	3.78E-01	*rny*	-0.37	2.57E-01	*murE*
-0.34	1.88E-01	*dnaE*	-0.37	4.52E-01	*rnmV_1*
-0.34	1.24E-01	*pbpH*	-0.37	5.23E-01	*mnhG1*
-0.35	7.57E-01	*lctP_1*	-0.37	1.43E-01	*plsC*
-0.35	3.52E-01	*truB*	-0.37	6.35E-02	*priA*
-0.36	2.53E-01	*gnd*	-0.38	6.91E-01	*ssl4_1*
-0.36	2.63E-01	*lysS*	-0.38	2.48E-01	*queG*
-0.36	4.79E-01	*mnmA*	-0.39	4.42E-01	*deoC1*
-0.36	8.14E-01	*psuG*	-0.40	1.21E-01	*yacP*
-0.36	1.83E-01	*rsmA*	-0.40	1.84E-01	*cdd*
-0.36	5.97E-01	*ykoE*	-0.40	5.85E-01	*trmL*
-0.36	2.41E-01	*gltT*	-0.40	3.40E-01	*norB_4*
-0.37	6.00E-01	*citN*	-0.40	3.74E-02	*tarS*
-0.37	2.40E-01	*glpG*	-0.40	5.26E-01	*essC*
-0.37	3.65E-01	*ydeN*	-0.41	6.76E-01	*cntC*
-0.38	3.72E-01	*fadR*	-0.41	2.31E-01	*tadA_2*
-0.38	2.22E-01	*gtfA_1*	-0.41	6.23E-01	*bceA_2*
-0.38	1.00E-01	*gyrB*	-0.41	6.23E-01	*opuD_1*
-0.38	1.18E-01	*mro*	-0.42	2.30E-01	*ydeN*
-0.38	8.73E-01	*lacC_1*	-0.42	3.64E-01	*pspB*
-0.38	6.94E-01	*lpl2_7*	-0.42	2.38E-01	*rbfA*
-0.39	2.54E-01	*nrnA*	-0.42	1.90E-01	*catE_1*
-0.39	7.60E-01	*lpl2_5*	-0.43	4.25E-01	*csd*
-0.39	4.40E-01	*murJ_1*	-0.43	7.49E-02	*thiK*
-0.40	4.21E-01	*dinG*	-0.43	2.00E-01	*uvrC*
-0.40	2.07E-01	*mvaA*	-0.43	1.87E-01	*moaA_2*

-0.40	2.30E-01	*hepT*	-0.44	2.16E-01	*ftsL*
-0.40	3.63E-01	*bepA_2*	-0.45	2.74E-01	*map_3*
-0.40	3.56E-01	*sepF*	-0.45	2.97E-01	*splD*
-0.40	5.02E-01	*recF*	-0.45	2.57E-01	*ponA*
-0.41	5.05E-01	*fabZ*	-0.45	8.86E-02	*gltT*
-0.41	4.32E-01	*norB_4*	-0.46	4.30E-01	*ecfT_2*
-0.42	4.02E-01	*xerD_1*	-0.46	4.09E-01	*esaA*
-0.42	2.54E-01	*thyA*	-0.46	2.82E-01	*sepA*
-0.43	5.45E-01	*bshB2*	-0.46	1.69E-01	*accD*
-0.43	5.21E-01	*yciC_1*	-0.47	8.27E-02	*efp*
-0.43	2.35E-01	*tsaB*	-0.47	2.90E-01	*hslO*
-0.43	1.97E-01	*murA1*	-0.47	4.10E-01	*rsbU_2*
-0.44	1.85E-01	*asnS*	-0.47	1.15E-01	*der_1*
-0.44	5.90E-01	*isdE*	-0.48	4.12E-01	*ribD*
-0.44	3.50E-01	*metK*	-0.48	7.96E-02	*truA*
-0.45	4.91E-01	*ywqG*	-0.48	2.82E-01	*pxpB_2*
-0.45	1.78E-01	*mutY*	-0.48	3.43E-01	*dgk*
-0.45	2.77E-01	*saeS*	-0.48	1.32E-02	*pcrA*
-0.45	3.08E-01	*rnj1*	-0.48	1.02E-01	*nupC_1*
-0.45	4.87E-02	*folE2*	-0.49	3.84E-01	*rbgA*
-0.45	3.38E-01	*bceB_1*	-0.49	3.43E-02	*map_2*
-0.45	4.75E-01	*esaA*	-0.49	3.04E-01	*cntI*
-0.45	2.93E-01	*sdhC*	-0.49	6.08E-01	*essB*
-0.45	2.38E-01	*proS*	-0.49	3.05E-01	*splA*
-0.46	3.20E-01	*crtN*	-0.49	7.39E-02	*recN*
-0.46	1.61E-01	*argR_1*	-0.50	3.28E-01	*sarV*
-0.46	6.10E-01	*thiM*	-0.50	8.14E-02	*hemL2*
-0.47	6.70E-01	*yagU*	-0.50	1.90E-01	*purR*
-0.47	1.69E-01	*yydK*	-0.50	1.55E-01	*fabH*
-0.48	2.64E-01	*topB*	-0.50	6.13E-01	*lpl2_6*
-0.48	4.24E-01	*tagO*	-0.50	1.65E-01	*guaA*
-0.48	4.50E-01	*arcB*	-0.50	2.72E-01	*est_2*
-0.48	7.72E-02	*thiK*	-0.51	1.81E-02	*purB*
-0.48	3.67E-01	*tagG*	-0.51	2.01E-01	*cmk*
-0.49	1.89E-01	*folK*	-0.51	2.74E-01	*codY*
-0.49	1.79E-01	*mnmE*	-0.51	2.12E-01	*deoB*
-0.49	5.52E-01	*ltaA*	-0.51	5.31E-01	*focA*
-0.49	6.54E-01	*ssl4_1*	-0.51	7.92E-02	*yydK*
-0.49	3.55E-01	*rsbU_1*	-0.51	3.28E-01	*obg*
-0.49	5.02E-01	*nreC*	-0.52	1.79E-01	*accA*
-0.49	1.28E-01	*alr1_1*	-0.52	3.89E-02	*menF*
-0.49	3.65E-01	*cspA_1*	-0.52	2.39E-01	*veg*
-0.49	4.98E-01	*mprF*	-0.52	7.22E-01	*lacC_1*
-0.49	1.80E-01	*mqo2*	-0.52	3.03E-01	*hrtA_2*
-0.49	5.91E-01	*splB*	-0.52	2.39E-01	*rnhA*
-0.50	3.13E-01	*alaS*	-0.53	2.02E-01	*yciC_2*
-0.50	5.12E-01	*arcA*	-0.53	5.94E-02	*hepT*
-0.51	1.42E-01	*darA*	-0.53	4.51E-01	*ssl7_2*
-0.51	5.46E-01	*secE*	-0.54	2.34E-01	*bioY*
-0.52	8.85E-01	*comGA*	-0.54	3.91E-01	*arcA*
-0.52	3.60E-01	*glpT*	-0.54	1.12E-01	*gcvPA*
-0.52	1.77E-01	*fni*	-0.54	6.01E-02	*nirQ*
-0.52	5.89E-01	*scdA*	-0.55	9.96E-02	*proS*
-0.52	5.02E-01	*tyrS*	-0.55	6.45E-02	*tsaB*
-0.53	3.74E-02	*scpA*	-0.55	7.37E-01	*lacB*
-0.53	1.13E-01	*era*	-0.55	2.01E-01	*nos*
-0.54	1.96E-01	*rnhB*	-0.55	2.93E-02	*yycI*
-0.54	3.30E-01	*mtlD*	-0.55	3.46E-02	*glpG*
-0.55	3.77E-01	*sarA*	-0.55	2.80E-03	*pbpH*
-0.55	3.95E-02	*queE_1*	-0.55	1.86E-01	*mnmA*
-0.55	2.58E-01	*gpmI*	-0.55	6.29E-02	*dnaC*
-0.55	1.76E-01	*gatC_1*	-0.55	1.82E-01	*tarM*
-0.56	2.08E-02	*nupG*	-0.56	3.41E-02	*mreC*
-0.56	2.27E-01	*yycJ*	-0.56	1.37E-01	*saeR*
-0.56	1.29E-01	*ltaS*	-0.56	1.25E-01	*atl_1*
-0.56	1.23E-01	*catE_1*	-0.56	1.36E-01	*acpP*

-0.56	1.34E-01	*sigB*	-0.56	4.19E-02	*cvfB*
-0.56	3.14E-01	*menG*	-0.57	5.25E-01	*ctsR*
-0.56	1.89E-01	*saeR*	-0.57	1.08E-01	*dgkA*
-0.56	5.97E-01	*map_1*	-0.57	2.54E-02	*gtfA_1*
-0.56	4.40E-01	*nikB*	-0.57	4.33E-01	*ftnA*
-0.56	1.56E-01	*rbfA*	-0.57	1.32E-01	*tsaE*
-0.57	9.13E-02	*ywiB*	-0.57	2.58E-01	*xpt*
-0.57	4.27E-01	*lnrL_3*	-0.57	3.07E-01	*nikB*
-0.57	6.37E-01	*wbjC*	-0.57	2.80E-02	*yhfS*
-0.58	4.39E-01	*nreB*	-0.58	9.95E-02	*saeS*
-0.58	2.35E-01	*setA*	-0.58	3.17E-01	*citN*
-0.58	4.31E-01	*lysP_1*	-0.58	1.18E-01	*rluD_1*
-0.58	3.68E-01	*pip*	-0.59	1.33E-01	*moeA*
-0.58	3.69E-01	*comEA*	-0.60	1.08E-01	*coaD*
-0.59	3.43E-01	*bcr_1*	-0.60	8.94E-02	*acpS*
-0.59	1.57E-01	*dgkA*	-0.60	1.50E-02	*pgk*
-0.59	1.05E-01	*moaA_2*	-0.61	7.52E-01	*cap8A_1*
-0.61	5.34E-02	*rlmCD*	-0.61	3.04E-01	*lnrL_3*
-0.61	2.09E-01	*nos*	-0.61	5.57E-02	*glmU*
-0.61	2.25E-01	*mrpA*	-0.61	1.13E-01	*pxpC_2*
-0.62	6.03E-02	*def1*	-0.62	6.50E-01	*sarU*
-0.62	4.22E-01	*mutS_1*	-0.63	1.91E-01	*nth_2*
-0.62	2.02E-02	*yrrB*	-0.64	7.64E-02	*sufU*
-0.62	1.08E-01	*gcvPA*	-0.64	2.32E-01	*trpG_2*
-0.63	5.11E-01	*fhuD_1*	-0.64	1.62E-01	*cspA_1*
-0.63	1.42E-01	*dck*	-0.65	4.19E-03	*murT*
-0.63	2.47E-01	*suhB_1*	-0.65	2.68E-01	*plsY*
-0.63	2.64E-01	*rsmH_1*	-0.65	4.74E-01	*essG_4*
-0.63	4.11E-01	*isdG_2*	-0.65	1.95E-01	*lctP_2*
-0.63	4.28E-01	*feuC*	-0.65	4.05E-02	*sigB*
-0.63	4.06E-01	*srtB*	-0.66	1.09E-01	*ssl1*
-0.64	5.33E-02	*treA*	-0.66	1.32E-01	*queF*
-0.64	4.25E-01	*trmL*	-0.66	3.68E-02	*ispE*
-0.65	5.11E-01	*prs_1*	-0.66	7.25E-02	*dck*
-0.65	4.83E-02	*der_1*	-0.66	3.49E-01	*ywqE_2*
-0.65	3.36E-03	*prs_2*	-0.66	2.70E-03	*whiA*
-0.65	1.95E-01	*mshD_1*	-0.67	1.68E-03	*scpA*
-0.65	1.41E-01	*cmk*	-0.67	6.62E-01	*lacD*
-0.66	3.78E-03	*hemY*	-0.67	2.94E-01	*ribE*
-0.66	6.62E-02	*bioB_2*	-0.67	3.03E-01	*lrgB*
-0.66	1.18E-02	*rnz*	-0.67	5.68E-02	*gcvT*
-0.67	7.49E-02	*mutL*	-0.67	3.21E-01	*aldC_1*
-0.67	4.51E-01	*ywqE_2*	-0.68	1.34E-03	*dnaE*
-0.67	5.35E-02	*divIB*	-0.68	5.03E-02	*rnhB*
-0.68	1.41E-01	*alsS*	-0.68	7.94E-01	*ywqD_1*
-0.68	7.33E-03	*ydjM*	-0.68	2.02E-02	*smc_1*
-0.68	5.95E-02	*auaG*	-0.68	5.41E-02	*gltX*
-0.68	9.53E-02	*gcvT*	-0.68	6.96E-02	*mnhD1_2*
-0.68	4.13E-01	*thiD*	-0.68	1.70E-01	*yojF*
-0.69	6.13E-02	*farB_2*	-0.68	2.67E-01	*ribH*
-0.70	2.68E-01	*srrA*	-0.69	4.37E-02	*gmuF*
-0.70	9.63E-02	*accA*	-0.69	2.39E-01	*arsC*
-0.70	7.20E-02	*gmuF*	-0.69	8.51E-02	*gapA2*
-0.70	3.16E-02	*qoxA*	-0.69	1.48E-02	*gcvPB*
-0.70	2.79E-02	*cdaR*	-0.69	4.01E-01	*mcsB*
-0.71	5.52E-04	*ffh*	-0.69	2.96E-01	*cntD*
-0.71	2.52E-02	*yhfS*	-0.69	2.48E-03	*lipA_1*
-0.71	1.05E-01	*pxpC_2*	-0.69	6.50E-02	*bglA*
-0.71	2.79E-03	*polC*	-0.70	1.08E-01	*zur*
-0.71	2.07E-01	*sarV*	-0.70	4.83E-03	*rho*
-0.71	4.53E-02	*argS*	-0.71	2.80E-02	*ybiV*
-0.72	3.76E-02	*glcK*	-0.71	1.22E-02	*glmM*
-0.72	2.85E-01	*fadA*	-0.71	4.63E-01	*bceB_2*
-0.72	2.11E-01	*obg*	-0.71	8.30E-03	*gpsA*
-0.72	1.83E-01	*hhaIM*	-0.72	9.46E-03	*def1*
-0.72	1.52E-01	*prmA*	-0.72	2.20E-02	*mutS2_2*

-0.72	8.19E-02	*purR*	-0.72	2.19E-01	*ribBA*
-0.72	6.03E-02	*hemA*	-0.72	5.75E-02	*pucK*
-0.72	2.06E-01	*nusB*	-0.72	1.54E-01	*pbuG*
-0.73	1.02E-01	*mvaS*	-0.72	1.91E-01	*srrA*
-0.73	1.69E-01	*sufC*	-0.73	1.21E-03	*oatA_2*
-0.73	3.99E-02	*queG*	-0.73	3.81E-01	*thiE*
-0.74	3.63E-01	*isdF*	-0.73	2.38E-01	*ptsG_1*
-0.74	9.66E-03	*ligA*	-0.73	1.18E-01	*menE_2*
-0.74	7.35E-02	*yhaP*	-0.73	3.15E-02	*sodM*
-0.74	1.07E-02	*efp*	-0.73	1.76E-02	*auaG*
-0.75	3.42E-02	*glmU*	-0.73	2.53E-01	*fib*
-0.76	5.96E-01	*mrpB*	-0.73	6.34E-02	*bceA_1*
-0.76	3.92E-03	*scpB*	-0.74	6.62E-02	*sdhA*
-0.76	6.48E-02	*fgd_2*	-0.75	6.55E-05	*folE2*
-0.77	4.96E-01	*ldhD_2*	-0.75	4.70E-02	*bioB_1*
-0.77	2.35E-01	*rnpA*	-0.75	8.37E-03	*fmt*
-0.77	3.38E-01	*rpoE*	-0.76	2.37E-02	*hemA*
-0.77	1.89E-01	*cspLA*	-0.76	1.34E-02	*argS*
-0.77	1.52E-02	*nupC_1*	-0.76	1.06E-02	*glcK*
-0.77	3.34E-02	*pgsA*	-0.76	2.24E-01	*cntF*
-0.77	2.85E-01	*ureG*	-0.76	1.01E-02	*mnmE*
-0.79	1.18E-01	*rsmD*	-0.77	4.99E-04	*scpB*
-0.79	7.15E-01	*cap8A_1*	-0.78	1.30E-01	*fba*
-0.80	2.41E-01	*crcB_2*	-0.78	2.67E-01	*rpoE*
-0.80	4.57E-02	*atl_1*	-0.78	2.97E-02	*tap*
-0.80	4.03E-02	*fruA_1*	-0.78	2.90E-01	*ssl4_3*
-0.80	3.13E-01	*epsL*	-0.78	3.55E-03	*ywiB*
-0.80	1.42E-01	*menE_2*	-0.78	1.43E-02	*mutL*
-0.81	1.01E-03	*mnmG*	-0.79	3.01E-02	*rnj1*
-0.81	1.74E-02	*murE*	-0.79	1.71E-01	*bshB2*
-0.81	5.47E-02	*ponA*	-0.79	2.52E-02	*bepA_2*
-0.81	1.34E-01	*mepA*	-0.79	4.20E-01	*bioK*
-0.82	8.34E-02	*cshB*	-0.80	4.28E-03	*era*
-0.82	1.76E-01	*purC*	-0.81	3.35E-02	*yhgF*
-0.82	1.33E-01	*uvrA*	-0.81	1.26E-05	*gyrB*
-0.83	1.16E-03	*oatA_2*	-0.81	9.43E-03	*pgsA*
-0.83	6.33E-02	*bglA*	-0.81	3.81E-01	*secY_2*
-0.83	1.74E-01	*ktrA*	-0.82	5.57E-02	*pbuE*
-0.83	5.46E-02	*ptsI*	-0.82	2.83E-01	*flr*
-0.84	8.64E-02	*ligJ*	-0.82	2.39E-01	*esxA*
-0.84	3.23E-02	*thrS*	-0.82	1.64E-01	*sigS*
-0.84	1.69E-02	*ispE*	-0.83	7.85E-03	*queH*
-0.84	1.04E-03	*lipA_1*	-0.83	6.87E-03	*rsbW*
-0.85	8.34E-02	*ribF*	-0.83	2.28E-01	*znuB*
-0.85	1.78E-02	*mutS2_1*	-0.83	1.89E-01	*cntE_1*
-0.85	4.02E-02	*sdrE*	-0.84	3.15E-02	*recQ_2*
-0.85	9.30E-02	*sbcC*	-0.84	1.82E-01	*yajC*
-0.85	8.51E-02	*rimI*	-0.85	2.41E-01	*yfmC_2*
-0.86	6.83E-01	*bioD*	-0.86	5.71E-03	*farB_2*
-0.86	2.21E-01	*nirC*	-0.86	2.22E-01	*arsB*
-0.87	8.13E-02	*zur*	-0.86	7.06E-03	*btuD_2*
-0.87	6.99E-03	*cdd*	-0.86	8.60E-02	*pip*
-0.87	1.97E-02	*garK_2*	-0.86	3.39E-03	*divIB*
-0.87	2.53E-02	*yabJ*	-0.86	3.10E-02	*setA*
-0.87	1.13E-02	*ruvA*	-0.87	6.47E-07	*ffh*
-0.87	3.93E-03	*srrB*	-0.88	9.22E-03	*sspP*
-0.89	7.17E-02	*lnrL_1*	-0.88	2.46E-02	*dinG*
-0.89	3.79E-02	*sbcD*	-0.88	7.83E-02	*murB*
-0.89	1.89E-01	*lip2*	-0.88	4.01E-03	*ltaS*
-0.89	7.01E-02	*splC*	-0.88	1.64E-01	*isdG_2*
-0.89	2.35E-01	*rocD2_2*	-0.88	2.55E-01	*mrpC*
-0.90	3.37E-01	*accB_1*	-0.88	4.01E-02	*rsmD*
-0.90	2.90E-01	*ssl7_2*	-0.88	7.79E-04	*trmB*
-0.90	7.36E-03	*treP_2*	-0.89	8.63E-03	*yabJ*
-0.90	1.47E-01	*trpG_2*	-0.89	4.38E-02	*rplO*
-0.91	1.83E-01	*ansA*	-0.89	2.95E-06	*hemY*

-0.91	2.79E-02	*tap*	-0.89	5.35E-04	*rlmCD*
-0.91	4.55E-03	*fmt*	-0.89	8.74E-02	*comEA*
-0.91	5.56E-02	*yciC_2*	-0.89	7.68E-02	*ptsH*
-0.92	1.97E-01	*yajC*	-0.90	1.49E-02	*sbcD*
-0.92	5.94E-03	*treR_2*	-0.90	1.50E-02	*cfiB*
-0.92	7.12E-02	*glcU_1*	-0.90	1.66E-01	*bioW*
-0.92	1.82E-01	*comFA*	-0.90	2.12E-04	*ligA*
-0.93	6.01E-02	*noc*	-0.91	3.38E-01	*nikA*
-0.93	7.06E-01	*leuA_3*	-0.92	8.26E-02	*yciC_1*
-0.94	5.88E-02	*yclN*	-0.92	7.91E-03	*yhaP*
-0.94	1.21E-01	*ecfT_2*	-0.92	2.48E-01	*ndhB*
-0.94	3.53E-01	*crcB_1*	-0.92	1.18E-01	*mprF*
-0.94	3.47E-02	*mnhD1_2*	-0.93	2.85E-01	*qoxD*
-0.94	1.66E-03	*gpsA*	-0.93	5.50E-03	*gatD_1*
-0.95	8.88E-03	*parE*	-0.93	2.41E-02	*lnrL_1*
-0.95	9.41E-02	*trmFO*	-0.93	8.98E-02	*mrpE*
-0.95	4.75E-03	*hflX*	-0.94	1.25E-01	*scn_2*
-0.96	1.79E-02	*rluD_1*	-0.94	1.16E-03	*hflX*
-0.97	1.09E-01	*pckA*	-0.95	1.53E-01	*ltaA*
-0.97	6.80E-02	*gyrA*	-0.95	4.37E-07	*prs_2*
-0.98	3.28E-02	*pchA*	-0.96	1.76E-02	*frdA*
-0.98	7.33E-02	*csd*	-0.96	2.23E-02	*ribF*
-0.99	5.69E-04	*mtaB*	-0.96	2.14E-06	*polC*
-0.99	3.13E-01	*cntL*	-0.96	1.81E-02	*fmtA*
-0.99	6.11E-03	*rsfS*	-0.96	2.15E-03	*rsfS*
-0.99	2.64E-02	*ndk*	-0.96	7.06E-02	*rpsU*
-1.01	5.93E-02	*rluB*	-0.96	3.87E-02	*atpE*
-1.01	5.36E-03	*xseA*	-0.96	1.33E-01	*xseB*
-1.02	9.50E-04	*rsmB*	-0.97	2.00E-02	*cdaA*
-1.02	7.43E-02	*ptsH*	-0.97	5.10E-02	*trmFO*
-1.02	2.44E-01	*focA*	-0.98	2.46E-03	*prfC*
-1.02	7.55E-03	*sspP*	-0.98	8.97E-02	*fhuD_2*
-1.03	8.95E-03	*nusG*	-0.98	2.97E-02	*alsT*
-1.04	7.10E-03	*ybiV*	-0.99	7.55E-04	*thyA*
-1.04	4.49E-01	*hlgC*	-0.99	1.69E-03	*arcR*
-1.04	1.08E-02	*greA*	-0.99	8.04E-04	*nnrD*
-1.04	1.40E-02	*yhgF*	-1.00	4.69E-02	*ywqG*
-1.06	8.39E-07	*recD2*	-1.00	4.64E-02	*entA*
-1.07	2.81E-05	*glpP*	-1.00	4.55E-02	*sarA*
-1.07	2.50E-02	*dnaJ*	-1.00	6.79E-04	*ruvA*
-1.08	7.35E-03	*coaD*	-1.01	9.50E-03	*accB_2*
-1.08	2.27E-02	*ruvB_1*	-1.01	1.72E-01	*lpl2_7*
-1.09	2.11E-03	*fabF*	-1.01	4.73E-08	*tpiA*
-1.09	5.08E-02	*fba*	-1.01	1.71E-01	*splB*
-1.10	5.79E-05	*ftsK*	-1.01	9.25E-03	*ndk*
-1.10	1.84E-04	*cvfB*	-1.02	2.79E-05	*recO*
-1.11	5.39E-05	*yabA*	-1.02	1.89E-01	*sarT*
-1.11	4.43E-09	*ackA*	-1.03	1.07E-03	*apt*
-1.12	1.74E-02	*arcD*	-1.03	9.10E-03	*splC*
-1.13	9.53E-02	*qoxB*	-1.03	1.15E-03	*xseA*
-1.14	1.29E-03	*ftsY*	-1.03	1.01E-05	*pnp_1*
-1.14	8.22E-02	*rpmJ*	-1.04	3.86E-02	*nikC*
-1.14	1.62E-03	*apt*	-1.05	5.55E-06	*yabA*
-1.15	8.96E-02	*yfiY*	-1.06	4.28E-03	*yycJ*
-1.15	1.95E-01	*flr*	-1.06	6.52E-05	*cdaR*
-1.16	1.06E-05	*pnp_1*	-1.06	1.10E-02	*sdrM*
-1.16	3.24E-02	*pbuG*	-1.06	6.75E-05	*rsmB*
-1.17	1.92E-02	*menB*	-1.07	2.84E-01	*mrpB*
-1.17	1.94E-02	*cntI*	-1.07	1.03E-05	*ung*
-1.17	3.50E-01	*sbnD*	-1.07	8.63E-03	*mshD_1*
-1.17	4.28E-03	*cfiB*	-1.07	1.98E-03	*pdxT*
-1.17	8.52E-02	*fhuD_2*	-1.08	4.26E-03	*mvaS*
-1.19	1.26E-04	*gcvPB*	-1.08	3.39E-03	*ptsI*
-1.19	6.17E-02	*ymdB*	-1.08	5.25E-02	*comFA*
-1.19	1.38E-06	*murT*	-1.08	3.14E-04	*mutS2_1*
-1.20	9.18E-02	*cntA*	-1.08	5.73E-04	*parE*

