Mucosal environment induces phage susceptibility in *Streptococcus mutans*

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Authorship contribution statement

Conceptualization: GMFA, L-RS. Methodology: GMFA, NR, L-RS. Data curation: GMFA, NR, L-RS. Writing- Original draft preparation: LRS. Writing – review and editing: : GMFA, NR, L-RS. Visualization: GMFA, L-RS. Supervision, project administration, and funding acquisition: L-RS.

30

31 **Abstract**

32 Pathogenic bacteria are attracted towards mucosa, as it is their way of entry into the body. Yet,
33 we know surprisingly little about the phage-bacterium interactions in the mucosal environment.
34 Here, we explored the effect of the mucosal environment on growth characteristics and phage-
35 bacterium interactions in *Streptococcus mutans*, causative agent of dental caries. We found that
36 while mucin supplementation increased bacterial growth and survival, it decreased *S. mutans*
37 biofilm formation. More importantly, presence of mucin had a significant effect on *S. mutans*
38 phage susceptibility. In two experiments done in BHI, phage M102 replication was detected
39 only with 0.2% mucin supplementation. In 0.1xTSB 0.5% mucin supplementation led to 4-log
40 increase in phage titers compared to control. These results suggest that the mucosal
41 environment can have a major role for growth, phage sensitivity and phage resistance of *S.*
42 *mutans*, and underline the