-1.21	2.11E-01	*opuD_1*	-1.09	4.98E-02	*mtlA*
-1.21	1.17E-02	*arcC2*	-1.09	7.46E-02	*thiM*
-1.22	1.01E-05	*recO*	-1.09	8.82E-05	*leuS*
-1.23	1.37E-05	*ctaB2*	-1.09	1.01E-04	*udk*
-1.23	4.90E-04	*tsaD*	-1.09	5.08E-04	*lytR*
-1.24	6.07E-04	*lepA*	-1.09	2.68E-07	*yrrB*
-1.24	2.73E-02	*qoxC*	-1.09	1.33E-03	*sdhC*
-1.24	4.61E-03	*gapA2*	-1.10	1.58E-02	*tuf*
-1.25	1.10E-01	*isdA*	-1.10	1.47E-04	*ychF*
-1.25	1.91E-01	*qoxD*	-1.10	1.71E-02	*rluB*
-1.25	1.78E-01	*isdG_1*	-1.11	2.72E-02	*rpsK*
-1.26	4.90E-04	*prfC*	-1.11	9.95E-03	*sbcC*
-1.26	1.03E-03	*sufU*	-1.11	3.18E-02	*glpK*
-1.26	3.69E-03	*accB_2*	-1.11	3.52E-03	*yhhQ*
-1.26	1.04E-06	*secDF*	-1.12	1.25E-01	*prs_1*
-1.27	1.21E-05	*ung*	-1.12	5.96E-03	*pdxS*
-1.27	4.28E-01	*phnE_1*	-1.12	1.21E-01	*phoB*
-1.27	3.47E-02	*tcaR*	-1.13	2.96E-02	*rplQ*
-1.27	6.33E-02	*fib*	-1.13	1.54E-02	*menA*
-1.27	2.60E-04	*atpH*	-1.13	7.96E-03	*ybiT*
-1.27	9.67E-03	*hemW*	-1.13	1.96E-03	*bceB_1*
-1.29	6.00E-06	*murC*	-1.14	1.01E-05	*srrB*
-1.29	2.20E-06	*bfmBAA*	-1.15	2.93E-02	*adk*
-1.29	4.66E-03	*clpX*	-1.16	2.39E-02	*rpoA*
-1.29	3.24E-05	*leuS*	-1.17	2.92E-02	*secY_1*
-1.30	4.70E-02	*nikC*	-1.17	3.91E-03	*rimI*
-1.30	6.44E-05	*ychF*	-1.18	7.42E-03	*infA*
-1.31	2.01E-01	*thiE*	-1.18	2.68E-07	*rlmN*
-1.31	4.39E-10	*gatB_1*	-1.19	3.71E-03	*rpsN2*
-1.31	2.03E-04	*yfiC*	-1.20	5.67E-03	*menB*
-1.31	3.85E-04	*hemL1*	-1.21	2.52E-04	*thrS*
-1.31	2.00E-03	*ssaA*	-1.21	2.30E-01	*ywqD_2*
-1.32	1.08E-02	*stp*	-1.21	6.87E-03	*fapR*
-1.32	3.15E-03	*yhhQ*	-1.21	2.13E-02	*rnpA*
-1.32	2.63E-04	*arcR*	-1.21	1.91E-03	*gpmI*
-1.32	9.05E-03	*fapR*	-1.22	4.31E-04	*ytkD*
-1.32	1.95E-01	*cntM*	-1.22	1.33E-02	*qoxC*
-1.34	6.17E-05	*atpF*	-1.23	2.75E-04	*nusG*
-1.34	1.45E-02	*murB*	-1.23	1.25E-01	*map_1*
-1.34	8.83E-03	*tam*	-1.24	2.88E-11	*recD2*
-1.34	6.33E-06	*rsmI*	-1.25	7.68E-04	*ssaA*
-1.35	3.07E-05	*rsmE*	-1.25	2.22E-02	*wbnH*
-1.35	9.57E-10	*dnaG*	-1.26	6.13E-05	*garK_2*
-1.36	1.74E-03	*recU*	-1.26	3.55E-06	*qoxA*
-1.36	2.67E-07	*gatA*	-1.27	1.63E-14	*ackA*
-1.37	2.13E-04	*gatD_1*	-1.27	4.78E-03	*tam*
-1.37	3.42E-03	*sdrM*	-1.28	1.82E-08	*secDF*
-1.38	7.77E-08	*pdhC_1*	-1.28	7.68E-04	*recU*
-1.38	1.09E-04	*btuD_2*	-1.28	3.03E-01	*mrpF*
-1.39	3.21E-02	*arsC*	-1.28	1.06E-05	*atpC*
-1.39	3.49E-02	*mrpE*	-1.28	5.10E-02	*secE*
-1.40	7.65E-03	*yfmC_1*	-1.28	9.59E-03	*rpmG2_1*
-1.40	2.91E-03	*queF*	-1.28	2.12E-03	*noc*
-1.40	1.66E-03	*trmK*	-1.29	4.57E-10	*mnmG*
-1.40	8.24E-06	*udk*	-1.30	9.66E-04	*cshB*
-1.40	8.00E-08	*rlmN*	-1.30	1.41E-05	*smpB*
-1.40	3.62E-02	*dltC*	-1.31	2.47E-02	*nreB*
-1.40	8.73E-05	*fabH*	-1.31	1.02E-01	*ssl4_2*
-1.41	1.59E-05	*nfo*	-1.31	3.41E-10	*glpP*
-1.41	7.46E-02	*yfmC_2*	-1.32	4.31E-10	*coaW*
-1.42	2.78E-03	*iscS_1*	-1.33	5.59E-06	*atpF*
-1.42	3.28E-02	*scn_2*	-1.33	2.19E-05	*lepA*
-1.42	3.16E-04	*pbpB*	-1.34	3.50E-02	*scn_1*
-1.42	5.97E-02	*ctaA*	-1.34	5.79E-09	*atpB*
-1.43	5.59E-04	*glmS*	-1.34	1.02E-05	*rnr*
-1.43	5.31E-02	*znuB*	-1.35	1.08E-08	*ftsK*

-1.44	1.55E-06	*atpA*	-1.35	8.56E-06	*tsaD*
-1.46	2.96E-03	*est_1*	-1.35	3.68E-08	*ctaB2*
-1.47	2.17E-01	*tdcB*	-1.35	9.66E-04	*arcC2*
-1.47	1.45E-08	*atpB*	-1.36	2.36E-06	*nfo*
-1.48	2.56E-03	*parC*	-1.37	5.83E-06	*ftsY*
-1.48	2.73E-03	*alsT*	-1.38	7.19E-04	*ruvB_1*
-1.48	1.86E-03	*rplO*	-1.38	5.56E-03	*rpsE*
-1.49	2.45E-03	*infA*	-1.38	2.95E-02	*cap8A_2*
-1.49	9.36E-03	*glpK*	-1.39	1.20E-08	*mtaB*
-1.49	2.89E-09	*ptpB*	-1.40	3.19E-02	*rpmA*
-1.50	3.31E-03	*plc*	-1.40	4.43E-05	*alsS*
-1.51	1.04E-03	*pbuE*	-1.41	1.14E-02	*rpmJ*
-1.51	3.64E-10	*prkC*	-1.42	1.55E-03	*uvrA*
-1.52	8.34E-03	*rpmH*	-1.42	5.54E-04	*iscS_1*
-1.52	4.80E-02	*sbnE*	-1.42	4.01E-03	*rpsM*
-1.53	4.10E-04	*sdhA*	-1.42	2.23E-01	*leuA_2*
-1.53	7.38E-06	*rnr*	-1.42	9.09E-03	*rpsO*
-1.54	2.29E-01	*narT*	-1.43	3.24E-04	*clpX*
-1.54	6.89E-06	*yqeH*	-1.44	2.33E-03	*nagP*
-1.55	4.28E-05	*queA*	-1.44	1.91E-07	*yqeN*
-1.55	2.21E-03	*atpE*	-1.45	2.72E-04	*valS*
-1.56	1.32E-03	*rpsT*	-1.46	6.77E-04	*est_1*
-1.56	8.15E-02	*cntK*	-1.46	1.31E-02	*thiD*
-1.56	2.16E-09	*tig*	-1.47	9.30E-04	*rpmD*
-1.57	2.76E-02	*xseB*	-1.47	3.79E-04	*plc*
-1.57	3.47E-02	*fecD*	-1.47	8.59E-06	*atpD*
-1.57	5.24E-04	*bfmBAB*	-1.48	4.37E-05	*glmS*
-1.57	8.08E-04	*ybiT*	-1.49	1.22E-10	*gatA*
-1.58	9.43E-03	*plsY*	-1.49	3.33E-06	*gatC_1*
-1.58	3.74E-03	*nadD*	-1.49	4.20E-02	*chp*
-1.58	1.64E-06	*nnrD*	-1.49	6.89E-08	*mscS*
-1.59	1.00E-01	*wbpI*	-1.50	1.25E-08	*atpA*
-1.59	2.57E-03	*sbnF*	-1.51	1.81E-10	*bfmBAA*
-1.60	1.92E-06	*trmR*	-1.51	7.35E-04	*gyrA*
-1.60	9.21E-07	*dltA_1*	-1.52	2.92E-02	*hel*
-1.60	1.53E-09	*rho*	-1.53	1.98E-01	*hlgA*
-1.60	1.37E-06	*tilS*	-1.53	1.08E-07	*plsX*
-1.60	1.93E-03	*lctP_2*	-1.55	1.78E-07	*yfiC*
-1.61	4.28E-03	*rpoA*	-1.55	6.82E-03	*nirC*
-1.62	4.07E-07	*yqeN*	-1.55	1.76E-02	*ctaA*
-1.62	5.79E-03	*secY_1*	-1.55	3.18E-06	*queA*
-1.63	3.36E-03	*rpsM*	-1.55	6.78E-10	*rsmI*
-1.64	4.95E-07	*atpC*	-1.55	7.58E-08	*tgt*
-1.64	3.86E-03	*rpmF*	-1.56	1.40E-08	*rsmE*
-1.64	4.55E-03	*opuD_3*	-1.56	3.82E-05	*rpsG*
-1.65	1.48E-03	*engB*	-1.56	8.02E-02	*opuCD*
-1.65	4.63E-07	*tgt*	-1.58	8.44E-07	*rpsL*
-1.66	4.20E-03	*adk*	-1.58	1.52E-03	*opuD_3*
-1.66	2.24E-02	*cntB*	-1.58	1.61E-04	*hemW*
-1.66	3.44E-04	*galK*	-1.58	2.84E-03	*rplF*
-1.66	2.26E-09	*mtnN*	-1.58	3.68E-04	*stp*
-1.66	2.06E-08	*pdhA*	-1.58	3.31E-08	*dltA_1*
-1.67	2.00E-13	*sigA*	-1.58	1.44E-07	*atpH*
-1.67	7.50E-06	*rsmG*	-1.59	3.35E-03	*nreC*
-1.67	8.06E-06	*yhbY*	-1.60	6.59E-07	*fabG*
-1.67	2.79E-02	*nupC_2*	-1.60	1.76E-04	*parC*
-1.67	7.91E-07	*smpB*	-1.61	1.18E-03	*rpmF*
-1.68	3.31E-04	*bioY*	-1.61	2.80E-03	*dnaK*
-1.68	9.73E-02	*pflA*	-1.63	1.99E-08	*atpG*
-1.68	6.48E-02	*yfiZ_2*	-1.63	2.12E-05	*fusA*
-1.68	2.11E-03	*rpsK*	-1.63	1.54E-02	*sceD*
-1.68	2.56E-07	*plsX*	-1.64	4.72E-12	*mtnN*
-1.69	2.50E-07	*atpG*	-1.64	3.23E-03	*grpE*
-1.70	4.70E-01	*lacB*	-1.64	1.46E-06	*fabD*
-1.70	6.46E-08	*yidC*	-1.64	3.58E-08	*yqeH*
-1.71	2.76E-02	*cap8A_2*	-1.64	4.08E-03	*dltC*

-1.71	5.11E-04	*scn_3*	-1.65	2.64E-15	*ptpB*
-1.72	1.04E-03	*rlhA*	-1.66	2.36E-09	*yidC*
-1.72	2.40E-02	*norB_3*	-1.66	4.23E-03	*potA*
-1.73	5.23E-08	*mscS*	-1.68	8.05E-05	*ywlC*
-1.74	1.84E-08	*tsf*	-1.68	5.42E-07	*catD*
-1.75	2.32E-06	*atpD*	-1.68	3.69E-20	*gatB_1*
-1.75	1.06E-03	*nagP*	-1.68	2.94E-05	*prmA*
-1.76	2.66E-03	*patA*	-1.68	6.47E-02	*tenA*
-1.78	2.16E-02	*lacC_2*	-1.70	6.53E-07	*pbpB*
-1.80	3.01E-02	*sceD*	-1.71	6.06E-02	*nikD*
-1.81	1.86E-07	*hemC*	-1.71	4.58E-01	*esaB*
-1.81	9.40E-14	*coaW*	-1.72	1.83E-05	*dnaJ*
-1.82	8.12E-02	*cidA*	-1.72	7.30E-03	*ribU*
-1.82	3.44E-02	*yclP*	-1.73	1.76E-02	*scdA*
-1.83	4.39E-08	*pdhB*	-1.74	1.44E-06	*fpgS*
-1.83	4.05E-08	*hemB*	-1.74	2.15E-03	*qoxB*
-1.84	3.47E-04	*menA*	-1.76	7.32E-06	*bfmBAB*
-1.84	2.68E-05	*sbnI*	-1.77	5.06E-05	*thiI*
-1.85	1.39E-06	*fabD*	-1.78	1.19E-09	*ileS*
-1.86	3.33E-08	*thrB_2*	-1.78	3.90E-04	*rpsR*
-1.86	9.10E-04	*rplQ*	-1.78	2.77E-03	*tyrS*
-1.86	2.32E-05	*valS*	-1.79	2.51E-09	*queE_2*
-1.88	1.37E-04	*rpmD*	-1.79	1.69E-17	*prkC*
-1.91	4.40E-09	*menD*	-1.82	3.86E-06	*rsmC*
-1.91	1.14E-02	*yclO*	-1.82	2.53E-02	*phnE_2*
-1.91	3.33E-08	*dltD*	-1.82	1.73E-15	*tig*
-1.94	1.63E-02	*yfiZ_1*	-1.83	1.35E-08	*rsmG*
-1.94	8.25E-08	*fabG*	-1.83	2.05E-09	*dltD*
-1.95	1.43E-09	*pdhC_2*	-1.85	2.87E-05	*rpsH*
-1.96	1.84E-02	*ndhB*	-1.86	1.11E-12	*pdhA*
-1.96	1.01E-02	*pyrD*	-1.86	2.96E-02	*puuR*
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-1.99	3.08E-03	*sigS*	-1.86	2.21E-02	*lctP_1*
-1.99	4.10E-03	*scn_1*	-1.88	9.97E-03	*emp*
-1.99	5.96E-05	*thiI*	-1.88	6.15E-06	*rplR*
-1.99	1.16E-11	*mdrP_1*	-1.89	2.13E-04	*patA*
-1.99	1.50E-14	*frr*	-1.89	8.92E-14	*mdrP_1*
-2.00	8.45E-06	*rsmC*	-1.89	1.46E-21	*sigA*
-2.00	1.02E-15	*hpt*	-1.90	2.21E-02	*cidA*
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-2.02	1.38E-07	*pdhD*	-1.90	9.40E-10	*yhbY*
-2.03	2.49E-01	*lacD*	-1.91	8.60E-13	*rluD_2*
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-2.04	2.37E-15	*uppS*	-1.92	1.78E-04	*potB*
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-2.06	1.26E-07	*menH*	-1.95	7.83E-05	*rpmH*
-2.06	5.15E-05	*odh*	-1.96	8.71E-07	*rpsI*
-2.07	1.07E-04	*sbnH*	-1.96	1.43E-03	*groL*
-2.09	2.95E-05	*rpsP*	-1.96	3.26E-02	*clpB*
-2.11	1.18E-07	*pyrH*	-1.96	1.39E-06	*queD*
-2.11	3.98E-04	*rpsO*	-1.97	4.99E-07	*mleN_2*
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-2.17	7.65E-06	*tuf*	-1.99	1.16E-11	*pdhB*
-2.18	1.84E-08	*pheT*	-1.99	2.75E-14	*aroE*
-2.19	4.98E-13	*aroE*	-2.00	2.43E-12	*pdhC_2*
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-2.22	3.69E-04	*lspA*	-2.03	1.96E-03	*treP_1*
-2.23	4.97E-04	*cntE_2*	-2.04	7.81E-07	*rplE*
-2.24	1.94E-04	*entA*	-2.05	3.65E-20	*uppS*
-2.25	1.37E-05	*pheS*	-2.05	2.16E-04	*rplU*
-2.30	2.74E-07	*rpsI*	-2.05	6.03E-10	*rplK*
-2.30	3.75E-04	*cntE_1*	-2.06	1.24E-09	*pdhD*

-2.31	1.63E-05	*rpsE*	-2.07	3.21E-06	*engB*
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-2.33	6.26E-05	*rplF*	-2.07	7.20E-07	*rpsZ*
-2.33	9.98E-07	*ywlC*	-2.08	3.34E-06	*odh*
-2.35	2.14E-11	*rplGB*	-2.08	6.01E-08	*rplX*
-2.35	1.21E-25	*phaB*	-2.09	3.68E-04	*ydcV*
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-2.48	8.16E-05	*garL*	-2.22	2.85E-07	*brnQ_1*
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-2.53	1.68E-09	*fusA*	-2.27	1.55E-05	*cntE_2*
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-2.71	1.17E-14	*rpsL*	-2.40	1.07E-09	*galK*
-2.71	1.16E-23	*nusA*	-2.40	7.89E-16	*hemC*
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-2.74	4.73E-07	*hisS*	-2.46	1.14E-11	*pyrG*
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-2.76	6.44E-06	*rplU*	-2.48	5.12E-28	*frr*
-2.78	6.30E-14	*catD*	-2.48	5.67E-09	*rpsF*
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-2.83	1.12E-04	*treP_1*	-2.50	6.73E-11	*isaA*
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-3.06	8.31E-06	*rpmA*	-2.71	1.42E-10	*rplJ*
-3.07	2.38E-12	*aspS*	-2.71	2.95E-06	*lrgA*

-3.15	4.08E-12	*rplA*	-2.74	6.13E-49	*phaB*
-3.16	3.66E-09	*fnbB*	-2.74	1.06E-16	*menH*
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-3.21	1.18E-19	*rpsD*	-2.75	2.73E-06	*ythA*
-3.21	5.05E-11	*rplC*	-2.77	6.85E-18	*tdk*
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-3.29	1.45E-12	*rpmC*	-2.80	1.17E-41	*infB*
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-3.37	6.47E-05	*yclQ*	-2.81	1.19E-10	*rplC*
-3.49	1.16E-10	*rpsR*	-2.82	5.29E-24	*prfA*
-3.50	9.98E-07	*fnbA*	-2.86	6.59E-04	*ldhD_2*
-3.51	1.28E-13	*rpsF*	-2.87	1.79E-10	*ssaA2*
-3.53	4.67E-12	*rplD*	-2.94	6.93E-21	*rpsD*
-3.56	5.68E-12	*ssbA_2*	-2.95	2.65E-08	*rplT*
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-3.60	6.50E-07	*rpmI*	-3.01	2.48E-10	*fnbB*
-3.62	2.10E-11	*potD*	-3.04	3.62E-13	*opuD_2*
-3.62	3.97E-16	*pyrR*	-3.08	1.54E-23	*rplGA*
-3.63	2.83E-09	*sbi*	-3.10	1.52E-06	*rpmI*
-3.64	2.74E-02	*leuA_2*	-3.10	2.80E-07	*nucH*
-3.65	1.23E-32	*ptsG_3*	-3.10	5.71E-11	*rpsJ*
-3.69	9.88E-15	*rplP*	-3.11	1.48E-14	*scn_3*
-3.70	5.01E-08	*isdB*	-3.14	1.10E-13	*guaC*
-3.72	2.69E-15	*rplJ*	-3.16	3.04E-14	*infC*
-3.73	3.24E-10	*rplT*	-3.28	4.03E-28	*rpsB*
-3.73	2.20E-02	*nasE*	-3.37	5.00E-21	*purA*
-3.76	9.87E-16	*infC*	-3.45	5.90E-08	*pyrD*
-3.76	1.92E-15	*cshA*	-3.45	2.10E-16	*cshA*
-3.81	3.96E-09	*ydcV*	-3.72	3.09E-09	*fnbA*
-3.89	1.33E-15	*rplL*	-3.77	2.72E-44	*ptsG_3*
-3.96	4.86E-16	*rimM*	-4.02	7.33E-32	*pyrE*
-3.99	6.55E-23	*hlb_1*	-4.03	1.29E-18	*pyrF*
-4.00	1.81E-12	*trmD*	-4.07	6.01E-26	*pyrR*
-4.23	1.26E-04	*mrpC*	-4.08	2.57E-07	*pflA*
-4.27	2.90E-16	*pyrF*	-4.15	1.38E-23	*rimM*
-4.37	1.37E-22	*carB*	-4.21	1.75E-17	*trmD*
-4.37	4.97E-28	*pyrE*	-4.28	1.87E-27	*carB*
-4.55	7.75E-21	*carA_1*	-4.40	4.16E-06	*narT*
-4.59	1.94E-12	*lrgA*	-4.72	5.38E-19	*pyrB*
-4.75	1.69E-16	*pyrC*	-4.82	1.21E-07	*tdcB*
-5.06	7.86E-24	*ssaA2*	-4.94	4.40E-32	*carA_1*
-5.21	3.82E-17	*pyrB*	-5.11	2.37E-24	*pyrC*
-5.21	3.80E-21	*pyrP*	-5.23	6.44E-29	*pyrP*
-5.75	1.09E-10	*ssl5_1*	-5.29	1.54E-11	*ssl5_1*

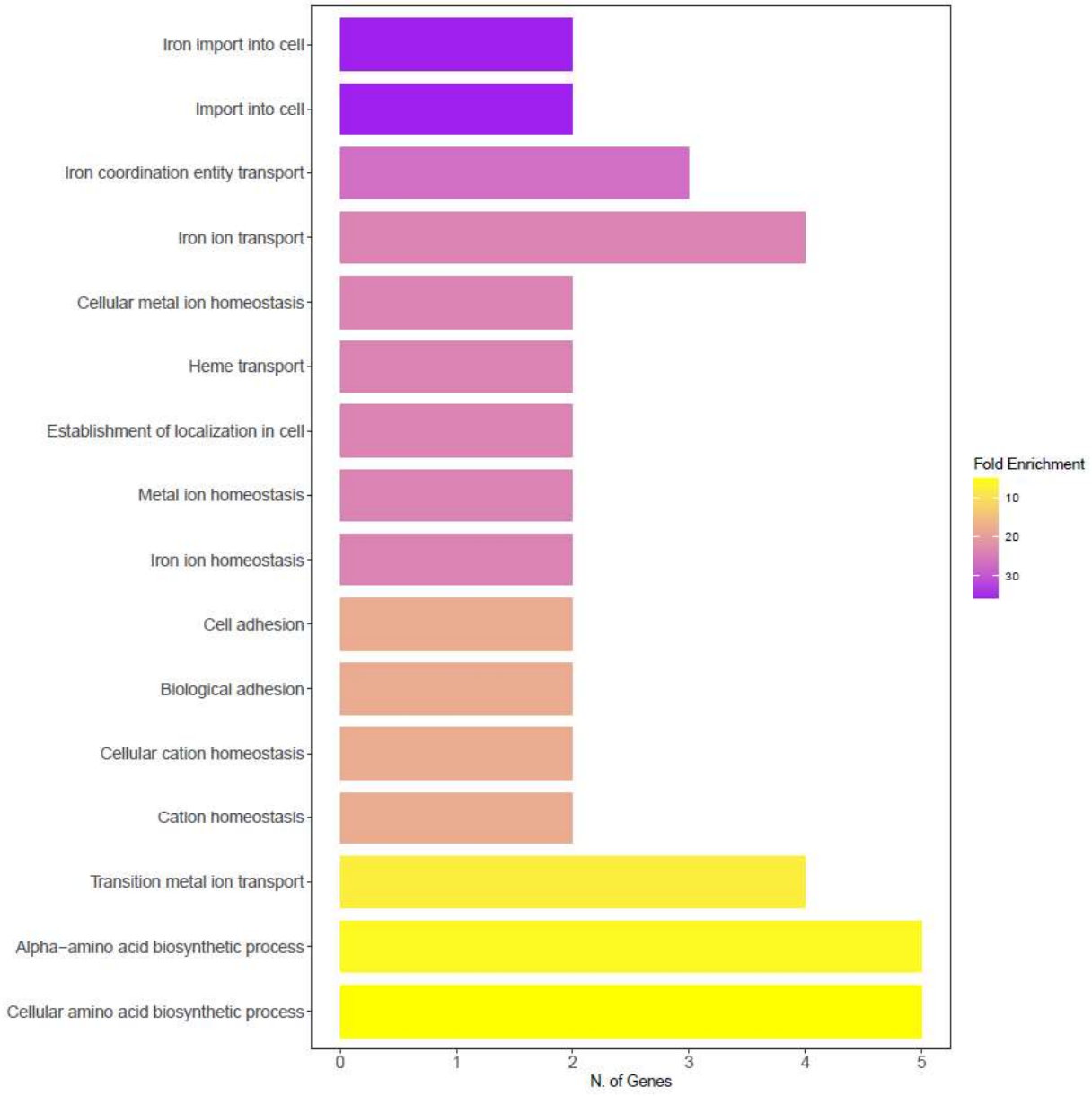

Figure S1: Top 16 significantly enriched GO terms involved in biological process analyzed from differentially expressed genes uniquely upregulated at 3h of exposure to host cells. *Genes were significantly involved (FDR < 0.05) in the respective pathways detected by GO enrichment analysis.*

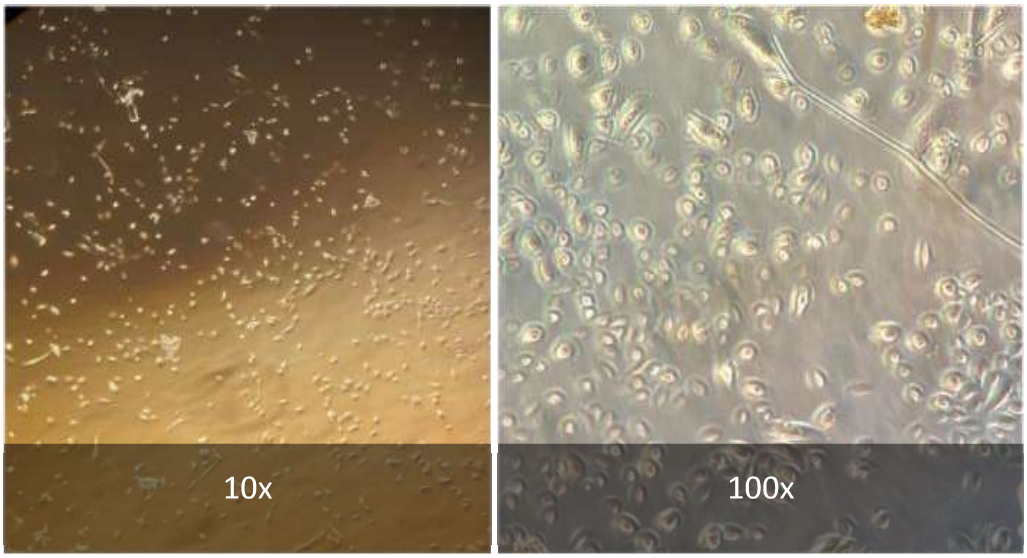

Figure S2: Microscopic view of Human Tonsil Epithelial Cells (HTEpiC) at 10x and 100x magnifications*. Images shows healthy and dividing HTEpiC at passage 4, which is ready to be infected.*

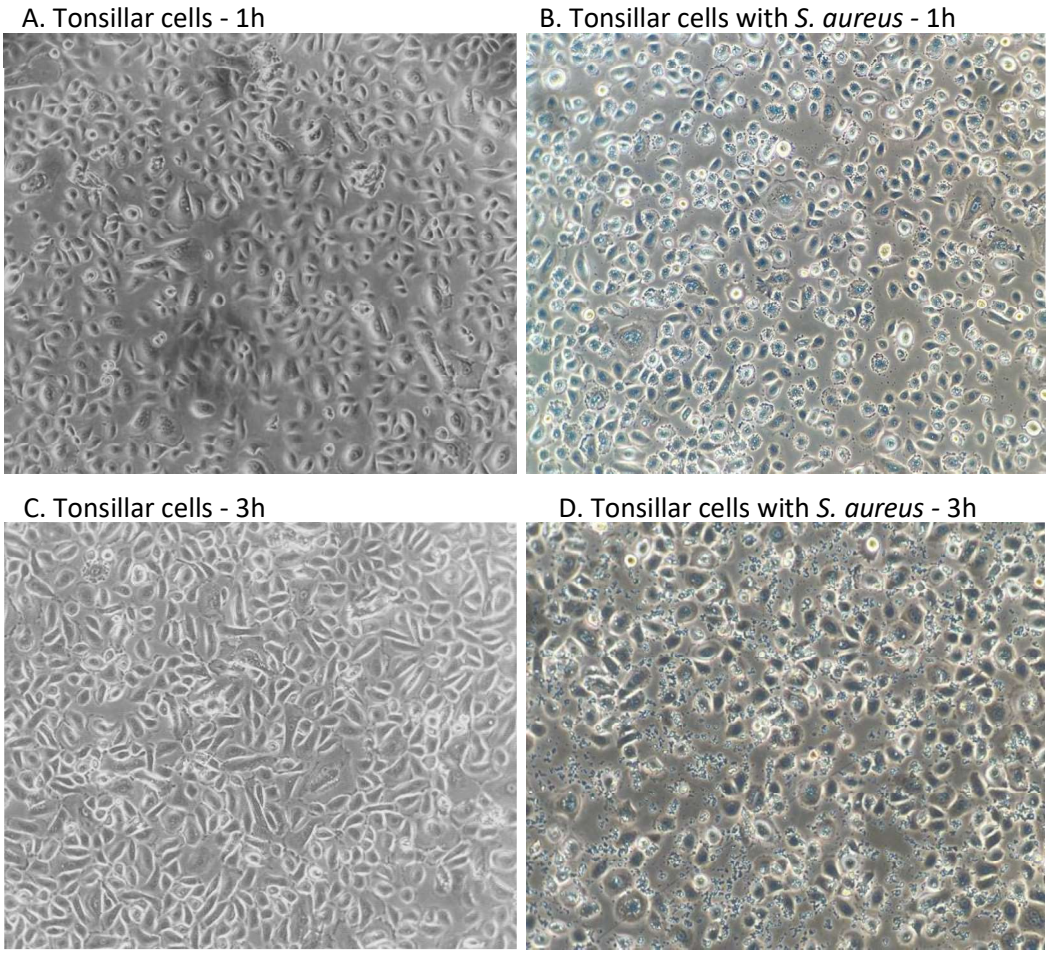

Figure S3: Microscopic view of Human Tonsil Epithelial Cells (HTEpiC) in the absence or presence of S. aureus. A) *Tonsillar cells incubated for 1h in the absence of S. aureus.* ***B)*** *Tonsillar cells incubated for 1h of in the presence of S. aureus.* ***C)*** *Tonsillar cells incubated for 3h in the absence of S. aureus.* ***D)*** *Tonsillar cells incubated for 3h with S. aureus. Bacteria are seen as dots or aggregates in B and D.*

Paper II

Co-culturing with *Streptococcus anginosus* alters *Staphylococcus aureus* transcriptome when exposed to tonsillar cells

Srijana Bastakoti*, Maiju Pesonen, Clement Ajayi, Kjersti Julin, Jukka Corander, Mona Johannessen, Anne-Merethe Hanssen*

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EDITED BY
Simon Clarke,
University of Reading, United Kingdom

REVIEWED BY
Atmika Paudel,
Hokkaido University, Japan
Fangchao Song,
Lawrence Livermore National Laboratory (DOE), United States

*CORRESPONDENCE
Srijana Bastakoti
Srijana.bastakoti@uit.no
Anne-Merethe Hanssen
anne-merethe.hanssen@uit.no

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Co-culturing with *Streptococcus anginosus* alters *Staphylococcus aureus* transcriptome when exposed to tonsillar cells

Srijana Bastakoti[1]*, Maiju Pesonen[2], Clement Ajayi[1], Kjersti Julin[1], Jukka Corander[3,4,5], Mona Johannessen[1] and Anne-Merethe Hanssen[1]*

[1]Department of Medical Biology, Research group for Host-Microbe Interaction (HMI), UiT – The Arctic University of Norway, Tromsø, Norway, [2]Oslo Centre of Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway, [3]Department of Biostatistics, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway, [4]Parasites and Microbes, Wellcome Sanger Institute, Cambridgeshire, United Kingdom, [5]Helsinki Institute of Information Technology, Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland

Introduction: Improved understanding of *Staphylococcus aureus* throat colonization in the presence of other co-existing microbes is important for mapping *S. aureus* adaptation to the human throat, and recurrence of infection. Here, we explore the responses triggered by the encounter between two common throat bacteria, *S. aureus* and *Streptococcus anginosus*, to identify genes in *S. aureus* that are important for colonization in the presence of human tonsillar epithelial cells and *S. anginosus*, and further compare this transcriptome with the genes expressed in *S. aureus* as only bacterium.

Methods: We performed an *in vitro* co-culture experiment followed by RNA sequencing to identify interaction-induced transcriptional alterations and differentially expressed genes (DEGs), followed by gene enrichment analysis.

Results and discussion: A total of 332 and 279 significantly differentially expressed genes with p-value < 0.05 and $\log_2$ FoldChange ($\log_2$FC) ≥ |2| were identified in *S. aureus* after 1 h and 3 h co-culturing, respectively. Alterations in expression of various *S. aureus* survival factors were observed when co-cultured with *S. anginosus* and tonsillar cells. The serine-aspartate repeat-containing protein D (*sdrD*) involved in adhesion, was for example highly upregulated in *S. aureus* during co-culturing with *S. anginosus* compared to *S. aureus* grown in the absence of *S. anginosus*, especially at 3 h. Several virulence genes encoding secreted proteins were also highly upregulated only when *S. aureus* was co-cultured with *S. anginosus* and tonsillar cells, and iron does not appear to be a limiting factor in this environment. These findings may be useful for the development of interventions against *S. aureus* throat colonization and could be further investigated to decipher the roles of the identified genes in the host immune response in context of a throat commensal landscape.

KEYWORDS

co-culture, *Staphylococcus aureus*, transcriptome, tonsillar cells, throat colonization

1 Introduction

The influence of bacterial composition and interactions in the throat during frequent recurrence of staphylococcal colonization and infection are poorly understood. The anterior nares are considered the primary site of *Staphylococcus aureus* colonization (Wertheim et al., 2005; Hanssen et al., 2017) but several studies in healthy individuals indicate that *S. aureus* pharyngeal or throat carriage may be equally, or even more common (Ringberg et al., 2006; Mertz et al., 2007; Hamdan-Partida et al., 2010; Hamdan et al., 2018; Erikstrup et al., 2019). Indeed, the prevalence of *S. aureus* has been shown to be significantly higher in the throat (45%) than in the nose (40%) (Erikstrup et al., 2019), and the throat is considered to represent an important reservoir for methicillin-resistant *S. aureus* (MRSA) (Ringberg et al., 2006; Ide et al., 2009; Jang et al., 2014).

Aside from *S. aureus*, other aerobic and anaerobic opportunistic pathogens have also been found to colonize the throat regions, such as alpha and beta-hemolytic streptococci (group A, C, G), *Haemophilus influenzae, Haemophilus parainfluenzae, Enterococcus spp, Klebsiella pneumoniae, Corynebacterium* spp., *Peptostreptococcus, Fusobacteria, Bacteroides and Veillonella* (Dickinson et al., 2020; Katkowska et al., 2020; Buname et al., 2021). The *Streptococcus anginosus* group (SAG), is the most common beta-hemolytic group C streptococci isolated from the human throat (Al-Charrakh et al., 2011). *S. anginosus* is a part of the normal human flora, commonly colonizing tonsils, and the upper respiratory, gastrointestinal, and reproductive tracts (Jiang et al., 2020; Buname et al., 2021). *S. anginosus* can cause dental abscesses and is associated with pharyngitis and tonsilitis (Shinzato and Saito, 1994; Mukae et al., 2016).

The interaction between *S. aureus* and *S. anginosus* is not well understood and, the influence in the growth and metabolism of both species during throat colonization is unclear. A bacterial co-culture can induce interspecies competition for nutrients, space, and attachment sites in their environment, and eventually enhances antibiotic resistance and virulence (Pajon et al., 2023). For instance, an interaction study between *S. aureus* and *Pseudomonas aeruginosa* indicates expression of virulence factors that can reduce metabolism in *S. aureus* through multiple mechanisms (Pastar et al., 2013; Nguyen and Oglesby-Sherrouse, 2016; Noto et al., 2017) and ultimately result in an enhanced virulence capacity and increased antibiotic tolerance (DeLeon et al., 2014). *S. aureus* can enhance virulence of *P. aeruginosa* through the release and assimilation of peptidoglycan component N-acetyl glucosamine (GlcNAc) (Korgaonkar et al., 2013; Yang H. et al., 2020). Another study has also shown the promotion of *S. aureus* colonization of lung tissue in the presence of *P. aeruginosa* due to upregulation of cell receptors in the lung tissue, which are absent in *S. aureus* infection alone (Millette et al., 2019).

During colonization of the respiratory tract, pathogens compete with pre-existing commensal bacteria. Bacteria adapted to particular hosts appear to be more capable of displacing a host's microbiota (Iwase et al., 2010; Siegel and Weiser, 2015). The Interaction between two commensals can benefit colonization and increase the survival of both species in a specific site of the body (Jenkinson et al., 1990). This interaction can enable bacteria to adhere to host cells and can even increase their resistance to the host's innate immune system (Asam and Spellerberg, 2014). Colonization with *S. aureus* constitute a significant risk factor for recurrent episodes of disease e.g., rhinosinusitis (Plouin-Gaudon et al., 2006), tonsillitis (Zautner et al., 2010) and osteomyelitis (Ellington et al., 2003) after the successful adhesion and invasion of the host cell. The success of *S. aureus* depends not only on adhesins and/or virulence genes and the ability to escape antibiotic treatment, but also on the coordinated and timely expression of genes upon infection of its host (Xu et al., 2016), which may change in the presence of another microbe. We have previously identified differentially expressed key determinants in *S. aureus* in the presence of primary human tonsillar epithelial cell using RNA sequencing and pathway analysis (Bastakoti et al., 2023). In the present study, we aimed to identify differentially expressed genes (DEGs) in *S. aureus* when co-cultured with *S. anginosus* in the presence or absence of a tonsillar cell line using the same experimental set up. This allowed us to observe an alteration in the gene expression landscape in *S. aureus* during co-culture with another frequent throat colonizer and compare the DEGs results between the present and our previous study.

2 Materials and methods

2.1 Experimental design

The experimental setup is schematically illustrated in Figure 1. In this study, a *Staphylococcus aureus* throat isolate was co-cultured with *Streptococcus anginosus* and a tonsillar cell line to study the alteration in the transcriptome in *S. aureus*.

2.2 Handling of cells

Human Tonsil Epithelial Cells (HTEpiC, Cat #2560, Sciencell, United States) used in this study were handled and sub-cultured according to the manufacturer´s instructions (Sciencell Research Laboratories, California). The tonsillar cells were grown in poly-L-lysin (PLL) coated flask. Briefly, tonsillar cells were incubated at 37°C in a 5% CO_2 incubator together with Tonsil Epithelial Cell Medium (TEpiCM), 1% Tonsil Epithelial Cell Growth Supplement (TEpiCGS) and penicillin/streptomycin solution (P/S).

A *Staphylococcus aureus* strain TR145 (SAMEA112465883) was isolated from a healthy human throat (Sangvik et al., 2011) and was studied for the first-time concerning co-culturing. The

Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus*; SAG, *Streptococcus anginosus* group; CFU, Colony forming units; BHI, Brain heart infusion broth; LDH, Lactate dehydrogenase; PCA, Principal component analysis; VST, Variance stabilizing transformation; DEGs, Differentially expressed genes; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; HTEpiC, Human tonsil epithelial cells; MOI, Multiplicity of infection; PLL, Poly-L-lysin; DPBS, Dulbecco's Phosphate-Buffered Saline; TEpiCM, Tonsil Epithelial Cell Medium; NGS, Next generation sequencing; FDR, False discovery rate; Isd, Iron-regulated surface determinants; RNA-seq, RNA-sequencing.

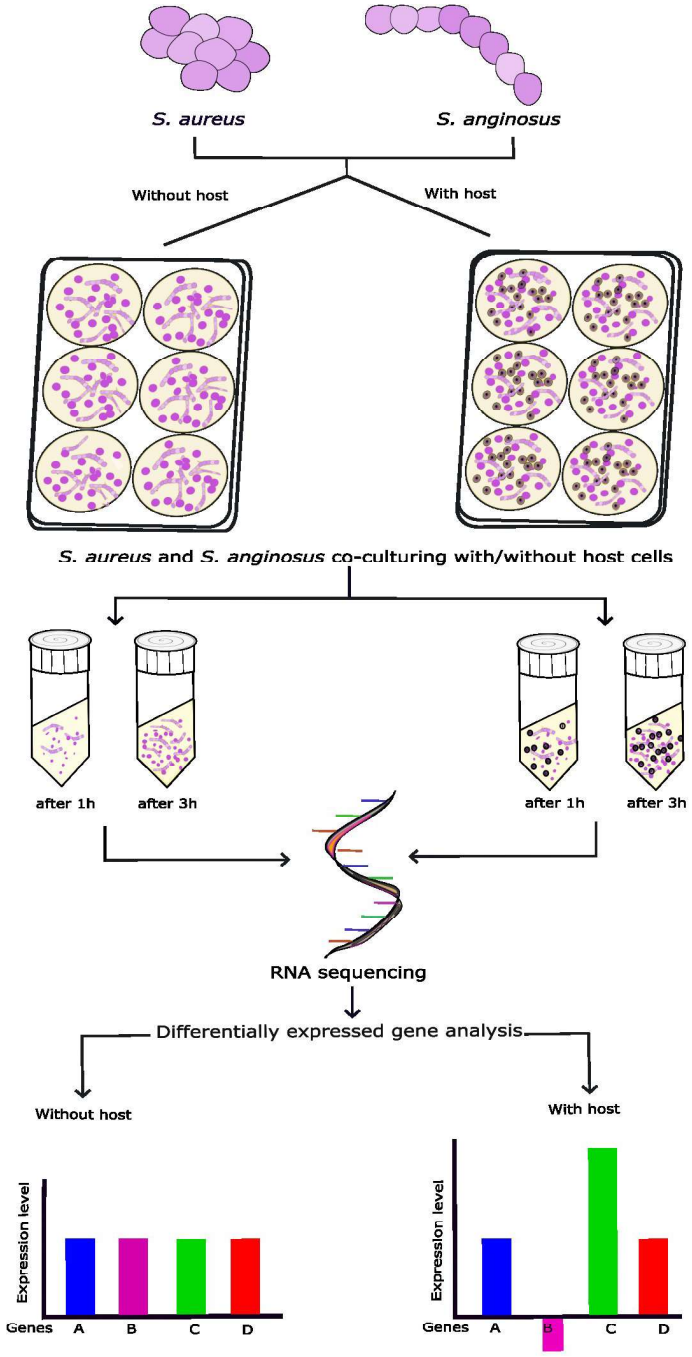

FIGURE 1
Schematic representation of the *in vitro* co-culturing of *S. aureus* and *S. anginosus* with or without tonsillar cells. *S. aureus* and *S. anginosus* were added to poly-L-lysin (PLL)-coated wells containing host media without tonsillar cells or with the presence of monolayer of host cells and incubated for 1h or 3h. Three independent experiments were run in triplicates. The adhered bacteria were collected for RNA extraction. The RNA samples were further processed for RNA-seq followed by differentially expressed genes (DEGs) analysis.

Streptococcus anginosus (ATCC 33397) strain originated from human throat tissue, it is ß-hemolytic and typed as Lancefield's group G (Bauer et al., 2020; Kuryłek et al., 2022). *S. anginosus* was grown according to the handling information provided by ATCC and *S. aureus* was grown as described previously (Bastakoti et al., 2023). Briefly, bacterial cultures were incubated separately in Brain Heart Infusion (BHI) media overnight at 37°C with shaking at 220 revolutions per minute (rpm). At first, both bacteria were grown on BHI overnight at 37°C, and then the fresh bacterial culture was prepared (1:10) for monoculture and co-culturing (*S. aureus* grown in the presence of *S. anginosus*) to get an overview of the bacterial growth pattern for up to 3 hours (h).

2.3 *In-vitro* co-culturing of *S. aureus* with *S. anginosus* and tonsillar cells

HTEpiC was cultured until passage four and seeded at a density of ~ 4×10^5 viable cells per well in six-well plates or ~ 7×10^4 viable cells per well in 24 well plates coated with poly-L-lysin (PLL). The HTEpiC was grown until confluence, washed with Dulbecco's Phosphate-Buffered Saline (DPBS, Sciencell, Cat #SC0303) and added Tonsil Epithelial Cell Medium (TEpiCM) before being exposed to bacteria.

At first, both the bacterial strains, *S. aureus* and *S. anginosus*, were grown to log phase OD of ~ 1 and adjusted to OD of ~ 0.4 (corresponding to ~ 1 x 10^8 CFU/ml). The separate bacterial inoculum was prepared with Multiplicity of Infection (MOI) of 5. The co-culture experiment was conducted in 6 well plates with 2 ml working volume: a 1 ml *S. aureus* inoculum followed by 1 ml of *S. anginosus* inoculum was added into the well seeded with HTEpiC. Two different time points (1 h and 3 h) were selected for triplicate *in-vitro* experiments in 6-well plates. Following incubation at 37°C in the presence of 5% CO_2 with tonsillar cell medium (TEpiCM), bacteria in the presence or absence of host cells were collected and total RNA was subjected to RNA-seq according to a previously described protocol (Bastakoti et al., 2023). Briefly, all unbound co-cultured bacteria were washed away, host cells were trypsinized and only those bacteria which had managed to attach to tonsillar cells were collected and processed for RNA-seq. As a control, only those bacteria that managed to attach to the PLL-coated wells containing host media (TEpiCM, no tonsillar cells) were collected and processed for RNA-seq. Additionally, *S. aureus* and *S. anginosus* monocultures with/without tonsillar cells were performed in parallel for colony forming units (CFU) plate enumeration.

To confirm the CFUs from co-culture, serial dilution of the collected bacteria was performed, followed by plate enumeration in selective strep agar (COBA medium) (cat no. #C754532, ThomasScientifc, USA) to detect *S. anginosus*, and CHROMagar plate to detect *S. aureus*, before incubation for 24 h at 37°C The plating was done in triplicate, and the average CFU/ml was calculated. For better understanding of host stress levels during the exposure time points, cytotoxicity assays were performed on co-culturing plates, as described earlier (Bastakoti et al., 2023).

2.4 NGS library construction and RNA sequencing

The RNA extraction was performed according to a previously described protocol (Bastakoti et al., 2023) following the recommendations from the manufacturer (RNeasy Mini Kit, Cat. No. 74104). Briefly, all samples were lysed enzymatically using lysozyme and lysostaphin, as well as mechanically disrupted using a homogenizer (Precellys Evolution, Bertin technologies) before RNA extraction followed by DNase treatment. Total RNA extracted from three replicates of *S. aureus* co-cultured with *S. anginosus* in the absence/presence of host cells collected at the time point of 1 h and 3 h, were processed for RNA-seq library preparation, as described previously (Bastakoti et al., 2023), using Lexogen's CORALL™ Total RNA-Seq Kit with RiboCop (Cat.No.96; EU, CH, USA). No prior RNA fragmentation was needed in this protocol. The samples were sequenced on an Illumina 550 platform, with dual indexes, and paired end (PE) mode. The final sequencing concentration was 1.8 pM. The expected fragment length for PE reads was < 100 nucleotides.

2.5 Read quality control, trimming and mapping

The overall pipeline for RNA-seq data analysis included generating FASTQ-format files containing reads sequenced from a next-generation sequencing (NGS) platform, quality control and trimming, aligning reads to an annotated reference genome, and quantifying expression of genes (Figure 2). Each library produced between 45 – 184 million reads, and they were pre-processed for a quality check using FASTQC/0.11.9-Java-11. Filtering (removing adaptor dimer reads) and trimming (removing low-quality bases) were performed by Trimmomatic/0.39-Java-11. Then, only those sequences with a quality score Q > equal to 20 and a minimum of 55 nucleotide sequence length were retained in the dataset. The final quality check was performed in the trimmed file.

Strain *S. aureus* TR145 (SAMEA112465883) and *S. anginosus* (ATCC 33397) were used as two separate reference genomes for the mapping performed by Bowtie2/2.4.4-GCC-10.3.0. Clean and trimmed RNA-seq reads were first mapped to the *S. aureus* genome, and then again to *S. anginosus*, in order to determine how many reads mapped to each bacterium. The percentage of mapping efficacy was retrieved from Bowtie2 and sorted by Samtools/1.14-GCC-11.2.0. Further, the gene count matrix for gene expression analysis was identified using the featureCounts program implemented in the SourceForge Subread package (Liao et al., 2013).

2.6 Differentially expressed gene analysis

Differentially expressed genes (DEGs) of the test group (*S. aureus* and *S. anginosus* with host cell) versus the control group (*S. aureus* and *S. anginosus* without host cell) were analyzed using the DESeq R package (1.38.0). Each group has three biological replicates per condition. DESeq2 count data were transformed using the variance stabilizing transformation (VST) for negative binomial data distributions with dispersion-mean trends (Anders and Huber, 2010). DEGs were calculated under $\log_2$ FoldChange ($\log_2$FC) ≥ |2| and a false discovery rate (FDR) adjusted p (p_{adj}) < 0.05. The p values were adjusted using the Benjamini and Hochberg approach for controlling FDR. Genes with an adjusted p value less than 0.05 found by DESeq were assigned as differentially expressed. Any gene that followed this threshold was considered a good starting point for identifying significantly expressed genes. The DEGs exhibited by *S. aureus* from co-culturing with *S. anginosus* were then compared to *S. aureus* alone from our previous study (Bastakoti et al., 2023) to identify alteration in DEGs due to co-culturing with *S. anginosus* in the presence of tonsillar cells. An overview of total DEGs analyses and comparisons performed is shown in Table 1.

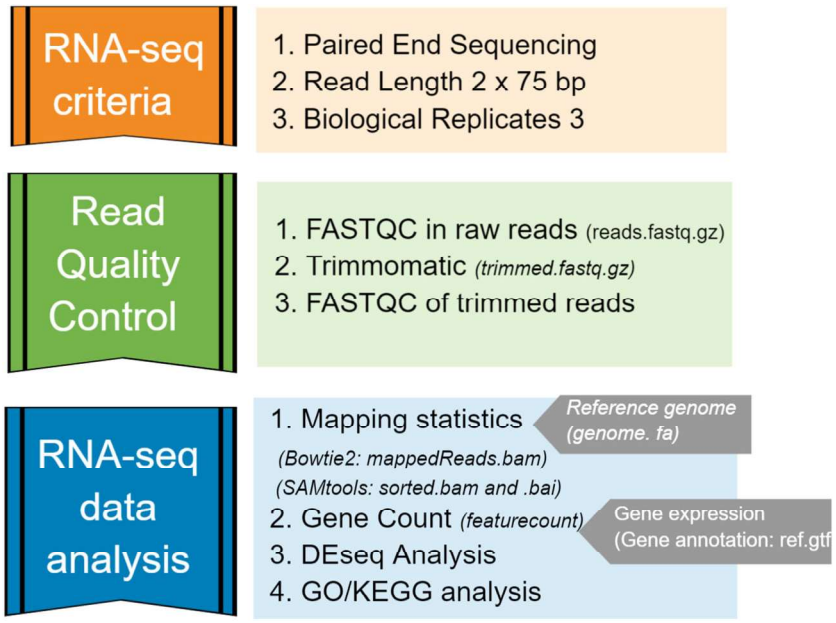

FIGURE 2
Bioinformatics pipeline used for RNA-seq data analysis in this study.

TABLE 1 An overview of total DEGs analyses and DEGs comparisons performed in this study.

Culture type	Sample type	Sample code	DEGs analysis
Co-culture (this study)	*S. aureus* + *S. anginosus* + host at 1h	$T_{co}1$	**$C_{co}1/T_{co}1$** and **$C_{co}3/T_{co}3$**
	S. aureus + *S. anginosus* + host at 3h	$T_{co}3$	
	S. aureus + *S. anginosus* at 1h	$C_{co}1$	
	S. aureus + *S. anginosus* at 3h	$C_{co}3$	
Monoculture (previous study, Bastakoti et al., 2023)	*S. aureus* at 3h	C3	**C1/T1** and **C3/ T3**
	S. aureus at 1h	C1	
	S. aureus + host at 3h	T3	
	S. aureus + host at 1h	T1	

Comparison of total DEGs between two studies

The red color shading represents sample type and RNA-seq data generated from the present co-culture study. The blue color shading represents sample type and RNA-seq data generated from the previous transcriptomics analysis of *S. aureus*.

2.7 GO/KEGG pathways enrichment analysis of DEGs

Gene Ontology (GO) enrichment analysis of the DEGs was performed by the ShinyGO 0.77 online software (http://bioinformatics.sdstate.edu/go/), in which pathways were sorted by fold enrichment. GO terms with a corrected FDR value of less than 0.05 were considered significantly enriched by DEGs. Pathway enrichment was determined using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways annotation. String Database (v 11.5) (https://string-db.org/) was used to test the statistical enrichment of DEGs in the KEGG pathways. Pathways were considered significantly enriched with an FDR of less than 0.05.

3 Results

3.1 Recovery of bound bacteria in the presence and absence of tonsillar cells

Both bacterial strains, *S. aureus and S. anginosus*, were originally isolated from the human throat and were chosen as representative throat bacteria in this study. As we aimed to analyze *S. aureus* transcriptome altered due to presence of *S. anginosus* in the tonsillar cells, *in-vitro* infection assay was performed, and only surface-bound bacterial strains were collected and plated.

The recovery of bound *S. aureus* when co-cultured with *S. anginosus* with host cells (referred to as test samples) was evaluated after 1 and 3 h and is presented in Figure 3. After 1 h, 6.4 log10 CFU/ml (corresponding to ~ 1.1 x 10^6 CFU/ml) of bound *S. aureus* was recovered when co-cultured with *S. anginosus* with host cells, and after 3 h it was 7.5 log10 CFU/ml (corresponding to ~ 3.9 x 10^7 CFU/ml). Whereas recovery of *S. aureus* co-cultured with *S. anginosus* in the absence of tonsillar cells (referred to as control samples) after 1 and 3 h was 5.9 log10 CFU/ml and 6.5 log10 CFU/ml, respectively. Likewise, the recovery of bound *S. anginosus* when co-cultured with *S. aureus* with host cells (referred to as test samples) was 4.60 log10 CFU/ml (corresponding to ~ 4.1 x 10^4 CFU/ml) at 1 h and 5.4 log10 CFU/ml (corresponding to ~ 2.7 x 10^5 CFU/ml) at 3 h (Figure 3). Whereas recovery of *S. anginosus* co-cultured with *S. aureus* without host cells (referred to as control samples) after 1 and 3 h was 5.5 log10 CFU/ml and 5.9 log10 CFU/ml, respectively (Figure 3). For the evaluation of the bacterial effect on tonsillar cell viability during *in-vitro* infection assay, the lactase dehydrogenase (LDH) release by the tonsillar cells was measured. The LDH release was found to be less than 5% (Supplementary Figure S1), indicating that the host cells remained viable during the co-incubation with *S. aureus* and *S. anginosus*.

Similar procedure was executed to identify the recovery of *S. aureus* during co-culturing with *S. anginosus* in the absence of host cell i.e., with host cell media (TEpiCM) in PLL-coated wells. A significant increase in the recovery of bound bacteria was observed for both *S. aureus* and *S. anginosus* from their individual growth when compared to recovery of both bacteria from co-culture set-up (Supplementary Figure S2). Taken together, despite a significant decrease in recovery of *S. aureus* when co-cultured with *S. anginosus* without a host cell, a significant increase in *S. aureus* recovery was identified when co-cultured with *S. anginosus* and host cells. The number of surface bound bacteria increased over time, and the recovery of *S. aureus* and *S. anginosus* indicated that all the samples could be proceeded for RNA seq.

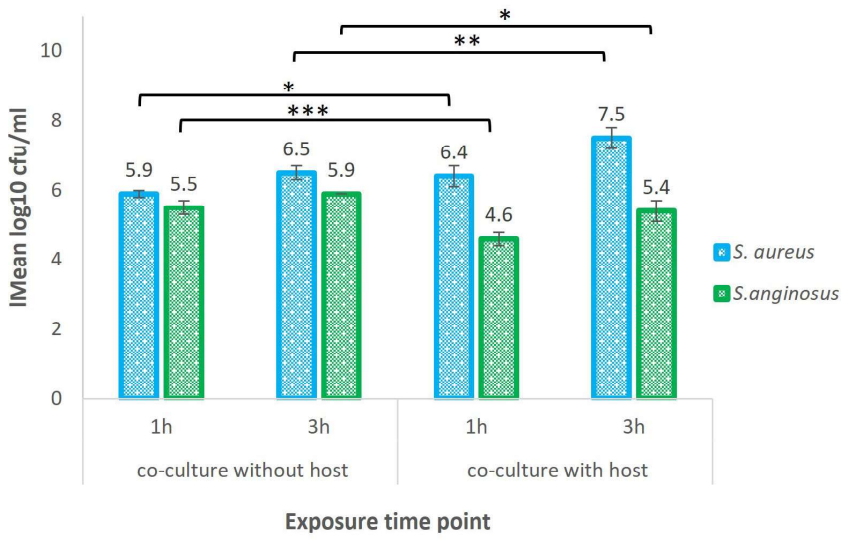

FIGURE 3
Recovery of *S. aureus and S. anginosus* co-cultured with or without tonsillar cells. S. aureus and S. anginosus were grown for 1 or 3 hours (h) in tonsillar cell medium, with or without tonsillar cells (host) at 37°C in the presence of 5% CO_2. All unbound bacteria were washed away, and remaining bacteria were collected for RNA-seq and plated on selected media for CFU enumeration. The results are presented as mean log 10 CFU/ml from three independent experiments. Error bars represent the +/- SD. Differences in the means between the groups were tested using a two-sample Student t-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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TABLE 2 Mapping efficacy of reads assigned against *S. aureus* genome.

Biological Replicates (BR)	Sample ID	Experimental conditions	Mapping efficacy (%)
1st	S1.ctr_1h.BR1	*S. aureus* + *S. anginosus*, control, 1h	73
	S2.trt_1h.BR1	*S. aureus* + *S. anginosus* + tonsillar cells, test, 1h	<20
	S3.ctr_3h.BR1	*S. aureus* + *S. anginosus*, control, 3h	64
	S4.trt_3h.BR1	*S. aureus* + *S. anginosus* + tonsillar cells, test, 3h	69
2nd	S5.ctr_1h.BR2	*S. aureus* + *S. anginosus*, control, 1h	64
	S6.trt_1h.BR2	*S. aureus* + *S. anginosus* + tonsillar cells, test 1h	47
	S7.ctr_3h.BR2	*S. aureus* + *S. anginosus*, control, 3h	63
	S8.trt_3h.BR2	*S. aureus* + *S. anginosus* + tonsillar cells, test, 3h	69
3rd	S9.ctr_1h.BR3	*S. aureus* + *S. anginosus*, control, 1h	66
	S10.trt_1h.BR3	*S. aureus* + *S. anginosus* + tonsillar cells, test, 1h	26
	S11.ctr_3h.BR3	*S. aureus* + *S. anginosus*, control, 3h	55
	S12.trt_3h.BR3	*S. aureus* + *S. anginosus* + tonsillar cells, test, 3h	61

Additionally, fresh bacterial culture prepared for *S. aureus* and *S. anginosus* in BHI was performed to visualize their growth pattern when grown alone and together, prior to *in-vitro* infection assay. The OD_{600nm} measured in 30-minute intervals for up to 3 h for alone and mix of both species, including the CFU/ml identified after 1 h and 3 h, are presented in Supplementary Figure S3. The results showed that the two species can co-exist when grown in BHI, especially at the time point chosen for infection assay.

3.2 Reads assigned to the *S. aureus* genome

RNA-seq was performed to study the transcriptome of *S. aureus* when exposed to *S. anginosus* with/without tonsillar cells. Total reads per library ranged from 45 – 184 million, with 44 - 183 million reads per library remaining after processing of the raw data.

The trimmed reads were aligned with the reference genome, and the mapping efficacies against the *S. aureus* reference genome ranged between 20 - 73% for all 1 h samples and between 55 - 69% for 3 h samples (Table 2). The detailed mapping efficacy for *S. aureus and S. anginosus* reference genome is presented in Supplementary Table S1. Overall, this finding revealed that most sequencing reads were mapped to the *S. aureus* reference genome, which corresponds to higher recovery of bound *S. aureus* compared to *S. anginosus.*

3.3 Normalization of the gene read counts

The normalization of read counts was used for gene count comparisons between *S. aureus* co-cultured with *S. anginosus* in the presence or absence of tonsillar cells. The representation of raw counts from all 12 samples (S1 to S12) is shown in Figure 4A. DEseq2 normalization has corrected variations in sequencing depths and biological replicates (Figure 4B). The results obtained after the normalization of gene counts are more reliable and accurate than raw counts, and thus normalized gene read counts are implemented for DEGs analysis.

3.4 Visualization of sample-to-sample distances in PCA plot

To visualize the between-group and between-time sample variance in the samples, a principal component analysis (PCA) plot was done before performing DEGs analysis. The PCA plot showed a clear clustering of RNA reads from the three biological replicas of *S. aureus* co-cultured with *S. anginosus* at 1 h or 3 h in the presence or absence of host cells (Figure 5). The difference between the two time points was considerable (PC2 explaining 17% of the overall variability), though not stronger than the differences due to exposure to host cells (PC1 explaining 72% of the overall variability). Hence, the results suggest that exposure to host cells induces more variability than temporal change.

3.5 Highest variance across sample and gene clustering

The overall gene clustering pattern of genes present in *S. aureus* co-cultured with *S. anginosus* in the absence (without host) and the presence of tonsillar cells (with host) is visualized in heatmaps (Figure 6). The 300 genes with the highest variation across samples are presented in Figure 6A. For a subset of genes (in the middle of the heatmap), the main source of variation was caused by the length of incubation (1 or 3 h). For a more detailed visualization of gene clustering, the top 65 genes with the highest variation across samples are presented in Figure 6B. It indicated two clear clusters of genes: the top 50 genes constructed one cluster where genes such as *glpD, dtpT, ilvB* and *sdrC* showed high variance in samples exposed to host cells, whereas the remaining 15 genes constructed the second separate cluster where genes such as *pyrC, irgA, emp* and *flr* showed high variance in samples with no exposure to host cells. Overall, the *S. aureus* transcript pattern is highly influenced by the

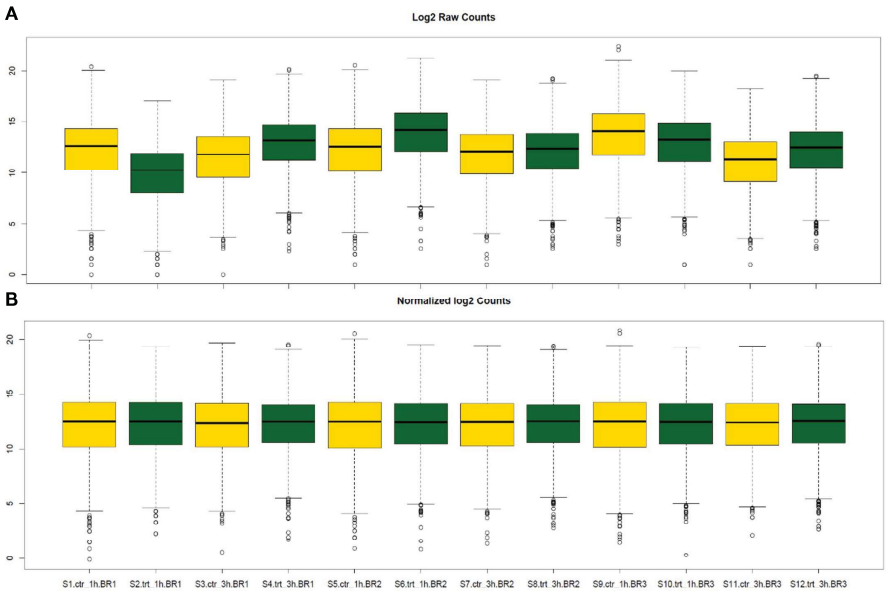

FIGURE 4
Box plot representation of raw read counts before and after DESeq2 normalization. The yellow and green box plots represent the *S. aureus* co-cultured with *S. anginosus* in the presence or absence of tonsillar cells, respectively (either at 1h or 3h). **(A)** The distribution of log2 raw counts before normalization. **(B)** The counts after DESeq2 normalization.

co-culturing with *S. anginosus* in the presence/absence of the tonsillar cells.

3.6 DEGs in *S. aureus* after co-culturing

To identify the differentially expressed genes (DEGs) present in *S. aureus* co-cultured with *S. anginosus* in the presence of tonsillar cells compared to *S. aureus* co-cultured with *S. anginosus* in the absence of tonsillar cells, the normalized gene reads counts were analyzed using DESeq2. A total of 332 (at 1 h) and 279 (at 3 h) significant DEGs in *S. aureus* co-cultured with *S. anginosus* in the presence of host in comparison to *S. aureus* co-cultured with *S. anginosus* in the absence of host were identified. There were 242 commonly shared DEGs, of which 155 were annotated. The volcano plots in Figure 7 represent annotated DEGs in *S. aureus* co-cultured with *S. anginosus* in the

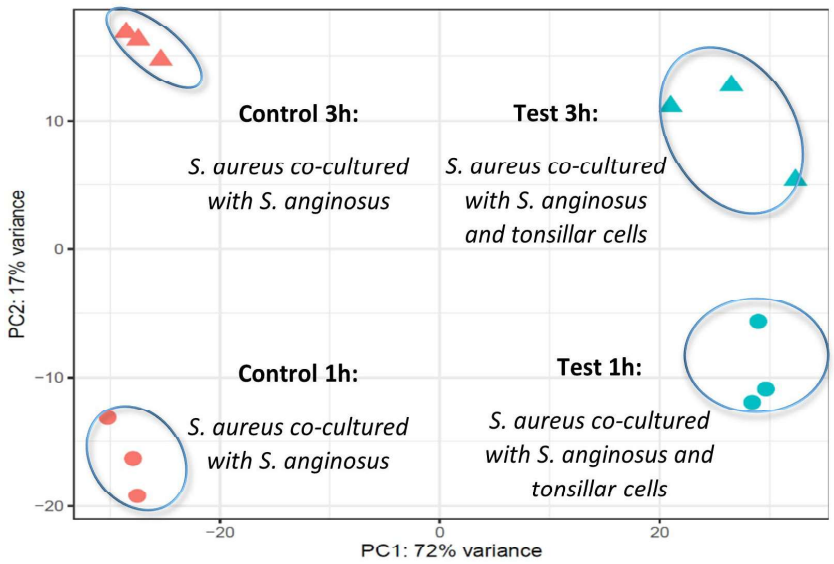

FIGURE 5
PCA plot using the variance stabilizing transformation (VST) values of the read counts. The circles and triangles represent 1 and 3 hours, respectively, while orange and blue color represent *S. aureus* co-cultured with *S. anginosus* without host cells (control) and *S. aureus* co-cultured with *S. anginosus* with host cells (test), respectively.

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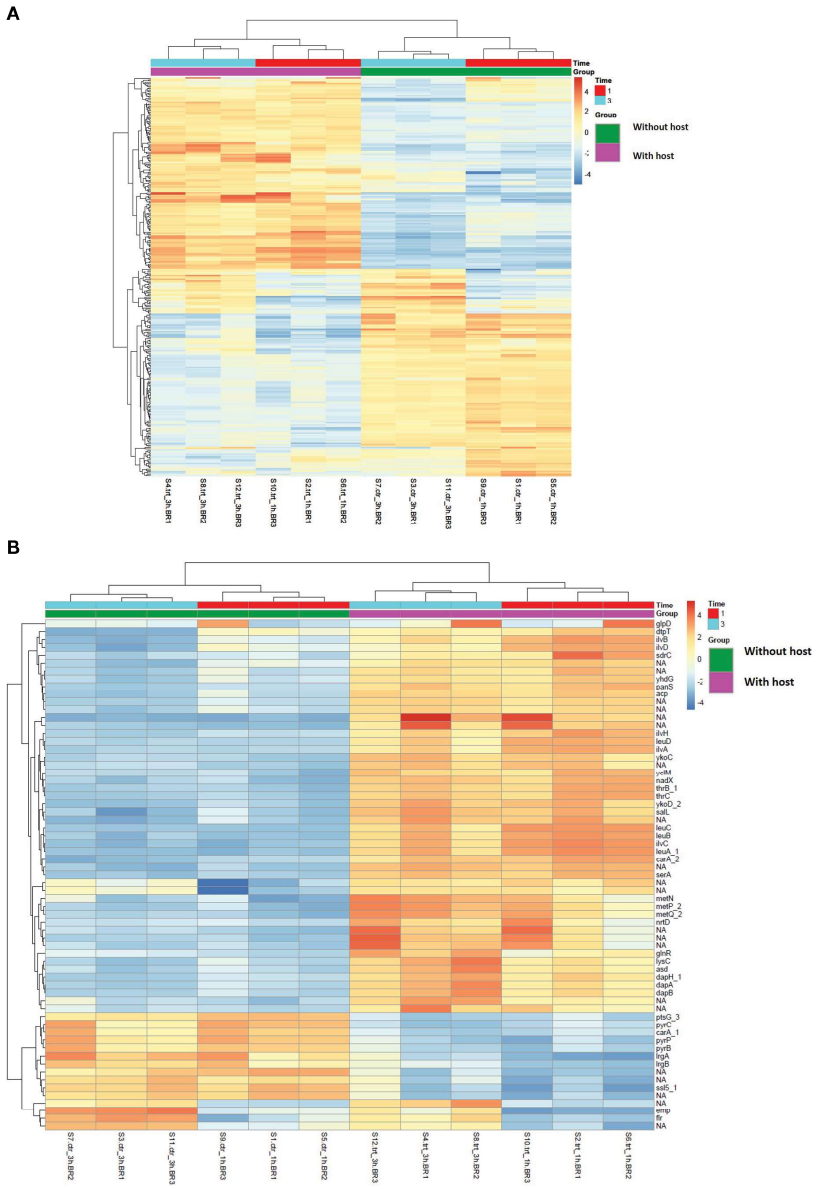

FIGURE 6
Heatmap showing gene clustering-based presence/absence of host cells for 1 and 3 hours in *S. aureus* using variance stabilizing transformation data. The values in the heatmap present mean-centered read counts after applying variance stabilizing transformation (VST). The gene clusters (pink and green) represent the gene clustering based on sample type i.e., in the absence and presence of tonsillar cells, while the different exposure times are represented in red (1h) and turquoise (3h). Blue-toned color indicates lower than average variation across samples, whereas orange-toned color indicates higher than average variation. **(A)** Clustering of the top 300 genes with the highest variation across the various conditions. **(B)** Clustering of the top 65 genes with the highest variation across the various conditions. Unannotated genes are marked with NA.

presence of tonsillar cells, i.e., 245 out of 332 DEGs at 1 h (Figure 7A) and 207 out of 279 DEGs at 3 h (Figure 7B). The total number of DEGs, excluding unknown genes, was 297.

Among the significantly upregulated genes with the highest $\log_2$FC at 1 h were *ilvC, leuA_1, leuB, leuC, leuD, ilvH, thrB_1, ilvA*, and *carA_2*, and among the highly downregulated genes were *lrgA, ssl5_1, ydjF, pyrP, sbi* and *fnbA* (Supplementary Table S2). Similarly, at 3 h, the top upregulated genes were *salL, metp_2, carA_2, lysC, ykoD_2, dapA, metQ_2, ilvC, leuA_1, dapB*, and *asd*, and while the top downregulated were *pyrC, lrgA, pyrP*, and *carA_1* (Supplementary Table S3).

S. aureus and *S. anginosus* genes may have some sequence similarity. Therefore, we evaluated whether the genes that were differentially expressed in *S. aureus*, could be found in S. *anginosus*. Of the 297 obtained DEGs, 185 genes were exclusively present in *S. aureus*. The remaining 112 DEGs could be a result of expression of both *S. aureus* and *S. anginosus* genes. These genes are listed in Supplementary Table S4. All DEGs were analyzed for functional enrichment analysis to identify any possible significant pathways involved by those genes in S. *aureus* during co-culturing.

In summary, the transcriptome of *S. aureus* revealed significant changes during co-culturing with *S. anginosus* upon

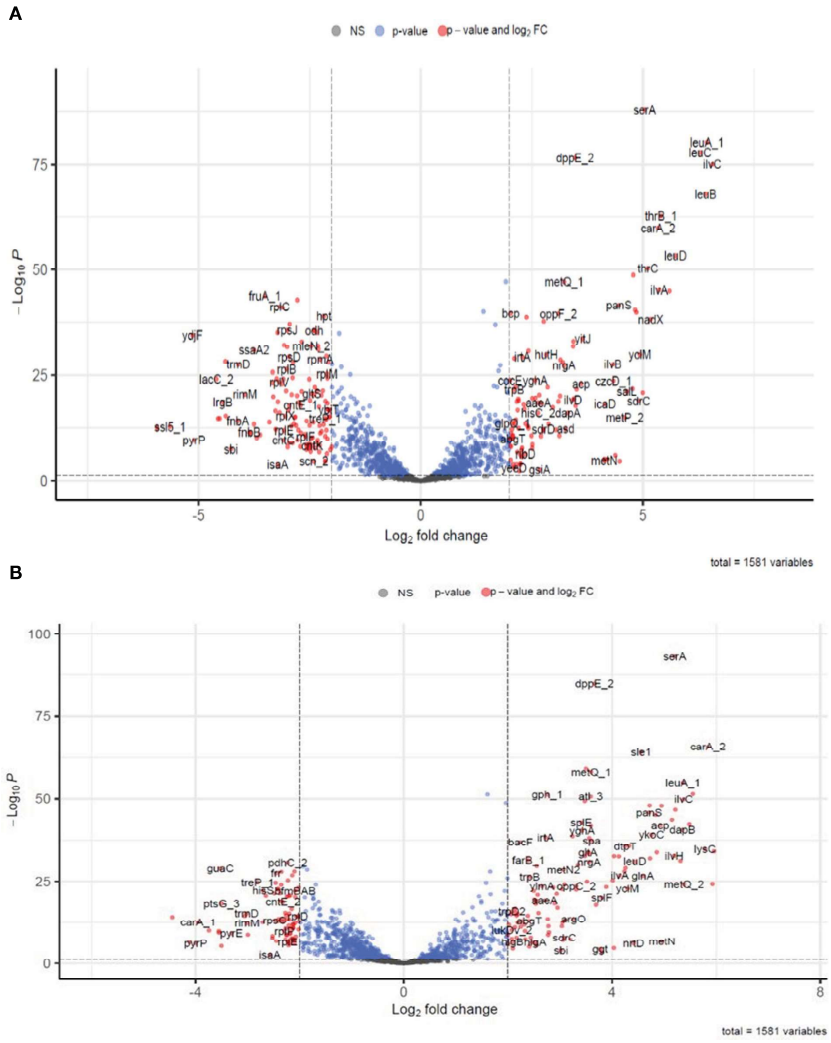

FIGURE 7
Differentially expressed genes identified in *S. aureus* co-cultured with *S. anginosus* and tonsillar cells at 1h and 3h. Only annotated genes were included in the plot. Blue dots represent genes with only significant p-value <0.05, red dots represent significantly differentially expressed genes (DEGs) with cut-off values with p-value < 0.05 and $\log_2$ fold change cut-off >2 and grey are non-significantly differentially expressed genes. **(A)** Significantly DEGs after 1h. **(B)** Significantly DEGs after 3h.

exposure to tonsillar cell lines. Several transcripts were found to be unique to the tested time points, while others were expressed at both time points.

3.7 GO term enrichment analysis

All upregulated and downregulated genes derived from RNA-seq data analysis were separately applied in gene ontology (GO) enrichment analysis to identify enriched GO terms involved during *S. aureus* co-cultured with *S. anginosus* and tonsillar cells compared to *S. aureus* co-cultured with *S. anginosus* but without tonsillar cells. The GO enrichment analysis selected by FDR and sorted by fold enrichment revealed that significantly enriched upregulated DEGs were mainly involved in biological processes, including the "amino acid metabolic process", "lysin biosynthesis", "cell adhesion" and "biological adhesion" (Figure 8A). The downregulated DEGs were mainly enriched in "cytosolic ribosome", "ribosomal protein", "rRNA binding", and "organelle" (Figure 8B).

Additionally, the uniquely upregulated DEGs in co-cultured *S. aureus* with *S. anginosus* and tonsillar cells compared to *S. aureus* co-cultured with *S. anginosus* but no tonsillar cells, at 1 h were enriched in "riboflavin biosynthesis" and "lumazine binding domain" (Figure 9A), whereas at 3 h the uniquely upregulated DEGs were enriched in the "Defense response" biological process (Figure 9B). The common DEGs between the two time points showed enrichment in the biological process group of branched-chain amino acid biosynthetic process, cell adhesion, and amide biosynthetic process (Figure 9C). The list of significantly enriched GO terms, after GO analysis of DEGs associated with *S. aureus* during co-culturing with *S. anginosus* and tonsillar cells, is in Supplementary Data Sheet S1.

In addition, a pathway enrichment analysis performed on the overlapping genes from *S. anginosus* and *S. aureus*, listed in Supplementary Table S4 is also presented in Figure 10. The top 10

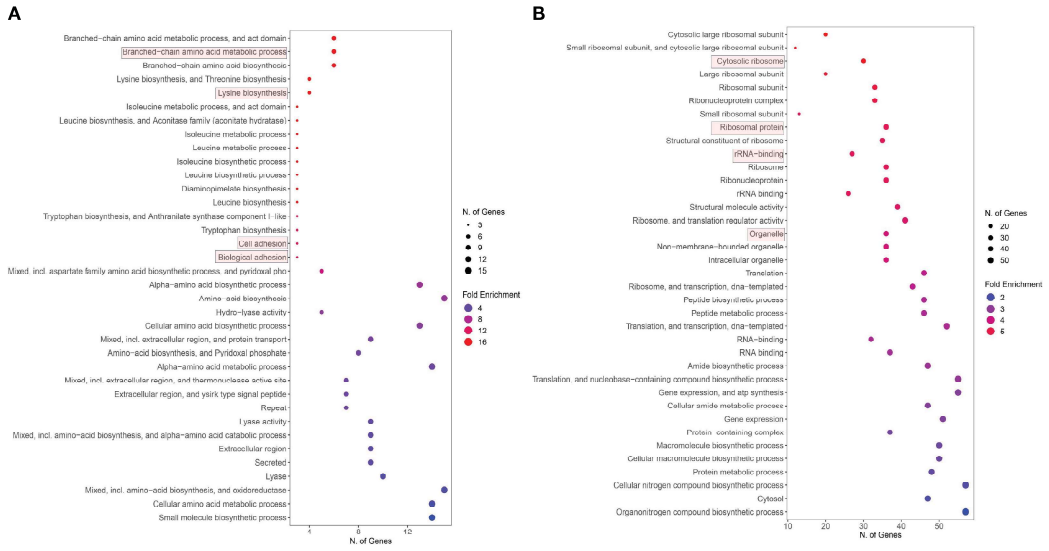

FIGURE 8
Enriched GO functions of DEGs. **(A)** Upregulated genes GO analysis. **(B)** Downregulated genes GO analysis.

GO enriched pathways involved in biological process, cellular component and molecular functions were mainly found to be responsible in biosynthesis processes (Figure 10A), ribosomal subunits (Figure 10B) and RNA binding (Figure 10C), respectively. The GO enrichment analysis of overlapping genes (Figure 10) does not show the involvement of some of the important GO terms (such as cell adhesion, riboflavin biosynthesis and defense response), that were significantly enriched in *S. aureus* during co-culturing with *S. anginosus* (Figure 9). Overall, these findings provide insight into the involvement of the majority of biological processes in *S. aureus* during co-culturing together with evidence for enrichment in defense response only after 3 h of co-culturing with *S. anginosus* in the presence of tonsillar cells. Some of the enriched GO terms detected in this study using overlapping genes remain the same for both *S. aureus* and S. *anginosus*.

3.8 KEGG pathway analysis

KEGG analysis also uses DEGs involved in co-cultured *S. aureus* with *S. anginosus* in the presence of tonsillar cells compared to co-

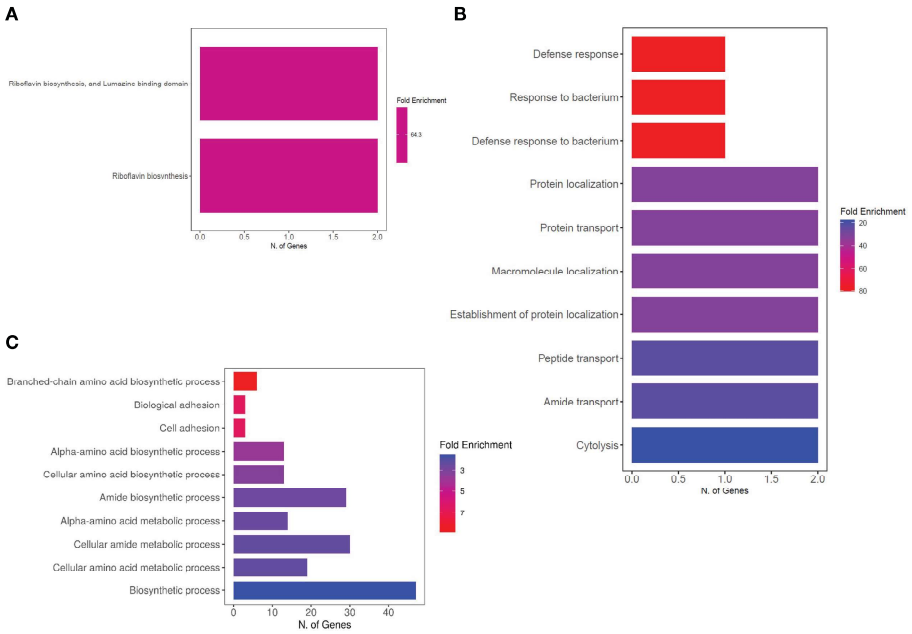

FIGURE 9
Enriched biological processes of DEGs. **(A)** Two biological processes identified from uniquely upregulated genes from 1 h of co-culturing. **(B)** Top 10 biological processes identified from uniquely upregulated genes from 3 h of co-culturing. **(C)** Top 10 biological processes identified from common DEGs after 1h and 3 h of co-culturing.

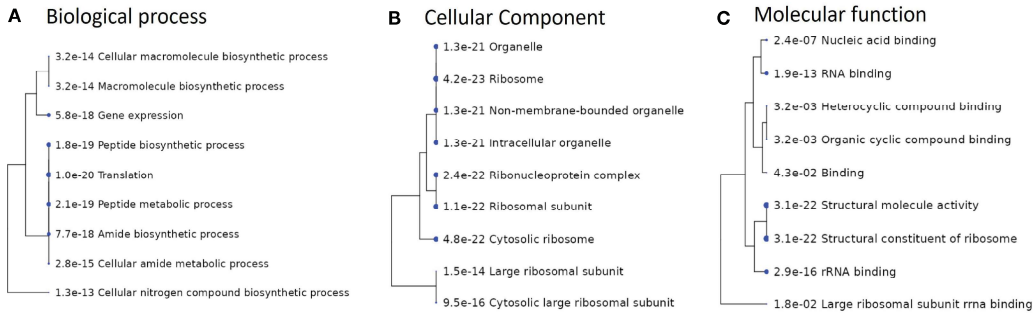

FIGURE 10
Enriched GO pathways of overlapping genes between *S. aureus* and *S. anginosus*. Hierarchical clustering trees summarize the correlation among pathways identified during enrichment analysis. The pathways with many shared genes are clustered together. A larger dot indicates a more significant P-value. **(A)** Top 10 enriched pathways involved in biological processes. **(B)** Top 10 enriched pathways involved in cellular component. **(C)** Top 10 enriched pathways involved in molecular functions.

cultured *S. aureus* with *S. anginosus* without tonsillar cells, to identify the enriched pathways involved by DEGs. The KEGG pathway analysis revealed that the upregulated DEGs were significantly (FDR < 0.05) associated with pathways including "Valine, leucine and isoleucine biosynthesis", "2- Oxocarboxylic acid metabolism", "Phenylalanine, tyrosine and tryptophan biosynthesis", "Biosynthesis of amino acids", "Biosynthesis of secondary metabolites", and "Metabolic pathways" (Figure 11). The percentage of enriched KEGG pathways identified from upregulated genes were ranging from 4 to 50% (Figure 11, red bar). The downregulated DEGs were significantly (FDR < 0.05) associated with "Ribosome" and "Pyrimidine metabolism" (Figure 11). The percentage of enriched KEGG pathways by downregulated genes were ranging from 15 to 60% (Figure 11, blue bar). Overall, the KEGG pathway analysis has provided valuable insights into the highly enriched significant pathways together with identification of ribosome shutdown by *S. aureus* during stress response.

3.9 Comparison of *S. aureus* DEGs upon mono- and co-culturing

To gain a better understanding of the alteration of the transcriptome in *S. aureus* upon co-culturing, a broad comparison was made between the identified DEGs in *S. aureus* co-cultured with *S. anginosus* and tonsillar cells (present study, $C_{co}1/T_{co}1$ and $C_{co}3/T_{co}3$) versus DEGs in *S. aureus* with tonsillar cells (previous study, C1/T1 and C3/T3) as described in Table 1. The list of DEGs identified in *S. aureus* without *S. anginosus* were retrieved from a previous study (Bastakoti et al., 2023). Several *S. aureus* transcripts were identified, and a

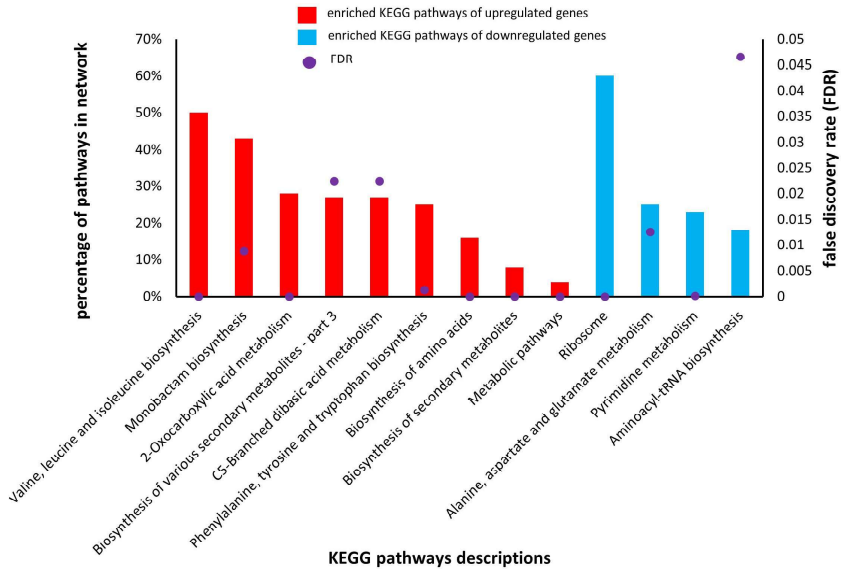

FIGURE 11
Enriched KEGG pathways of upregulated and downregulated genes in *S. aureus* co-culture with *S. anginosus* and tonsillar cells versus *S. aureus* co-cultured without tonsillar cells. Nine different KEGG pathways were associated with upregulated genes (red bars), whereas four different KEGG pathways were associated with downregulated genes (blue bars). Only significant pathways with a false discovery rate (FDR) < 0.05) are presented (purple dots).

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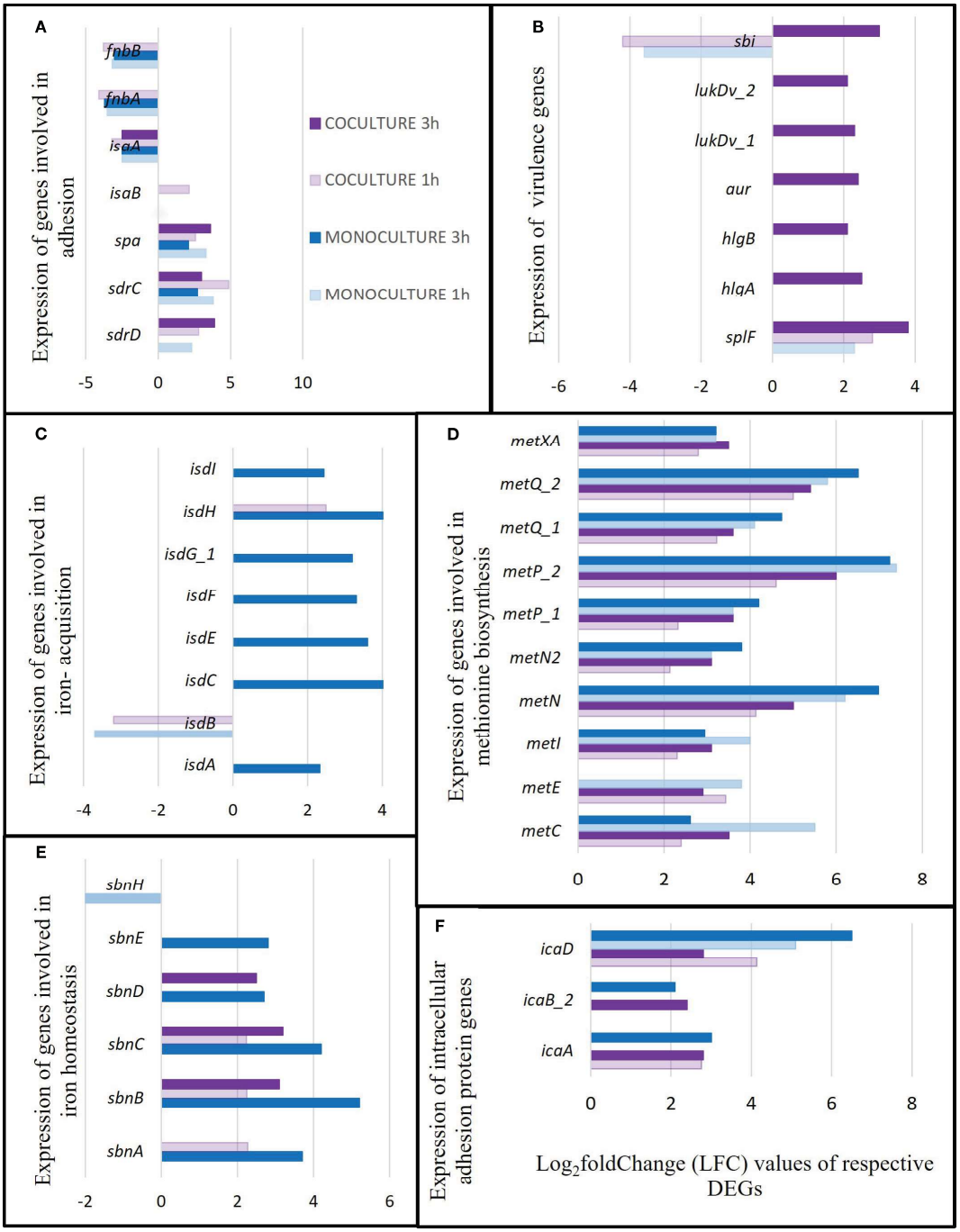

FIGURE 12
Several survival factors exhibited by *S. aureus* during co-culturing with *S. anginosus* and tonsillar cells compared to S. aureus monoculturing with tonsillar cells. The genes presented are the significant DEGs (p-value < 0.05 and $\log_2$FC ≥ |2|) identified from two separate datasets i.e., monoculturing was performed in a previous study and co-culturing in the present study. The dark and light purple color bars represent the DEGs from co-culture at 3 h and 1 hour, respectively, while the dark and light blue color bars represent the DEGs identified from monoculture at 3 h and 1 h, respectively. **(A)** Expression of genes encoding different adhesion factors. **(B)** Expression of virulence genes. **(C)** Expression of genes involved in iron-regulated surface genes. **(D)** Expression of genes involved in methionine biosynthesis. **(E)** Expression of genes involved in iron homeostasis. **(F)** Expression of genes involved in intracellular adhesion protein.

comparison of the DEGs identified from the present and previous study is presented in Figure 12; Table 3.

Several DEGs were identified only in *S. aureus* (Supplementary Table S4). Most of the adhesion genes were differentially expressed in both monoculture (DEGs from our previous study) and co-culture condition (present study with *S. anginosus*). For instance, a significant downregulation of fibronectin-binding protein A (*fnbA*), fibronectin-binding protein B (*fnbB*), and staphylococcal antigen A (*isaA*) was seen with LFC ≤ -2 for all conditions except *fnbA* and *fnbB* in co-culture 3 h (LFC < -1.37, not presented in Figure 12A). There was an upregulation

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TABLE 3 An overview of total DEGs in S. aureus influenced due to the presence of S. anginosus and tonsillar cells.

Culture type	Sample type	Sample code	DEGs analysis	Total DEGs at 1h and 3h
Co-culture (this study)	*S. aureus* + *S. anginosus* + host at 1h	$T_{co}1$	$C_{co}1/T_{co}1$ and $C_{co}3/T_{co}3$	**332** at 1h **279** at 3h
	S. aureus + *S. anginosus* + host at 3h	$T_{co}3$		
	S. aureus + *S. anginosus* at 1h	$C_{co}1$		
	S. aureus + *S. anginosus* at 3h	$C_{co}3$		
Monoculture (previous study, Bastakoti et al., 2023)	*S. aureus* at 3h	C3	C1/T1 and C3/ T3	**430** 1h **348** 3h
	S. aureus at 1h	C1		
	S. aureus + host at 3h	T3		
	S. aureus + host at 1h	T1		

The red color shading represents the DEGs, 332 at 1h and 279 at 3h, identified in the present co-culture study. The blue color shading represents DEGs from a previous transcriptomics study.

of staphylococcal protein A (*spa*) and serine-aspartate repeat-containing protein C (*sdrC*) regardless of culture conditions and time points. It appears that these genes are continuously differentially expressed in *S. aureus* when it meets tonsillar cells with or without *S. anginosus* (Figure 12A). *IsaB*, was upregulated only at 1 h of co-culturing, and *sdrD* was upregulated in both monoculture and co-culture after 1 h, but only after 3 h of co-culturing, being absent in 3 h monoculture. This indicates that *sdrD* expression is vital at 3 h of co-culturing with *S. anginosus*, compared to *S. aureus* alone, in the presence of host (Figure 12A).

Some of the genes encoding secreted toxins or enzymes were only expressed after 3 h of co-culturing of exposure to tonsillar cells, such as *lukD, aur*, *hlgA* and *hlgB* (Figure 12B). Most genes responsible for iron acquisition such as *isdA, isdB, isdC, isdE, isdF, isdG*, and *isdI* were not expressed in *S. aureus* during co-culturing with *S. anginosus* and host cells (Figure 12C). Expression of several genes involved in methionine biosynthesis was upregulated, with LFC ranging from 2 to 7, in all culturing conditions and time exposures (Figure 12D). Additionally, the genes responsible for iron homeostasis, *sbn*ABCDE, were found to be upregulated either in co-culture (1 h and 3 h) or in monoculture 3 h; however, *sbnH* was found to be downregulated only in monoculture after 1 h of exposure (Figure 12E). Some of the intercellular adhesion protein genes (*eg., icaA, icaB and icaD*) were also identified to be upregulated both in monoculture and co-culture in the presence of tonsillar cells (Figure 12F).

Similarly, the gene enrichment analysis of DEGs in *S. aureus* due to *S. anginosus* and tonsillar cells have revealed a small number of upregulated genes (15 genes, Figure 8A in x-axis) involved in GO terms in comparison to DEGs identified from monoculture study (25 genes (Bastakoti et al., 2023),). The pathways involved in "riboflavin biosynthesis" and "lumazine binding domain" were identified by upregulated genes only from 1 h datasets of co-culturing.

Taken together, the number of DEGs in *S. aureus* co-cultured with *S. anginosus* is found to be lower than DEGs identified in *S. aureus* without *S. anginosus*, both being analyzed in the presence of tonsillar cells. Nevertheless, several new sets of DEGs were identified when *S. aureus* was co-cultured, and some of the DEGs also present in *S. aureus* without being exposed to *S. anginosus* were also detected. This finding indicates that the presence of *S. anginosus* can influence the DEGs of *S. aureus* when exposed to tonsillar cells. Briefly, there are some core virulence factors exhibited by *S. aureus* that are expressed in every condition, and some are unique with respect to culturing condition and time point of exposure to host cells.

4 Discussion

A better understanding of the *S. aureus* throat colonization process together with other competing and/or coexisting microbes, may provide insight into *S. aureus* adaptation to throat and recurrence of colonization. In this work, we explored the responses triggered by the encounter of two common throat pathogens, *S. aureus* and *S. anginosus*, in the presence of human tonsil epithelial cells (HTEpiC). Previously, we verified the suitability of HTEpiC for studying the interaction between *S. aureus* and human tonsillar cells without compromising the viability of the host cells (Bastakoti et al., 2023). In this study, we aimed to identify the transcripts in *S. aureus* that are important when facing a potential competitor during throat colonization. We compared the transcriptome of *S. aureus* co-cultured with *S. anginosus* in the presence of tonsillar cells, and the transcriptome from *S. aureus* grown in monoculture (Bastakoti et al., 2023).

Several adhesion factors exhibited by *S. aureus* with/without *S. anginosus* in the presence/absence of tonsillar cells were identified. Many transcripts were differentially expressed in *S. aureus*, and these transcripts are likely to play an important role in *S. aureus* colonization in the presence of a competitor or they may be used by *S. aureus* to protect itself from competition. In contrast to a study by Hamamoto et al, which examined the virulent Newman strain to identify upregulated genes after infection in a mouse model (Hamamoto et al., 2022), our study focused on a strain colonizing the throat of a healthy individual.

The present co-culture study demonstrates the significant recovery of both bacteria, *S. aureus* and *S. anginosus*, when exposed to tonsillar cells. This is in contrast to another co-culture study which showed that *S. aureus* was outcompeted by *Pseudomonas aeruginosa* by producing inhibitory molecules (Filkins et al., 2015; Smith et al., 2017); however, the coexistence between these two pathogens could also be determined from the interactions between metabolism and growth (Woods et al., 2018; Price et al., 2020; Pajon et al., 2023). Thus, these studies indicate that certain bacterial strains can produce substances that give them a competitive advantage over other strains in a co-culture environment, but not necessarily kill the bacteria. The upregulation of several virulence genes during co-culture and expression of iron-regulatory genes mostly in *S. aureus* grown without *S. anginosus*, suggest that the presence of other bacteria, such as *S. anginosus*, could augment and affect the pathogenicity of *S. aureus*. Our observation is also consistent with the result of other co-culture studies with *S. aureus* and *P. aeruginosa* (Korgaonkar et al., 2013; Yang N. et al., 2020). Taken together, the presence of *S. anginosus* in the tonsillar cells could significantly impose a change in the transcriptomic level of *S. aureus* after co-culturing.

This study identified several DEGs in *S. aureus* when exposed to tonsillar cells and *S. anginosus* at 1 h and 3 h. Genes associated with the production of virulence factors, as well as genes involved in methionine biosynthesis, adhesion factors, iron-regulated surface genes, iron homeostasis genes, intercellular adhesion protein, defense response and other survival mechanisms were identified. Some of the genes encoding proteins involved in adhesion such as *isaB*, was upregulated in *S. aureus* at 1h of coculturing, and *sdrD*, which is commonly expressed after 1 h of monoculture and co-culture, was upregulated only after 3 h of co-culturing; virulence genes encoding secreted proteins were also highly upregulated; and iron-regulatory genes were not expressed in co-culture. This suggests that the presence of *S. anginosus* might create an environment in which *S. aureus* can better survive and express its virulence factors. Additionally, iron does not appear to be a limiting factor in the co-culturing environment as it is not upregulated. This suggests that *S. aureus* is able to adapt to changes in the environment by modulating the expression of specific genes in order to survive. In addition, the upregulation of virulence genes may suggest that *S. aureus* is able to use the resources from host or another bacterial species. Similarly, a previous co-culture transcriptomics study between *S. aureus* and *P. aeruginosa* indicated that *S. aureus* has a significant impact on the gene expression of genes involved in *P. aeruginosa* carbon and amino acid metabolism (Camus et al., 2020). A transcriptome study of *S. anginosus* when grown with *S. aureus* and *P. aeruginosa* in a biofilm has also shown the impact on the expression of genes involved in cell wall synthesis and on cell wall thickness (Tavernier et al., 2018).

Further, our transcriptome analysis of *S. aureus* co-cultured with *S. anginosus* and tonsillar cells indicated upregulation of genes involved in riboflavin biosynthesis and downregulation of Staphylococcal ribosomal protein-encoding genes. This contrasts with the recent finding from the transcriptional interplay between *S. aureus* and *Malassezia restricta* co-existing during skin colonization (Yang et al., 2023). Riboflavin is a precursor to essential co-enzymes, Flavin mononucleotide and flavin adenine dinucleotide (Fischer and Bacher, 2005). *S. aureus* generates riboflavin via *de novo* biosynthesis or obtains it from the host environment (Zhang et al., 2010; Gutiérrez-Preciado et al., 2015). It is possible that *S. aureus* and *S. anginosus* may compete for riboflavin uptake in the co-culture environment in the presence of host cells. The upregulation of genes involved in riboflavin biosynthesis might be one of the reasons for the increased growth of *S. aureus* (Yang et al., 2023).

The expression of iron acquisition genes in *S. aureus* during co-culturing with *S. anginosus* was not detected after being exposed to host cells. This expression was highly upregulated in the monoculture study, where *S. aureus* was not co-cultured with *S. anginosus* (Bastakoti et al., 2023). This is in contrast to other co-culture studies between *P. aeruginosa* and *S. aureus* where they detected that *P. aeruginosa* kills *S. aureus* to acquire iron using the LasA protease and disperses the *S. aureus* biofilm (Woods et al., 2018; Tognon et al., 2019). Thus, our study suggests that *S. aureus* may not be able to acquire iron from its environment during co-culturing. This indicates that *S. aureus* may acquire iron from other sources, or that iron is not a limiting factor in the co-culturing environment. However, further studies are needed to better understand the role of iron in the co-culturing of *S. aureus* and *S. anginosus* with tonsillar cells.

We identified alterations in the expression of various *S. aureus* survival factors (for instance adhesion, virulence, iron-acquisition, and iron homeostasis genes) upon mono and co-culturing with *S. anginosus* and tonsillar cells. To identify the transcripts that were affected due to presence of *S. anginosus*, we compared survival factors exhibited by *S. aureus* during co-culturing with *S. anginosus* in the presence of tonsillar cells with our previous study where *S. aureus* alone was exposed to tonsillar cells (Bastakoti et al., 2023). Most of the DEGs were common to monoculture but the expression

level varied, and some genes were uniquely up/down regulated only in presence of *S. anginosus*. Interestingly, *isaB* was only upregulated in co-culture (1 h) with *S. anginosus* and tonsillar cells, and *sdrD* was upregulated at all conditions, except in *S. aureus* grown alone (3 h). Furthermore, some of the genes involved in secretion of toxins, such as *lukD*, *aur, hlgA* and *hlgB* were also expressed only after 3 h of co-culturing with *S. anginosus* and tonsillar cells. The results indicate that the expression of adhesins and toxins is not simultaneous, but rather follows a precise temporal sequence. It is therefore likely that adhesins play an important role in the attachment of *S. aureus* to tonsillar cells, followed by the secretion of toxins for further infection. In line with this, a previous study has indicated that interaction between *S. aureus* and *P. aeruginosa* can be beneficial for colonization and further lead toward pathogenicity (Alves et al., 2018). Our results are similar to the results of a previous study performed on *S. aureus* and *P. aeruginosa*, where the presence of *P. aeruginosa* caused changes in the transcriptome of *S. aureus* during internalization into epithelial cells (Briaud et al., 2019). This suggests that the presence of different microbes can have a significant effect on the transcriptome of *S. aureus* and could potentially have significant implications for the spread and control of bacterial infections.

We have also identified a set of DEGs in *S. aureus* that are involved in various pathways, such as amino acid metabolism, biosynthesis of secondary metabolites, biological adhesion, ribosomal protein, and rRNA binding, which all play a key role in ensuring the proper functioning and regulation of the biological system. Each of these processes and pathways might play a critical role in making *S. aureus* as a potential colonizer to the host cells (Schoenfelder et al., 2013; Alreshidi et al., 2022; Samuel et al., 2022). For instance, secondary metabolites are essential for producing molecules that enable *S. aureus* to adhere to host cells and cause infection. Importantly, our study has shown that certain genes exhibited by *S. aureus* play a key role in the response to *S. anginosus* while meeting tonsillar cells. The number of genes involved in GO terms during co-culturing were found to be less than monoculture, indicating that some sets of genes are not necessary to be differentially expressed during co-culturing with *S. anginosus*. Nevertheless, there were some GO terms such as "riboflavin biosynthesis", "lumazine binding domain" and "defense response", which were identified to be upregulated only during co-culturing environment. Thus, these identified genes, involved in several GO terms, are of particular importance for *S. aureus* and may be essential for its survival and adaptation to changing environments. It is important to note that there are overlapping genes in *S. aureus* and *S. anginosus* and this was expected. Some of the potential overlapping DEGs between *S. aureus* and *S. anginosus* were also found to be significantly enriched in GO terms related to ribosomal subunits and RNA binding. These enriched GO terms suggest that the same genes contribute to the assembly and functioning of ribosomes, as well as the regulation of RNA metabolism in both *S. aureus* and *S. anginosus*. In general, our findings demonstrate the importance of these genes in responding to *S. anginosus* in the presence of tonsillar cells and may lead to new treatments of infections or other diseases associated with throat colonization by *S. aureus*.

There are some limitations to this study: (1) Current results could change if different strains were used in the experiments because of intra-strain variability of expression landscape and colonization ability in *S. aureus*. We know that some strains are better adapted to humans, but repeated host adaptation events have happened in both human and animal directions (Richardson et al., 2018). (2) The transcriptomics profiling is performed only for *S. aureus* but neither for *S. anginosus* nor the host cell. The investigation of DEGs in *S. anginosus* might have revealed the unique and common genes involved by each bacterium during exposure to tonsillar cells (3). The number of reads mapped against the *S. anginosus* reference genome was very low, which could have resulted due to low recovery of *S. anginosus* RNA during total RNA extraction from co-culture samples. In line with this, a previous transcriptomic study of *S. anginosus* growing in a multispecies biofilm has also indicated a lower proportion of mapped reads against *S. anginosus* (Tavernier et al., 2018). In the case of RNA-seq for the *S. anginosus* mixed sample, it might be appropriate to increase the sequencing depth, so that the number of reads and aligned reads can be increased. Moreover, in our study, the separate sample clustering observed in the PCA plot also indicates the clear variation and gene clustering. Additionally, some of the significant DEGs variations observed in co-culture compared to monoculture, indicates that the presence of a low number of *S. anginosus* reads is enough to identify transcriptomics alteration in *S. aureus* during co-culturing.

In conclusion, our study identified several transcripts in *S. aureus* that might be important when facing a potential competitor during throat colonization. Alterations in expression of various *S. aureus* survival factors were observed when co-cultured with *S. anginosus* and tonsillar cells, especially in genes encoding adhesion protein, secreted proteins, and iron-acquisitions. These findings may be useful in the development of interventions against *S. aureus* throat colonization and suggest that a further investigation of the expression landscape is warranted to gain an improved understanding of the role of co-colonization in the host immune response.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/, GSE234900; https://www.ebi.ac.uk/ena, PRJEB59355.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

SB: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. MP: Formal analysis, Validation, Writing – review &

editing, Visualization. CA: Supervision, Writing – review & editing. KJ: Writing – review & editing, Methodology. JC: Writing – review & editing. MJ: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing, Visualization. A-MH: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2024.1326730/full#supplementary-material

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Table S1: Summary of trimming, and mapping efficacy against S. aureus and S. anginosus.

Replicate number	Sample ID	Filtering and Trimming		Assigned reads (%)	
		Before	After	Against *S. aureus* from Co-culture	Against *S. anginosus* from Co-culture
1st Biological	S1.ctr_1h.BR1	47,016,592	45,770,846	33, 459, 476 (73%)	431, 467 (1%)
	S2.trt_1h.BR1	45,222,333	44,992,247	5, 874, 067 (<20%)	30, 662 (0.1%)
	S3.ctr_3h.BR1	61,867,100	61,438,533	40, 126,209 (64%)	3,768,354 (5%)
	S4.trt_3h.BR1	66,505,739	66,122,361	45, 955,501 (69%)	1,169,906 (2%)
2nd Biological	S5.ctr_1h.BR2	54,908,205	53,983,097	34, 777, 674 (64%)	257,839 (1%)
	S6.trt_1h.BR2	184,884,504	183,304,704	86, 153, 211 (47%)	198,639 (0.1%)
	S7.ctr_3h.BR2	79,128,254	78,290,310	87, 170, 895 (63%)	5,288,902 (5%)
	S8.trt_3h.BR2	52,707,452	52,438,667	36, 661, 392 (69%)	2,796,840 (4%)
3rd Biological	S9.ctr_1h.BR3	152,951,194	151,687,550	100, 793, 534 (66%)	709,462 (1%)
	S10.trt_1h.BR3	153,371,425	152,701,103	40, 063, 655 (26%)	31, 215 (0.1%)
	S11.ctr_3h.BR3	59,656,507	58,949,731	33, 144, 791 (55%)	3,779,296 (5%)
	S12.trt_3h.BR3	63,512,640	63,172,198	39, 167, 211 (61%)	2,919, 438 (4%)

Table S2.Three hundred and thirty-two differentially expressed genes (DEGs) were identified in *S. aureus co-cultured with S. anginosus with/without tonsillar cells* after 1 h of exposure identified by RNA-seq. Out of 332 only 310 genes were pre-annotated and the remaining were unknown (NA). From known genes, 155 were identified as commonly expressed genes and 90 as uniquely differentially expressed genes at 1 h. Red and blue color shades indicates all the upregulated (173) and downregulated gene (159), respectively.

log2FoldChange	pvalue	padj	transcript_id	gene_name
7,60	2,55E-15	2,31E-14	t145_t084_12690	*NA*
7,23	2,69E-16	2,81E-15	t145_t084_12680	*NA*
6,56	8,55E-76	4,13E-73	t145_t084_04420	*ilvC*
6,44	6,55E-81	7,91E-78	t145_t084_04410	*leuA_1*
6,41	1,18E-68	4,75E-66	t145_t084_04400	*leuB*
6,29	1,43E-78	1,15E-75	t145_t084_04390	*leuC*
5,73	5,92E-54	1,43E-51	t145_t084_04380	*leuD*
5,71	2,17E-09	1,04E-08	t145_t084_21530	*NA*
5,60	9,62E-46	1,37E-43	t145_t084_04430	*ilvH*
5,41	1,71E-63	5,17E-61	t145_t084_11050	*thrB_1*
5,39	9,20E-10	4,59E-09	t145_t084_21540	*NA*
5,37	5,41E-46	8,17E-44	t145_t084_04370	*ilvA*
5,34	1,18E-60	3,16E-58	t145_t084_21270	*carA_2*
5,18	6,26E-39	4,73E-37	t145_t084_11070	*nadX*
5,11	4,92E-51	1,08E-48	t145_t084_11060	*thrC*
5,09	4,15E-68	1,43E-65	t145_t084_07330	*NA*
5,05	4,45E-43	5,37E-41	t145_t084_23240	*NA*
5,04	1,18E-88	2,85E-85	t145_t084_07320	*serA*
5,00	1,33E-21	2,38E-20	t145_t084_19180	*metQ_2*
5,00	1,53E-19	2,21E-18	t145_t084_24480	*NA*
4,93	1,37E-30	5,71E-29	t145_t084_11080	*yclM*
4,91	1,21E-19	1,76E-18	t145_t084_18000	*sdrC*
4,88	5,92E-08	2,24E-07	t145_t084_21550	*NA*
4,85	1,31E-40	1,22E-38	t145_t084_23220	*ykoD_2*
4,83	3,29E-41	3,32E-39	t145_t084_23230	*ykoC*
4,82	1,06E-26	2,98E-25	t145_t084_23210	*NA*
4,78	2,15E-49	4,32E-47	t145_t084_21280	*lcfB*
4,77	1,51E-22	3,05E-21	t145_t084_03490	*czrA*
4,64	6,88E-22	1,27E-20	t145_t084_23200	*salL*
4,59	1,23E-15	1,17E-14	t145_t084_19190	*metP_2*
4,59	5,64E-23	1,21E-21	t145_t084_17830	*NA*
4,48	2,03E-05	5,45E-05	t145_t084_21810	*ssuC*
4,46	2,33E-42	2,68E-40	t145_t084_01780	*panS*
4,44	2,50E-05	6,57E-05	t145_t084_21800	*NA*
4,38	9,08E-07	2,93E-06	t145_t084_21830	*nrtD*
4,34	2,17E-24	5,04E-23	t145_t084_03480	*czcD_1*
4,32	3,11E-28	1,01E-26	t145_t084_04440	*ilvB*
4,20	7,73E-06	2,21E-05	t145_t084_21820	*cmpC*
4,15	7,21E-19	9,48E-18	t145_t084_23390	*icaD*
4,14	6,81E-06	1,96E-05	t145_t084_21520	*ggt*
4,13	1,54E-05	4,21E-05	t145_t084_19200	*metN*
3,93	2,82E-30	1,12E-28	t145_t084_19810	*NA*
3,90	7,34E-11	4,17E-10	t145_t084_04630	*NA*
3,88	5,67E-07	1,88E-06	t145_t084_21840	*NA*
3,85	3,38E-24	7,72E-23	t145_t084_23600	*NA*
3,83	2,68E-25	6,88E-24	t145_t084_21490	*NA*
3,81	1,37E-48	2,55E-46	t145_t084_22810	*NA*

3,71	6,71E-08	2,52E-07	t145_t084_11250	*NA*
3,65	2,23E-34	1,25E-32	t145_t084_19980	*yitJ*
3,64	9,26E-16	8,88E-15	t145_t084_24340	*NA*
3,61	9,75E-24	2,14E-22	t145_t084_14290	*acp*
3,51	1,87E-22	3,70E-21	t145_t084_10420	*dapH_1*
3,49	1,61E-18	2,07E-17	t145_t084_10430	*dapB*
3,47	2,48E-77	1,50E-74	t145_t084_14520	*dppE_2*
3,45	3,38E-20	5,24E-19	t145_t084_24040	*yhdG*
3,44	8,46E-34	4,55E-32	t145_t084_19990	*metE*
3,43	1,49E-32	6,93E-31	t145_t084_22270	*butA*
3,43	7,18E-20	1,08E-18	t145_t084_04450	*ilvD*
3,34	6,99E-14	5,43E-13	t145_t084_14140	*NA*
3,33	3,07E-22	5,98E-21	t145_t084_22820	*NA*
3,31	9,03E-17	9,69E-16	t145_t084_10440	*dapA*
3,29	2,97E-39	2,31E-37	t145_t084_10360	*NA*
3,28	1,63E-22	3,26E-21	t145_t084_24310	*NA*
3,26	5,07E-13	3,67E-12	t145_t084_10450	*asd*
3,23	7,61E-48	1,23E-45	t145_t084_15320	*metQ_1*
3,22	5,43E-28	1,68E-26	t145_t084_04550	*nrgA*
3,20	9,56E-29	3,28E-27	t145_t084_19100	*gltA*
3,19	1,18E-19	1,73E-18	t145_t084_06460	*NA*
3,15	2,25E-29	8,10E-28	t145_t084_07190	*aroA_1*
3,14	8,74E-21	1,43E-19	t145_t084_10400	*alr1_2*
3,12	1,92E-11	1,17E-10	t145_t084_10460	*lysC*
3,10	4,99E-20	7,53E-19	t145_t084_06450	*NA*
3,10	1,76E-19	2,51E-18	t145_t084_10410	*scmP_2*
3,10	2,79E-40	2,40E-38	t145_t084_14530	*oppF_2*
3,10	2,04E-25	5,30E-24	t145_t084_23250	*NA*
3,09	3,85E-19	5,20E-18	t145_t084_24320	*NA*
3,04	8,70E-13	6,11E-12	t145_t084_15470	*argO*
2,97	4,53E-18	5,56E-17	t145_t084_19090	*gltB*
2,92	4,60E-08	1,77E-07	t145_t084_19840	*NA*
2,88	4,03E-14	3,24E-13	t145_t084_11340	*glnR*
2,86	6,22E-23	1,30E-21	t145_t084_14540	*oppD_2*
2,83	1,45E-30	5,94E-29	t145_t084_22840	*hutH*
2,77	1,98E-38	1,45E-36	t145_t084_22800	*metXA*
2,76	6,12E-13	4,40E-12	t145_t084_17990	*sdrD*
2,76	1,12E-12	7,76E-12	t145_t084_23400	*icaA*
2,74	3,61E-10	1,89E-09	t145_t084_24530	*NA*
2,72	4,62E-19	6,20E-18	t145_t084_10390	*lysA*
2,71	2,43E-11	1,47E-10	t145_t084_14510	*oppB_1*
2,70	7,62E-07	2,49E-06	t145_t084_02850	*NA*
2,68	4,31E-28	1,37E-26	t145_t084_06470	*NA*
2,68	2,25E-03	0,004371369	t145_t084_21560	*gsiA*
2,67	5,13E-21	8,68E-20	t145_t084_19110	*hdfR_2*
2,65	3,73E-19	5,07E-18	t145_t084_01470	*aaeA*
2,64	9,65E-17	1,03E-15	t145_t084_16260	*hisC_2*
2,60	3,75E-17	4,18E-16	t145_t084_19610	*NA*
2,58	2,27E-15	2,10E-14	t145_t084_10740	*NA*
2,58	2,59E-20	4,11E-19	t145_t084_22420	*spa*
2,58	5,75E-15	5,02E-14	t145_t084_09320	*NA*
2,58	1,75E-24	4,15E-23	t145_t084_01710	*yghA*
2,57	4,91E-16	4,95E-15	t145_t084_15160	*NA*
2,56	9,76E-07	3,13E-06	t145_t084_21850	*NA*

2,54	7,10E-29	2,49E-27	t145_t084_24960	*NA*
2,51	2,86E-09	1,34E-08	t145_t084_11330	*glnA*
2,51	6,77E-21	1,13E-19	t145_t084_24670	*NA*
2,51	3,01E-20	4,72E-19	t145_t084_01480	*mdtD*
2,51	5,46E-15	4,80E-14	t145_t084_14100	*NA*
2,51	1,98E-08	8,12E-08	t145_t084_19400	*lpl2_4*
2,49	1,90E-11	1,16E-10	t145_t084_07250	*isdH*
2,47	8,85E-26	2,38E-24	t145_t084_06480	*NA*
2,46	2,46E-27	7,18E-26	t145_t084_03620	*NA*
2,43	3,38E-13	2,48E-12	t145_t084_14560	*oppB_2*
2,42	1,86E-31	8,01E-30	t145_t084_10660	*trpF*
2,41	1,25E-13	9,53E-13	t145_t084_14550	*oppC_2*
2,38	1,98E-39	1,59E-37	t145_t084_07310	*gph_1*
2,37	3,39E-03	0,006339333	t145_t084_11540	*NA*
2,36	3,31E-14	2,70E-13	t145_t084_19970	*metC*
2,36	4,50E-07	1,52E-06	t145_t084_06880	*ribD*
2,33	1,12E-17	1,32E-16	t145_t084_15330	*metP_1*
2,33	9,24E-22	1,68E-20	t145_t084_01790	*NA*
2,32	8,31E-19	1,09E-17	t145_t084_03610	*abgB*
2,32	1,50E-21	2,66E-20	t145_t084_05310	*NA*
2,31	1,06E-08	4,56E-08	t145_t084_14080	*NA*
2,29	3,68E-19	5,03E-18	t145_t084_07940	*NA*
2,29	4,99E-30	1,88E-28	t145_t084_00710	*irtA*
2,28	1,74E-06	5,33E-06	t145_t084_06900	*ribBA*
2,28	1,68E-08	7,00E-08	t145_t084_19960	*metI*
2,28	1,53E-18	1,98E-17	t145_t084_11040	*NA*
2,27	4,76E-08	1,83E-07	t145_t084_22370	*sbnA*
2,27	6,98E-28	2,11E-26	t145_t084_13790	*NA*
2,27	6,24E-10	3,18E-09	t145_t084_10950	*NA*
2,26	1,78E-25	4,68E-24	t145_t084_14870	*NA*
2,25	7,62E-05	0,000188889	t145_t084_22360	*sbnB*
2,25	7,30E-07	2,39E-06	t145_t084_06890	*ribE*
2,24	1,28E-04	0,000300657	t145_t084_22350	*sbnC*
2,22	4,30E-03	0,007883144	t145_t084_21160	*gatB_2*
2,21	1,11E-19	1,64E-18	t145_t084_24470	*copA*
2,21	3,83E-13	2,79E-12	t145_t084_20120	*efeM*
2,19	4,18E-20	6,36E-19	t145_t084_06150	*traP*
2,18	1,83E-17	2,10E-16	t145_t084_10670	*trpC*
2,18	1,64E-07	5,85E-07	t145_t084_20130	*ydaF*
2,16	1,77E-19	2,51E-18	t145_t084_23680	*isaB*
2,16	1,21E-04	0,000285927	t145_t084_20230	*ulaC*
2,14	4,13E-15	3,67E-14	t145_t084_15340	*metN2*
2,14	3,51E-20	5,41E-19	t145_t084_01420	*NA*
2,13	4,81E-06	1,41E-05	t145_t084_14090	*NA*
2,13	6,10E-23	1,28E-21	t145_t084_12760	*argF*
2,12	5,38E-16	5,33E-15	t145_t084_24680	*NA*
2,11	3,57E-14	2,90E-13	t145_t084_19730	*nfrA*
2,11	1,07E-29	3,99E-28	t145_t084_11010	*katA*
2,11	2,69E-22	5,28E-21	t145_t084_10650	*trpB*
2,10	2,97E-16	3,07E-15	t145_t084_21790	*NA*
2,10	7,29E-04	0,001520896	t145_t084_04540	*yeeD*
2,08	1,20E-04	0,000283697	t145_t084_19720	*tcyP*
2,08	7,63E-18	9,22E-17	t145_t084_20070	*NA*
2,08	1,00E-04	0,000242632	t145_t084_20220	*NA*

2,07	2,93E-11	1,75E-10	t145_t084_20930	*rbsD*
2,07	1,24E-10	6,87E-10	t145_t084_00290	*abgT*
2,07	1,63E-11	1,00E-10	t145_t084_14500	*oppC_1*
2,07	2,50E-08	1,01E-07	t145_t084_02360	*NA*
2,07	2,46E-14	2,01E-13	t145_t084_07350	*glpQ_1*
2,07	4,84E-08	1,86E-07	t145_t084_15310	*NA*
2,06	6,21E-13	4,44E-12	t145_t084_23990	*acsA_2*
2,05	8,93E-16	8,67E-15	t145_t084_01800	*NA*
2,03	8,43E-09	3,66E-08	t145_t084_14480	*oppF_1*
2,03	3,39E-07	1,17E-06	t145_t084_10380	*NA*
2,03	6,19E-12	3,97E-11	t145_t084_22260	*wbgU*
2,03	4,46E-08	1,73E-07	t145_t084_06350	*NA*
2,03	1,11E-05	3,08E-05	t145_t084_06910	*ribH*
2,02	2,03E-40	1,82E-38	t145_t084_05550	*bcp*
2,02	9,31E-11	5,21E-10	t145_t084_02890	*adhR*
2,01	1,77E-24	4,16E-23	t145_t084_24110	*cocE*
2,01	2,43E-10	1,30E-09	t145_t084_00640	*cpdA_1*
2,01	6,35E-09	2,83E-08	t145_t084_07220	*acuA*
-2,00	1,05E-08	4,50E-08	t145_t084_02650	*rplR*
-2,04	4,32E-08	1,68E-07	t145_t084_11110	*nucH*
-2,05	1,16E-09	5,75E-09	t145_t084_10970	*NA*
-2,05	6,85E-16	6,75E-15	t145_t084_13970	*menD*
-2,05	2,62E-17	2,96E-16	t145_t084_13450	*pdhD*
-2,07	9,42E-16	9,00E-15	t145_t084_07970	*rplU*
-2,07	1,49E-17	1,73E-16	t145_t084_10470	*ybiT*
-2,07	9,31E-05	0,000226621	t145_t084_24360	*NA*
-2,08	9,64E-29	3,28E-27	t145_t084_08450	*yqeH*
-2,10	1,04E-10	5,81E-10	t145_t084_11610	*phaB*
-2,10	4,77E-25	1,19E-23	t145_t084_08460	*aroE*
-2,10	1,28E-17	1,50E-16	t145_t084_13040	*pheT*
-2,10	4,67E-19	6,24E-18	t145_t084_22170	*tet(38)*
-2,11	3,94E-07	1,34E-06	t145_t084_07740	*rpmI*
-2,11	5,57E-26	1,53E-24	t145_t084_02810	*rplM*
-2,12	5,84E-10	3,00E-09	t145_t084_11740	*NA*
-2,12	3,05E-30	1,19E-28	t145_t084_13540	*ythB*
-2,12	1,14E-12	7,90E-12	t145_t084_03670	*pyrG*
-2,13	4,13E-14	3,29E-13	t145_t084_09960	*NA*
-2,13	4,08E-15	3,65E-14	t145_t084_11730	*rplGA*
-2,14	1,95E-05	5,26E-05	t145_t084_12860	*fib*
-2,14	1,53E-19	2,21E-18	t145_t084_13050	*pheS*
-2,15	2,72E-11	1,63E-10	t145_t084_18180	*rplGB*
-2,15	2,18E-15	2,03E-14	t145_t084_01230	*treP_1*
-2,16	1,85E-05	5,00E-05	t145_t084_21330	*scn_3*
-2,18	1,04E-04	0,000250568	t145_t084_00850	*NA*
-2,18	1,14E-39	9,48E-38	t145_t084_18680	*hpt*
-2,19	4,80E-14	3,76E-13	t145_t084_11750	*nusA*
-2,20	4,56E-22	8,68E-21	t145_t084_13550	*ythA*
-2,22	1,10E-06	3,51E-06	t145_t084_04800	*NA*
-2,23	2,15E-12	1,44E-11	t145_t084_03750	*tdk*
-2,23	5,69E-21	9,54E-20	t145_t084_12460	*lspA*
-2,26	5,12E-12	3,30E-11	t145_t084_18160	*rpsG*
-2,26	1,54E-29	5,65E-28	t145_t084_07990	*rpmA*
-2,28	4,27E-09	1,96E-08	t145_t084_18770	*rplY*
-2,28	8,95E-14	6,87E-13	t145_t084_18170	*rpsL*

-2,29	1,42E-07	5,11E-07	t145_t084_13420	*potA*
-2,29	3,03E-18	3,76E-17	t145_t084_11720	*infB*
-2,30	6,93E-32	3,10E-30	t145_t084_18250	*rplK*
-2,32	1,01E-32	4,99E-31	t145_t084_13990	*menA*
-2,32	2,15E-10	1,16E-09	t145_t084_07670	*gapA2*
-2,33	6,83E-36	4,23E-34	t145_t084_02820	*rpsI*
-2,33	4,79E-22	9,04E-21	t145_t084_11420	*glpK*
-2,33	8,52E-10	4,27E-09	t145_t084_00350	*cntA*
-2,37	9,40E-07	3,02E-06	t145_t084_04740	*NA*
-2,38	1,90E-08	7,87E-08	t145_t084_07730	*infC*
-2,38	5,95E-28	1,82E-26	t145_t084_16850	*NA*
-2,38	6,53E-23	1,35E-21	t145_t084_12490	*ileS*
-2,40	2,30E-36	1,47E-34	t145_t084_01980	*odh*
-2,40	3,89E-21	6,72E-20	t145_t084_12450	*rluD_2*
-2,40	2,30E-36	1,47E-34	t145_t084_07410	*yfcA*
-2,41	2,08E-05	5,56E-05	t145_t084_12850	*scn_2*
-2,42	1,43E-14	1,20E-13	t145_t084_03760	*prfA*
-2,43	5,23E-11	3,05E-10	t145_t084_14040	*catD*
-2,45	5,70E-09	2,55E-08	t145_t084_00320	*cntK*
-2,45	2,45E-21	4,29E-20	t145_t084_01550	*gltS*
-2,45	1,52E-06	4,73E-06	t145_t084_09720	*NA*
-2,47	1,36E-32	6,56E-31	t145_t084_01990	*mleN_2*
-2,47	7,41E-11	4,21E-10	t145_t084_02660	*rpsE*
-2,48	1,05E-07	3,82E-07	t145_t084_25000	*xylB*
-2,48	2,78E-24	6,40E-23	t145_t084_07980	*NA*
-2,49	9,96E-20	1,48E-18	t145_t084_08130	*hisS*
-2,50	1,20E-08	5,11E-08	t145_t084_00330	*cntL*
-2,50	2,11E-09	1,01E-08	t145_t084_00360	*cntB*
-2,50	7,79E-14	6,04E-13	t145_t084_20180	*glpT*
-2,51	9,53E-25	2,28E-23	t145_t084_19300	*yciC_2*
-2,53	2,38E-08	9,64E-08	t145_t084_04780	*scn_1*
-2,55	3,43E-09	1,58E-08	t145_t084_15590	*emp*
-2,57	7,46E-15	6,41E-14	t145_t084_18150	*fusA*
-2,57	3,88E-08	1,52E-07	t145_t084_14830	*argH*
-2,58	3,34E-09	1,55E-08	t145_t084_11430	*glpF*
-2,59	3,53E-11	2,08E-10	t145_t084_02640	*rplF*
-2,60	1,04E-11	6,51E-11	t145_t084_02620	*rpsZ*
-2,67	4,97E-21	8,46E-20	t145_t084_09970	*NA*
-2,67	3,37E-06	1,01E-05	t145_t084_24990	*NA*
-2,68	1,12E-33	5,88E-32	t145_t084_18200	*rpoB*
-2,68	7,25E-22	1,33E-20	t145_t084_08140	*aspS*
-2,69	4,72E-12	3,05E-11	t145_t084_02630	*rpsH*
-2,71	1,34E-18	1,75E-17	t145_t084_00400	*cntE_1*
-2,71	8,50E-09	3,67E-08	t145_t084_00340	*cntM*
-2,74	2,87E-19	3,96E-18	t145_t084_11850	*rpsB*
-2,75	1,05E-12	7,34E-12	t145_t084_04120	*cshA*
-2,77	2,31E-10	1,25E-09	t145_t084_13410	*potB*
-2,78	1,20E-43	1,53E-41	t145_t084_10870	*opuD_2*
-2,78	7,69E-21	1,27E-19	t145_t084_03770	*prmC*
-2,80	8,83E-14	6,80E-13	t145_t084_19290	*ndhB*
-2,82	9,01E-16	8,71E-15	t145_t084_12000	*rplS*
-2,84	1,09E-15	1,04E-14	t145_t084_07750	*rplT*
-2,85	3,59E-14	2,90E-13	t145_t084_03980	*sceD*
-2,88	1,01E-28	3,38E-27	t145_t084_18240	*rplA*

-2,88	1,19E-09	5,88E-09	t145_t084_24380	*ssaA*
-2,88	3,97E-25	9,99E-24	t145_t084_13320	*typA*
-2,91	4,10E-21	7,02E-20	t145_t084_02570	*rpmC*
-2,92	1,31E-13	1,00E-12	t145_t084_06660	*pckA*
-2,95	9,20E-38	6,54E-36	t145_t084_02530	*rpsS*
-2,96	1,70E-18	2,17E-17	t145_t084_02580	*rpsQ*
-2,96	4,17E-30	1,60E-28	t145_t084_07360	*rpsD*
-2,96	1,08E-09	5,35E-09	t145_t084_00390	*cntF*
-2,97	4,21E-33	2,16E-31	t145_t084_19280	*NA*
-2,98	2,12E-17	2,42E-16	t145_t084_18220	*rplL*
-3,00	1,00E-08	4,33E-08	t145_t084_00380	*cntD*
-3,00	2,21E-36	1,47E-34	t145_t084_02480	*rpsJ*
-3,01	2,05E-32	9,34E-31	t145_t084_02510	*rplW*
-3,02	4,65E-27	1,34E-25	t145_t084_02520	*rplB*
-3,03	5,24E-22	9,82E-21	t145_t084_18230	*rplJ*
-3,05	5,13E-16	5,10E-15	t145_t084_02600	*rplX*
-3,05	1,31E-08	5,54E-08	t145_t084_14070	*NA*
-3,08	7,75E-33	3,90E-31	t145_t084_18190	*rpoC*
-3,08	1,28E-12	8,83E-12	t145_t084_02610	*rplE*
-3,09	2,42E-10	1,30E-09	t145_t084_00370	*cntC*
-3,09	9,13E-25	2,21E-23	t145_t084_19890	*ssbA_2*
-3,10	8,16E-42	8,58E-40	t145_t084_22750	*purA*
-3,12	1,14E-23	2,47E-22	t145_t084_19880	*rpsR*
-3,16	1,77E-15	1,66E-14	t145_t084_24940	*NA*
-3,17	7,87E-17	8,49E-16	t145_t084_02590	*rplN*
-3,18	4,56E-42	5,01E-40	t145_t084_02490	*rplC*
-3,19	4,26E-24	9,61E-23	t145_t084_02540	*rplV*
-3,22	2,05E-19	2,88E-18	t145_t084_02560	*rplP*
-3,22	1,33E-04	0,000309382	t145_t084_24350	*isaA*
-3,22	7,99E-36	4,83E-34	t145_t084_02500	*rplD*
-3,24	7,66E-25	1,89E-23	t145_t084_19900	*rpsF*
-3,26	6,11E-13	4,40E-12	t145_t084_13400	*ydcV*
-3,27	2,97E-17	3,34E-16	t145_t084_12440	*pyrR*
-3,32	1,36E-20	2,17E-19	t145_t084_22180	*deoD*
-3,33	2,21E-26	6,14E-25	t145_t084_10980	*guaC*
-3,34	5,77E-24	1,28E-22	t145_t084_02550	*rpsC*
-3,41	1,38E-14	1,16E-13	t145_t084_13390	*potD*
-3,46	3,08E-10	1,63E-09	t145_t084_15600	*NA*
-3,46	9,42E-20	1,41E-18	t145_t084_14980	*NA*
-3,47	1,43E-32	6,79E-31	t145_t084_03160	*NA*
-3,51	1,49E-44	2,00E-42	t145_t084_16490	*fruA_1*
-3,54	3,13E-22	6,04E-21	t145_t084_15000	*NA*
-3,57	3,83E-25	9,75E-24	t145_t084_15300	*NA*
-3,62	2,49E-05	6,57E-05	t145_t084_12890	*NA*
-3,62	1,26E-11	7,87E-11	t145_t084_12370	*pyrE*
-3,69	6,70E-11	3,84E-10	t145_t084_12380	*pyrF*
-3,75	1,17E-31	5,16E-30	t145_t084_02000	*ssaA2*
-3,76	4,28E-14	3,39E-13	t145_t084_14820	*argG*
-3,79	2,51E-30	1,01E-28	t145_t084_22460	*NA*
-3,82	4,17E-12	2,72E-11	t145_t084_12390	*carB*
-3,86	3,45E-12	2,26E-11	t145_t084_00030	*fnbB*
-3,96	3,28E-21	5,70E-20	t145_t084_12020	*rimM*
-4,11	3,15E-28	1,01E-26	t145_t084_12010	*trmD*
-4,12	6,93E-15	6,02E-14	t145_t084_00020	*fnbA*

-4,27	3,28E-08	1,30E-07	t145_t084_00820	*sbi*
-4,29	7,35E-28	2,19E-26	t145_t084_00950	*NA*
-4,39	4,98E-16	4,98E-15	t145_t084_12400	*carA_1*
-4,40	5,58E-29	1,99E-27	t145_t084_21660	*ptsG_3*
-4,46	2,47E-19	3,43E-18	t145_t084_20990	*lrgB*
-4,53	2,11E-15	1,97E-14	t145_t084_12410	*pyrC*
-4,56	2,29E-15	2,12E-14	t145_t084_12420	*pyrB*
-4,59	1,44E-17	1,68E-16	t145_t084_21410	*NA*
-4,60	8,61E-25	2,10E-23	t145_t084_16500	*lacC_2*
-5,11	2,52E-10	1,34E-09	t145_t084_12430	*pyrP*
-5,14	3,16E-35	1,82E-33	t145_t084_16510	*ydjF*
-5,15	1,67E-19	2,39E-18	t145_t084_19450	*NA*
-5,64	1,52E-13	1,14E-12	t145_t084_19460	*ssl5_1*
-5,82	1,15E-30	4,89E-29	t145_t084_21320	*NA*
-5,93	2,89E-13	2,13E-12	t145_t084_21000	*lrgA*

Table S3.Two hundred and seventy-nine differentially expressed genes (DEGs) were identified in S. aureus co-cultured with S. anginosus with/without tonsillar cells after 3 h of exposure identified by RNA-seq. Out of 279, only 207 genes were pre-annotated and the remaining were unknown (NA). From known genes, 155 were identified as commonly expressed genes and 52 as uniquely differentially expressed genes at 3 h. Red and blue color shades indicates all the upregulated (176) and downregulated (103) genes, respectively.

log2FoldChange	pvalue	padj	transcript_id	gene_name
8,14	3,95E-17	5,72E-16	t145_t084_12690	*NA*
6,29	1,12E-12	9,85E-12	t145_t084_12680	*NA*
6,22	2,60E-42	2,03E-40	t145_t084_23210	*NA*
6,04	7,14E-12	5,66E-11	t145_t084_21540	*NA*
5,96	8,13E-35	4,27E-33	t145_t084_23200	*salL*
5,93	6,38E-25	1,68E-23	t145_t084_19190	*metP_2*
5,88	7,27E-11	4,98E-10	t145_t084_21550	*NA*
5,84	1,67E-66	8,06E-64	t145_t084_21270	*carA_2*
5,78	1,89E-35	1,02E-33	t145_t084_10460	*lysC*
5,58	4,40E-81	3,55E-78	t145_t084_07330	*NA*
5,55	2,89E-52	5,82E-50	t145_t084_23220	*ykoD_2*
5,49	5,50E-43	4,74E-41	t145_t084_10440	*dapA*
5,38	8,93E-25	2,32E-23	t145_t084_19180	*metQ_2*
5,37	1,36E-50	2,05E-48	t145_t084_04420	*ilvC*
5,36	1,11E-55	2,43E-53	t145_t084_04410	*leuA_1*
5,35	3,02E-41	2,28E-39	t145_t084_10430	*dapB*
5,31	5,92E-32	2,51E-30	t145_t084_10450	*asd*
5,22	2,10E-47	2,41E-45	t145_t084_10420	*dapH_1*
5,21	7,59E-94	1,83E-90	t145_t084_07320	*serA*
5,19	1,98E-33	9,56E-32	t145_t084_04430	*ilvH*
5,15	2,56E-44	2,47E-42	t145_t084_04400	*leuB*
5,15	7,01E-08	2,96E-07	t145_t084_21530	*NA*
4,96	2,10E-07	8,19E-07	t145_t084_19200	*metN*
4,95	1,34E-48	1,62E-46	t145_t084_04390	*leuC*
4,92	1,32E-42	1,10E-40	t145_t084_14290	*acp*
4,86	1,70E-34	8,74E-33	t145_t084_11070	*nadX*
4,84	8,74E-46	8,80E-44	t145_t084_11060	*thrC*
4,80	7,62E-74	4,60E-71	t145_t084_22810	*NA*
4,76	8,38E-40	5,79E-38	t145_t084_23230	*ykoC*
4,73	1,12E-32	4,92E-31	t145_t084_04440	*ilvB*
4,72	1,09E-48	1,38E-46	t145_t084_11050	*thrB_1*
4,71	1,56E-46	1,71E-44	t145_t084_01780	*panS*
4,62	7,96E-41	5,66E-39	t145_t084_22820	*NA*
4,59	2,19E-27	6,71E-26	t145_t084_11330	*glnA*
4,56	6,86E-65	2,76E-62	t145_t084_19170	*sle1*
4,54	3,36E-14	3,64E-13	t145_t084_04630	*NA*
4,54	2,25E-37	1,33E-35	t145_t084_06460	*NA*
4,44	8,82E-32	3,67E-30	t145_t084_04380	*leuD*
4,41	7,80E-07	2,76508E-06	t145_t084_21830	*nrtD*
4,41	3,23E-33	1,44E-31	t145_t084_23240	*NA*
4,33	2,20E-36	1,23E-34	t145_t084_21280	*lcfB*

4,30	1,55E-23	3,60E-22	t145_t084_11080	*yclM*
4,27	3,96E-21	7,65E-20	t145_t084_24340	*NA*
4,26	7,00E-30	2,52E-28	t145_t084_24040	*yhdG*
4,25	2,47E-36	1,36E-34	t145_t084_16230	*dtpT*
4,24	8,74E-29	2,98E-27	t145_t084_11340	*glnR*
4,22	3,07E-21	6,09E-20	t145_t084_14140	*NA*
4,18	1,07E-31	4,36E-30	t145_t084_06450	*NA*
4,15	1,55E-27	4,80E-26	t145_t084_04370	*ilvA*
4,13	2,85E-33	1,30E-31	t145_t084_10410	*scmP_2*
4,04	2,35E-33	1,09E-31	t145_t084_10400	*alr1_2*
4,04	1,79128E-05	5,2479E-05	t145_t084_21820	*cmpC*
4,01	5,81E-26	1,63E-24	t145_t084_04450	*ilvD*
3,99	1,20E-57	2,90E-55	t145_t084_06470	*NA*
3,89	4,44E-24	1,07E-22	t145_t084_17990	*sdrD*
3,81	0,000297574	0,000723037	t145_t084_21810	*ssuC*
3,80	9,86E-21	1,83E-19	t145_t084_06420	*splF*
3,77	4,09448E-05	0,000112972	t145_t084_21520	*ggt*
3,77	6,92E-61	2,39E-58	t145_t084_03620	*NA*
3,72	2,30E-30	8,68E-29	t145_t084_14100	*NA*
3,69	1,33E-18	2,13E-17	t145_t084_14510	*oppB_1*
3,66	1,34E-85	1,62E-82	t145_t084_14520	*dppE_2*
3,61	4,71E-38	2,92E-36	t145_t084_22420	*spa*
3,60	7,47E-59	2,01E-56	t145_t084_15320	*metQ_1*
3,59	1,54E-42	1,24E-40	t145_t084_03610	*abgB*
3,59	1,79E-51	2,89E-49	t145_t084_13880	*atl_3*
3,58	4,18E-21	7,95E-20	t145_t084_23600	*NA*
3,56	7,74E-39	4,92E-37	t145_t084_15330	*metP_1*
3,56	1,27E-31	5,12E-30	t145_t084_04550	*nrgA*
3,55	2,73E-34	1,35E-32	t145_t084_23860	*bsaA_2*
3,54	2,00E-34	1,00E-32	t145_t084_19100	*gltA*
3,52	1,17E-25	3,24E-24	t145_t084_19970	*metC*
3,50	5,37E-60	1,62E-57	t145_t084_22800	*metXA*
3,48	2,25E-33	1,07E-31	t145_t084_22270	*butA*
3,48	5,22E-50	7,42E-48	t145_t084_14530	*oppF_2*
3,44	4,32E-46	4,54E-44	t145_t084_06480	*NA*
3,43	4,86E-41	3,56E-39	t145_t084_01710	*yghA*
3,41	1,90E-43	1,70E-41	t145_t084_06410	*splE*
3,35	7,60E-31	2,96E-29	t145_t084_14540	*oppD_2*
3,34	1,75103E-05	5,15497E-05	t145_t084_21840	*NA*
3,31	2,67E-24	6,58E-23	t145_t084_14550	*oppC_2*
3,31	3,24E-23	7,33E-22	t145_t084_14560	*oppB_2*
3,26	2,65E-14	2,92E-13	t145_t084_15470	*argO*
3,24	6,80E-44	6,32E-42	t145_t084_01420	*NA*
3,24	2,11E-39	1,42E-37	t145_t084_22840	*hutH*
3,21	4,58E-27	1,37E-25	t145_t084_23250	*NA*
3,20	4,82E-08	2,09E-07	t145_t084_22350	*sbnC*
3,13	0,002929017	0,006040473	t145_t084_21800	*NA*
3,11	3,99E-14	4,23E-13	t145_t084_19960	*metI*

3,09	9,12E-25	2,34E-23	t145_t084_19980	*yitJ*
3,08	1,22E-08	5,79E-08	t145_t084_18000	*sdrC*
3,07	2,60E-29	8,99E-28	t145_t084_15340	*metN2*
3,06	7,47E-08	3,13E-07	t145_t084_22360	*sbnB*
3,05	2,73E-13	2,53E-12	t145_t084_20130	*ydaF*
3,04	3,26E-10	2,02E-09	t145_t084_04740	*NA*
3,04	2,35E-23	5,35E-22	t145_t084_20120	*efeM*
3,04	3,96E-12	3,22E-11	t145_t084_23540	*asp1*
3,02	9,38266E-05	0,000246499	t145_t084_00820	*sbi*
2,95	9,80E-18	1,50E-16	t145_t084_19090	*gltB*
2,94	5,93E-22	1,25E-20	t145_t084_10390	*lysA*
2,90	4,83E-19	7,99E-18	t145_t084_01700	*scmP_1*
2,87	6,30E-24	1,49E-22	t145_t084_19990	*metE*
2,79	2,57E-10	1,61E-09	t145_t084_23390	*icaD*
2,78	4,21E-12	3,41E-11	t145_t084_23400	*icaA*
2,77	1,57E-09	8,61E-09	t145_t084_19400	*lpl2_4*
2,77	2,40E-14	2,66E-13	t145_t084_20110	*efeN*
2,75	4,99E-52	8,61E-50	t145_t084_07310	*gph_1*
2,75	1,14E-24	2,85E-23	t145_t084_24670	*NA*
2,72	4,51E-39	2,94E-37	t145_t084_00710	*irtA*
2,70	6,71E-20	1,18E-18	t145_t084_01470	*aaeA*
2,66	1,18E-27	3,76E-26	t145_t084_05310	*NA*
2,66	1,56E-18	2,49E-17	t145_t084_16240	*NA*
2,66	5,18E-24	1,24E-22	t145_t084_24680	*NA*
2,66	2,79E-24	6,82E-23	t145_t084_03440	*ylmA*
2,64	2,21E-14	2,47E-13	t145_t084_24320	*NA*
2,62	4,99E-08	2,16E-07	t145_t084_14090	*NA*
2,59	3,99E-16	5,27E-15	t145_t084_16260	*hisC_2*
2,57	1,15E-21	2,36E-20	t145_t084_10670	*trpC*
2,56	1,66E-10	1,08E-09	t145_t084_10380	*NA*
2,55	1,91E-30	7,33E-29	t145_t084_10660	*trpF*
2,55	7,81E-20	1,37E-18	t145_t084_07190	*aroA_1*
2,55	2,38E-06	7,85E-06	t145_t084_22340	*sbnD*
2,55	1,04E-36	6,00E-35	t145_t084_09910	*NA*
2,54	7,31E-19	1,19E-17	t145_t084_19110	*hdfR_2*
2,52	3,77E-07	1,41E-06	t145_t084_00810	*hlgA*
2,51	3,18E-20	5,66E-19	t145_t084_01480	*mdtD*
2,51	9,82E-14	9,81E-13	t145_t084_24310	*NA*
2,50	7,76E-23	1,69E-21	t145_t084_08360	*pxpC_1*
2,48	1,72E-11	1,30E-10	t145_t084_21490	*NA*
2,46	4,34E-07	1,60E-06	t145_t084_03490	*czrA*
2,44	9,93E-09	4,78E-08	t145_t084_03480	*czcD_1*
2,44	7,47E-11	5,10E-10	t145_t084_20510	*NA*
2,42	3,84E-27	1,16E-25	t145_t084_10650	*trpB*
2,41	6,81E-06	2,09466E-05	t145_t084_23380	*icaB_2*
2,41	8,93E-14	9,03E-13	t145_t084_00290	*abgT*
2,40	3,60E-32	1,56E-30	t145_t084_00860	*farB_1*
2,38	2,51E-15	3,05E-14	t145_t084_23690	*aur*

2,33	3,61E-08	1,59E-07	t145_t084_06310	*lukDv_1*
2,33	1,07E-10	7,12E-10	t145_t084_07220	*acuA*
2,32	4,52E-12	3,64E-11	t145_t084_00870	*NA*
2,32	1,22E-11	9,51E-11	t145_t084_19810	*NA*
2,32	2,17E-20	3,94E-19	t145_t084_10360	*NA*
2,32	1,39E-12	1,20E-11	t145_t084_24850	*mhqR*
2,28	9,29E-14	9,36E-13	t145_t084_08380	*accC*
2,25	5,74E-12	4,60E-11	t145_t084_14500	*oppC_1*
2,24	1,25E-15	1,57E-14	t145_t084_00070	*NA*
2,24	1,48E-37	8,92E-36	t145_t084_24440	*bacF*
2,23	3,08E-30	1,13E-28	t145_t084_01870	*NA*
2,21	2,26E-13	2,14E-12	t145_t084_15620	*NA*
2,19	7,32E-16	9,41E-15	t145_t084_07350	*glpQ_1*
2,19	3,64E-12	2,99E-11	t145_t084_20100	*NA*
2,19	3,46E-13	3,18E-12	t145_t084_04730	*dapE*
2,18	5,55E-07	2,01E-06	t145_t084_04750	*NA*
2,17	1,11E-08	5,34E-08	t145_t084_20810	*esxA*
2,17	1,94E-15	2,38E-14	t145_t084_24760	*pitA*
2,15	2,36E-16	3,20E-15	t145_t084_23550	*asp2*
2,14	1,76E-17	2,66E-16	t145_t084_24830	*catE_2*
2,13	8,94E-12	7,02E-11	t145_t084_02890	*adhR*
2,12	3,46E-18	5,42E-17	t145_t084_01790	*NA*
2,12	1,37E-08	6,48E-08	t145_t084_02360	*NA*
2,09	2,77436E-05	7,82454E-05	t145_t084_02130	*ydbM*
2,09	2,95E-07	1,12E-06	t145_t084_00790	*hlgB*
2,08	5,05E-06	1,58625E-05	t145_t084_11320	*NA*
2,08	1,30E-16	1,81E-15	t145_t084_10680	*trpD2*
2,07	6,04E-11	4,24E-10	t145_t084_06320	*lukDv_2*
2,07	2,46E-08	1,11E-07	t145_t084_15570	*nuc*
2,06	7,55E-16	9,65E-15	t145_t084_08350	*pxpB_1*
2,05	9,81E-13	8,62E-12	t145_t084_05320	*NA*
2,04	5,49E-07	1,99E-06	t145_t084_14080	*NA*
2,04	0,000173554	0,000436957	t145_t084_21850	*NA*
2,03	3,35E-25	8,90E-24	t145_t084_04600	*NA*
2,02	1,64E-12	1,39E-11	t145_t084_23990	*acsA_2*
2,01	2,26E-09	1,21E-08	t145_t084_24920	*NA*
2,01	4,32E-17	6,22E-16	t145_t084_04610	*NA*
2,01	1,50E-06	5,07E-06	t145_t084_19220	*mccA*
1,44	2,20E-05	4,82E-05	t145_t084_12690	*NA*
-2,02	3,81E-11	2,74E-10	t145_t084_11760	*rimP*
-2,02	1,63E-13	1,56E-12	t145_t084_12130	*plsX*
-2,02	7,92E-24	1,86E-22	t145_t084_10870	*opuD_2*
-2,04	2,97E-15	3,57E-14	t145_t084_02500	*rplD*
-2,06	2,86E-11	2,08E-10	t145_t084_07140	*NA*
-2,06	4,43E-15	5,30E-14	t145_t084_11720	*infB*
-2,07	5,16E-09	2,58E-08	t145_t084_22180	*deoD*
-2,08	3,96E-10	2,39E-09	t145_t084_02340	*NA*
-2,08	5,33E-21	1,01E-19	t145_t084_18200	*rpoB*

-2,09	7,27E-23	1,60E-21	t145_t084_09240	*bfmBAB*
-2,09	5,51E-13	4,95E-12	t145_t084_12110	*fabG*
-2,09	6,43E-08	2,73E-07	t145_t084_18770	*rplY*
-2,10	5,95E-07	2,15E-06	t145_t084_12030	*rpsP*
-2,10	8,99E-29	3,02E-27	t145_t084_13480	*pdhA*
-2,11	1,89E-06	6,31E-06	t145_t084_24940	*NA*
-2,12	1,21E-12	1,06E-11	t145_t084_12120	*fabD*
-2,12	1,46E-11	1,13E-10	t145_t084_03760	*prfA*
-2,12	1,42E-10	9,32E-10	t145_t084_07130	*NA*
-2,13	3,73E-08	1,64E-07	t145_t084_04120	*cshA*
-2,13	1,45E-11	1,12E-10	t145_t084_19900	*rpsF*
-2,13	1,38E-12	1,20E-11	t145_t084_19890	*ssbA_2*
-2,13	2,46E-08	1,11E-07	t145_t084_02620	*rpsZ*
-2,14	5,33E-08	2,29E-07	t145_t084_06660	*pckA*
-2,14	1,49E-27	4,68E-26	t145_t084_13470	*pdhB*
-2,15	2,20E-17	3,27E-16	t145_t084_02510	*rplW*
-2,15	1,63E-08	7,62E-08	t145_t084_02590	*rplN*
-2,18	5,54E-10	3,27E-09	t145_t084_18220	*rplL*
-2,18	2,60E-11	1,90E-10	t145_t084_12140	*fapR*
-2,18	1,76E-21	3,55E-20	t145_t084_22750	*purA*
-2,19	6,34E-10	3,71E-09	t145_t084_07750	*rplT*
-2,20	3,70E-14	3,96E-13	t145_t084_11750	*nusA*
-2,20	5,18E-16	6,73E-15	t145_t084_11730	*rplGA*
-2,20	1,03E-06	3,58167E-06	t145_t084_18420	*pdxT*
-2,21	2,45E-12	2,05E-11	t145_t084_12600	*mraY*
-2,21	3,20E-21	6,29E-20	t145_t084_02490	*rplC*
-2,21	3,02E-06	9,80E-06	t145_t084_24380	*ssaA*
-2,22	1,65E-07	6,64E-07	t145_t084_07730	*infC*
-2,22	5,20E-26	1,48E-24	t145_t084_09250	*pdhC_1*
-2,23	2,72E-07	1,05E-06	t145_t084_02610	*rplE*
-2,23	2,04E-31	8,09E-30	t145_t084_13460	*pdhC_2*
-2,23	3,33E-09	1,75E-08	t145_t084_17700	*NA*
-2,24	5,71E-11	4,02E-10	t145_t084_11740	*NA*
-2,25	2,26E-09	1,21E-08	t145_t084_02600	*rplX*
-2,25	4,12E-21	7,90E-20	t145_t084_02480	*rpsJ*
-2,25	3,39E-14	3,65E-13	t145_t084_03770	*prmC*
-2,26	1,38E-09	7,65E-09	t145_t084_03980	*sceD*
-2,26	1,80E-15	2,22E-14	t145_t084_09950	*rlmL*
-2,27	1,76E-11	1,33E-10	t145_t084_02580	*rpsQ*
-2,27	3,21E-16	4,29E-15	t145_t084_13320	*typA*
-2,28	4,58E-08	2,00E-07	t145_t084_07740	*rpmI*
-2,29	9,68E-14	9,71E-13	t145_t084_02570	*rpmC*
-2,30	1,27E-10	8,32E-10	t145_t084_02560	*rplP*
-2,32	1,83E-19	3,09E-18	t145_t084_02330	*cntE_2*
-2,33	6,90E-06	2,11627E-05	t145_t084_09720	*NA*
-2,34	1,02E-13	1,01E-12	t145_t084_02540	*rplV*
-2,34	1,68E-14	1,91E-13	t145_t084_11850	*rpsB*
-2,35	1,18E-28	3,86E-27	t145_t084_11830	*pyrH*

-2,35	1,37E-19	2,35E-18	t145_t084_07360	*rpsD*
-2,35	1,14E-24	2,85E-23	t145_t084_02530	*rpsS*
-2,38	4,14E-22	8,85E-21	t145_t084_13040	*pheT*
-2,38	2,24E-17	3,30E-16	t145_t084_09960	*NA*
-2,40	2,13E-14	2,40E-13	t145_t084_18230	*rplJ*
-2,40	1,31E-13	1,27E-12	t145_t084_11610	*phaB*
-2,41	3,29E-23	7,37E-22	t145_t084_13450	*pdhD*
-2,42	1,76E-23	4,04E-22	t145_t084_12490	*ileS*
-2,43	4,43E-13	4,04E-12	t145_t084_10970	*NA*
-2,44	2,17E-11	1,63E-10	t145_t084_15000	*NA*
-2,45	1,45E-07	5,87E-07	t145_t084_18430	*pdxS*
-2,45	3,34E-28	1,08E-26	t145_t084_11820	*frr*
-2,46	2,94E-25	7,90E-24	t145_t084_13050	*pheS*
-2,48	6,16E-14	6,36E-13	t145_t084_02550	*rpsC*
-2,49	4,39E-22	9,30E-21	t145_t084_18190	*rpoC*
-2,50	9,33E-18	1,44E-16	t145_t084_03160	*NA*
-2,52	3,62E-09	1,88E-08	t145_t084_15590	*emp*
-2,52	1,86E-08	8,65E-08	t145_t084_07270	*tyrS*
-2,57	0,002262689	0,004776349	t145_t084_24350	*isaA*
-2,63	1,39E-14	1,60E-13	t145_t084_11620	*NA*
-2,65	3,57E-17	5,23E-16	t145_t084_06570	*NA*
-2,65	2,23E-21	4,45E-20	t145_t084_08140	*aspS*
-2,70	5,81E-23	1,29E-21	t145_t084_08130	*hisS*
-2,71	2,53E-13	2,38E-12	t145_t084_11110	*nucH*
-2,73	1,56E-15	1,93E-14	t145_t084_15300	*NA*
-2,78	5,78E-15	6,85E-14	t145_t084_11630	*NA*
-2,80	3,48E-07	1,32E-06	t145_t084_15600	*NA*
-2,81	1,90E-25	5,22E-24	t145_t084_01230	*treP_1*
-2,98	3,48E-09	1,82E-08	t145_t084_21320	*NA*
-3,00	1,43E-09	7,90E-09	t145_t084_20990	*lrgB*
-3,01	5,65E-13	5,06E-12	t145_t084_12020	*rimM*
-3,02	4,86E-16	6,35E-15	t145_t084_12010	*trmD*
-3,05	1,27E-08	6,01E-08	t145_t084_21410	*NA*
-3,05	2,77E-15	3,35E-14	t145_t084_12440	*pyrR*
-3,31	5,46E-10	3,24E-09	t145_t084_12370	*pyrE*
-3,41	2,18E-09	1,17E-08	t145_t084_19450	*NA*
-3,50	5,82E-19	9,57E-18	t145_t084_21660	*ptsG_3*
-3,50	4,45E-06	1,40967E-05	t145_t084_19460	*ssl5_1*
-3,53	1,11E-29	3,94E-28	t145_t084_10980	*guaC*
-3,55	1,21E-10	8,02E-10	t145_t084_12390	*carB*
-3,55	3,30E-10	2,03E-09	t145_t084_12380	*pyrF*
-3,74	7,71E-11	5,25E-10	t145_t084_12420	*pyrB*
-3,95	2,68E-13	2,50E-12	t145_t084_12400	*carA_1*
-4,00	7,04E-07	2,52E-06	t145_t084_12430	*pyrP*
-4,11	4,14E-07	1,53E-06	t145_t084_21000	*lrgA*
-4,45	6,49E-15	7,61E-14	t145_t084_12410	*pyrC*

Table S4: Separate gene information of *S. aureus* and *S. anginosis* . The genes identified as differentially expressed in *S. aureus* were compared against the annotation file of *S. anignosis* and those genes only exhibited by *S. aureus* were differentiated manually.

Genes also present in S. anginosis (N=112)			**DEGs only present in S. aureus (N=185)**		
Both at 1h and 3h (n=68)	**only at 1h (n=29)**	**only at 3h (n=15)**	**Both at 1h and 3h (n=81)**	**only at 1h (n=65)**	**only at 3h (n=39)**
asd	*aroE*	*accC*	*abgB*	*argF*	*asp1*
aspS	*copA*	*bfmBAB*	*abgT*	*argG*	*asp2*
carA_1	*fruA_1*	*dapE*	*acp*	*argH*	*atl_3*
carB	*glpF*	*fabD*	*acsA_2*	*bcp*	*aur*
cshA	*glpK*	*fabG*	*acuA*	*catD*	*bacF*
czcD_1	*hpt*	*plsX*	*adhR*	*cntA*	*bsaA_2*
dapA	*lacC_2*	*pyrH*	*alr1_2*	*cntB*	*catE_2*
dapB	*oppF_1*	*rimP*	*argO*	*cntC*	*cntE_2*
dapH_1	*potA*	*yclM*	*aroA_1*	*cntD*	*dtpT*
glnA	*potB*	*frr*	*butA*	*cntE_1*	*efeN*
glnR	*potD*	*mhqR*	*carA_2*	*cntF*	*esxA*
gph_1	*pyrG*	*pdhC_1*	*cmpC*	*cntK*	*fapR*
guaC	*rplA*	*rlmL*	*czrA*	*cntL*	*farB_1*
hisS	*rplU*	*rpsP*	*deoD*	*cntM*	*hdfR_2*
infC	*rplK*	*tyrS*	*dppE_2*	*cocE*	*hisC_2*
leuA_1	*rpmA*		*efeM*	*cpdA_1*	*hlgA*
lysA	*rpsG*		*emp*	*fib*	*hlgB*
mdtD	*rpsL*		*ggt*	*fnbA*	*icaB_2*
metE	*rplF*		*glpQ_1*	*fnbB*	*lukDv_1*
metI	*rplM*		*gltA*	*fusA*	*mccA*
metN	*rplR*		*gltB*	*gapA2*	*metC*
metP_1	*rplS*		*hutH*	*gatB_2*	*mraY*
metQ_1	*rpsE*		*icaA*	*glpT*	*pdhA*
nadX	*rpsH*		*icaD*	*gltS*	*pdhB*
nusA	*rpsR*		*ileS*	*gsiA*	*pdhC_2*
oppC_1	*tdk*		*ilvA*	*hdfR_2*	*pitA*
pheS	*ulaC*		*ilvB*	*hisC_2*	*pdxS*
pheT	*ydjF*		*ilvC*	*isaB*	*pdxT*
prfA	*yqeH*		*ilvD*	*isdH*	*pxpB_1*
prmC			*ilvH*	*katA*	*pxpC_1*
purA			*infB*	*lspA*	*rplY*
pyrB			*irtA*	*menA*	*sbnD*
pyrC			*isaA*	*menD*	*scmP_1*
pyrE			*lcfB*	*metC*	*sle1*
pyrF			*leuB*	*mleN_2*	*splE*
pyrP			*leuC*	*ndhB*	*splF*
pyrR			*leuD*	*nfrA*	*trpD2*
rimM			*lpl2_4*	*odh*	*ylmA*

rplC
rplD
rplE
rplGA
rplJ
rplL
rplN
rplP
rplT
rplV
rplW
rplX
rpmC
rpmI
rpoB
rpoC
rpsB
rpsC
rpsD
rpsF
rpsJ
rpsQ
rpsS
salL
thrB_1
thrC
trmD
typA
yhdG
ykoD_2

lrgA	*rbsD*	*ydbM*
lrgB	*ribBA*	
lysC	*ribD*	
metP_2	*ribE*	
metQ_2	*ribH*	
metXA	*rluD_2*	
nrgA	*rplB*	
nrtD	*rplGB*	
nucH	*rplY*	
oppB_1	*sbnA*	
oppB_2	*scn_1*	
oppC_2	*scn_2*	
oppD_2	*scn_3*	
oppF_2	*ssaA2*	
opuD_2	*tcyP*	
panS	*tet(38)*	
pckA	*traP*	
pdhD	*wbgU*	
phaB	*xylB*	
ptsG_3	*ybiT*	
rpsZ	*yciC_2*	
sbi	*yclM*	
sbnB	*ydcV*	
sbnC	*yeeD*	
sceD	*yfcA*	
scmP_2	*ythA*	
sdrC	*ythB*	
sdrD		
serA		
spa		
ssaA		
ssbA_2		
ssl5_1		
ssuC		
treP_1		
trpB		
trpC		
trpF		
ydaF		
yghA		
yitJ		
ykoC		
aaeA		

Data Sheet 1: Gene ontology analysis of differentially expressed genes associated with *S. aureus* during coculturing with *S. anginosus* and tonsillar cells.

Gene name	Term/gene function	Gene count	Enrichment FDR
SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:1901607/Alpha-amino acid biosynthetic process	13	< 0.05
SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0008652/Cellular amino acid biosynthetic process	13	< 0.05
ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0009081/Branched-chain amino acid metabolic process	6	< 0.05
ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0009082/Branched-chain amino acid biosynthetic process	6	< 0.05
HUTH, SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:1901605/Alpha-amino acid metabolic process	14	< 0.05
SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0046394/Carboxylic acid biosynthetic process	13	< 0.05
SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0016053/Organic acid biosynthetic process	13	< 0.05
HUTH, SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0006520/Cellular amino acid metabolic process	14	< 0.05
HUTH, SBNA, TRPC, TRPB, DAPA, ILVD, LEUC, LEUD, ILVA	GO:0016829/Lyase activity	9	< 0.05
ILVD, ILVC, ILVA	GO:0006549/Isoleucine metabolic process	3	< 0.05
LEUB, LEUC, LEUD	GO:0006551/Leucine metabolic process	3	< 0.05
ILVD, ILVC, ILVA	GO:0009097/Isoleucine biosynthetic process	3	< 0.05
LEUB, LEUC, LEUD	GO:0009098/Leucine biosynthetic process	3	< 0.05
ESXA, SLE1, SDRC, SDRD, ISDH, SPLF, SPLE, SBI, HLGB	GO:0005576/Extracellular region	9	< 0.05

SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, RIBH, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0044283/Small molecule biosynthetic process	14	< 0.05
TRPB, DAPA, ILVD, LEUC, LEUD	GO:0016836/Hydro-lyase activity	5	< 0.05
SDRC, SDRD, ICAA	GO:0007155/Cell adhesion	3	< 0.05
SDRC, SDRD, ICAA	GO:0022610/Biological adhesion	3	< 0.05
SBNA, TRPB, ILVA	IPR001926/Pyridoxal-phosphate dependent enzyme	3	< 0.05
SBNA, TRPB, ILVA	IPR036052/Tryptophan synthase beta subunit-like PLP-dependent enzyme	3	< 0.05
HUTH, SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0019752/Carboxylic acid metabolic process	14	< 0.05
HUTH, SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0043436/Oxoacid metabolic process	14	< 0.05
TRPB, DAPA, ILVD, LEUC, LEUD	GO:0016835/Carbon-oxygen lyase activity	5	< 0.05
METE, DAPA, DAPB, ILVA	GO:0009066/Aspartate family amino acid metabolic process	4	< 0.05
TRPC, TRPF, TRPB	GO:0000162/Tryptophan biosynthetic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0006568/Tryptophan metabolic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0006586/Indolalkylamine metabolic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0042430/Indole-containing compound metabolic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0042435/Indole-containing compound biosynthetic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0046219/Indolalkylamine biosynthetic process	3	< 0.05
SDRC, SDRD, ISDH	PF04650/YSIRK type signal peptide	3	< 0.05
METE, DAPA, DAPB	GO:0009067/Aspartate family amino acid biosynthetic process	3	< 0.05
SDRC, SDRD, ISDH	IPR019931/LPXTG cell wall anchor domain	3	< 0.05

DAPA, DAPB	GO:0019877/Diaminopimelate biosynthetic process	2	< 0.05
SDRC, SDRD	PR013783/Immunoglobulin-like fold	2	< 0.05
SDRC, SDRD	PF13620/Carboxypeptidase regulatory-like domain	2	< 0.05
SDRC, SDRD	PF17210/SdrD B-like domain	2	< 0.05
HUTH, SBNA, METE, TRPC, TRPF, TRPB, DAPA, DAPB, ILVD, ILVC, LEUB, LEUC, LEUD, ILVA	GO:0006082/Organic acid metabolic process	14	< 0.05
SDRC, SDRD, ISDH	GO:0005618/Cell wall	3	< 0.05
TRPC, TRPF, TRPB	GO:0006576/Cellular biogenic amine metabolic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0009308/Amine metabolic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0009309/Amine biosynthetic process	3	< 0.05
SDRC, SDRD, ISDH	GO:0030312/External encapsulating structure	3	< 0.05
TRPC, TRPF, TRPB	GO:0042401/Cellular biogenic amine biosynthetic process	3	< 0.05
TRPC, TRPF, TRPB	GO:0044106/Cellular amine metabolic process	3	< 0.05
ESXA, SLE1, SPLF, SPLE, HLGB	GO:0005576/Extracellular region	5	< 0.05
RIBH, RIBBA	Riboflavin biosynthesis, and Lumazine binding domain	2	< 0.05
SLE1, HLGB	GO:0019835/Cytolysis	2	< 0.05
SLE1	GO:0042742/Defense response to bacterium	1	< 0.05
RPSF, RPSR, RPLK, RPLA, RPLJ, RPLL, RPSG, RPLS, RPSB, RPMA, RPLT, RPMI, RPSI, RPLM, RPSE, RPLR, RPLF, RPSH, RPLE, RPLX, RPLN, RPSQ, RPMC, RPLP, RPSC, RPLV, RPSS, RPLB, RPLW, RPLC	GO:0022626/Cytosolic ribosome	30	< 0.05

RPSF, RPSR, RPLK, RPLA, RPLJ, RPLL, RPSL, RPSG, RPSP, RIMM, RPLS, RPSB, RPMA, RPLU, RPLT, RPMI, RPSD, RPSI, RPLM, RPSE, RPLR, RPLF, RPSH, RPLE, RPLX, RPLN, RPSQ, RPMC, RPLP, RPSC, RPLV, RPSS, RPLB, RPLW, RPLD, RPLC	GO:0043226/Organelle	36	< 0.05
RPSF, RPSR, RPLK, RPLA, RPLJ, RPLL, RPOB, RPOC, RPSL, RPSG, FUSA, PHES, ILES, PYRR, RPSP, RIMM, TRMD, RPLS, RPSB, FRR, RIMP, INFB, ASPS, HISS, RPMA, RPLU, RPLT, RPMI, INFC, RPSD, TYRS, PRFA, RPSI, RPLM, RPSE, RPLR, RPLF, RPSH, RPLE, RPLX, RPLN, RPSQ, RPMC, RPLP, RPSC, RPLV, RPSS, RPLB, RPLW, RPLD, RPLC	GO:0010467/Gene expression	51	< 0.05
RPLK, RPLA, RPLJ, RPLL, RPLS, RPMA, RPLT, RPMI, RPLM, RPLR, RPLF, RPLE, RPLX, RPLN, RPMC, RPLP, RPLV, RPLB, RPLW, RPLC	GO:0015934/Large ribosomal subunit	20	< 0.05
RPSF, RPSR, RPSL, RPSG, RPSP, RPSB, RPSD, RPSI, RPSE, RPSH, RPSQ, RPSC, RPSS	GO:0015935/Small ribosomal subunit	13	< 0.05
RPSF, RPSR, PDXS, PDXT, RPLK, RPLA, RPLJ, RPLL, RPOB, RPOC, RPSL, RPSG, FUSA, ARGG, PHES, ILES, PYRR, PYRB, CARB, PYRF, RPSP, RIMM, TRMD, RPLS, RPSB, PYRH, FRR, RIMP, INFB, GUAC, ASPS, HISS, RPMA, RPLU, RPLT, RPMI, INFC, RPSD, TYRS, CSHA, PRFA, PYRG, RPSI, RPLM, RPSE, RPLR, RPLF, RPSH, RPLE, RPLX, RPLN, RPSQ, RPMC, RPLP, RPSC, RPLV, RPSS, RPLB, RPLW, RPLD, RPLC	GO:0034641/Cellular nitrogen compound metabolic process	61	< 0.05
CNTF, CNTD, CNTC, CNTB, CNTA	GO:0006824/Cobalt ion transport	5	< 0.05
CNTF, CNTD, CNTC, CNTB, CNTA	GO:0070838/Divalent metal ion transport	5	< 0.05

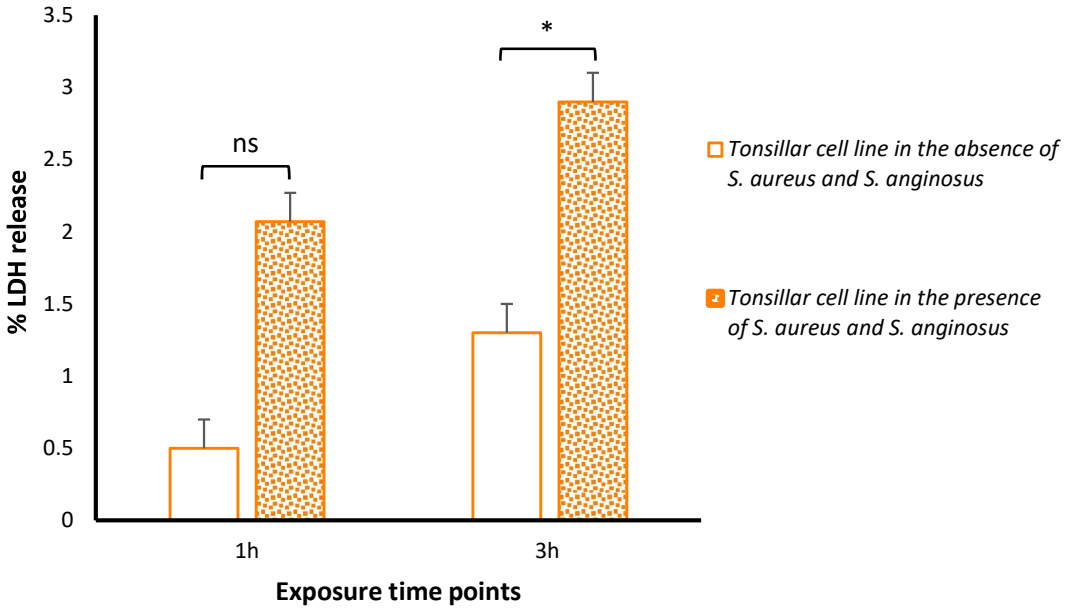

Figure S1. Co-culturing of S. aureus and S. anginosus shows minor cytotoxicity to the tonsillar cell line. *LDH release from tonsillar cells into the supernatant was measured following exposure with/without S. aureus and S. anginosus for 1 h and 3 h. The orange bar without dot represents the percentage (%) of LDH released by the tonsillar cell line in the absence of bacterial coculture (negative control) whereas dotted orange bar presents the LDH % release by the tonsillar cell line in the presence of bacterial co-culture. The bacterial cytotoxicity was calculated as a percentage of maximum LDH release control (positive control). The results are based on three independent experiments.*

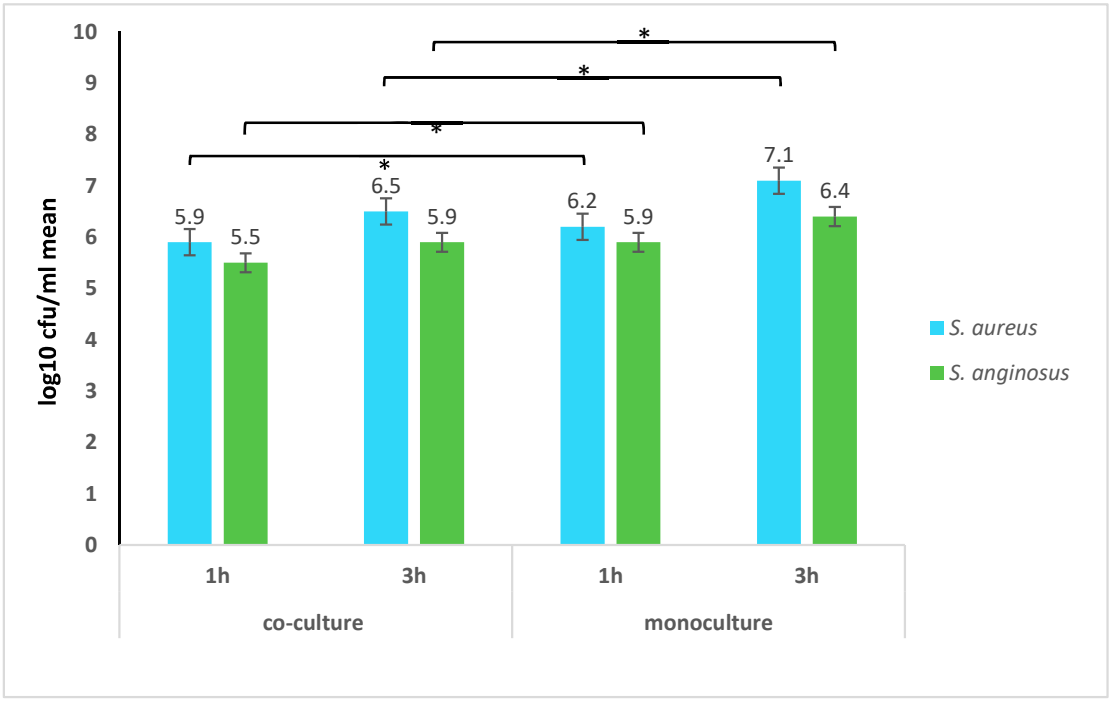

Figure S2. S. aureus and S. anginosus grown in the co-cultures and monocultures for 1 or 3 hours in the absence of host cells. *S. aureus and S. anginosus when grown alone is referred to as monoculture, and when grown together is referred to as co-cultured, both in tonsillar cell media for 1 and 3 hours, before being plated on selected media for CFU enumeration. The results are presented as mean log10 CFU/ml from three independent experiments. Error bars represent the +/- SD. Differences in the means between the groups were tested using a two-sample Student t-test. *P < 0.05.*

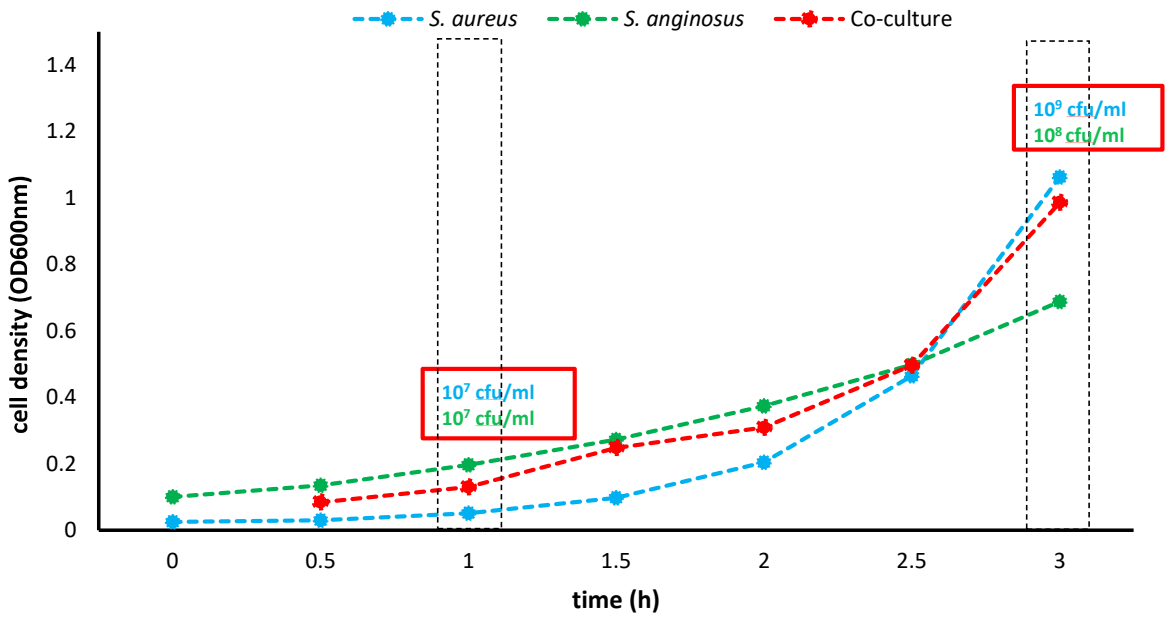

Figure S3. S. aureus and S. anginosus growth in bacteriological media. *Both the bacteria were grown on BHI overnight at 37 °C, and then the fresh bacterial culture was prepared (1:10) for monoculture (single bacterial strain growth) and co-culturing (S. aureus grown in the presence of S. anginosus). Cell density (OD600 nm) was measured every 30 mins for up to 3 hours (h). After 1 h and 3 h (marked with bars) bacteria were plated for colony-forming units (CFU) enumeration. CFU/ml of respective strains during co-culturing is indicated inside the red boxes, which is similar to enumeration from monoculture. Plate enumeration for monoculture samples was performed in BHI plate whereas selective media was used for co-cultured samples, such as CHROMagar™ for S. aureus counting and COBA plate for S. anginosus. The results show one representative experiment from a triplicate.*

Paper III

Transcriptomic responses of *Staphylococcus aureus* USA300 LAC during exposure to human Thp1 macrophages and host factors

Srijana Bastakoti*[1], Clement Ajayi [†1], Stephen Dela Ahator[†1], Mona Johannessen[1], Christian Stephan Lentz, Anne-Merethe Hanssen*[1]

Manuscript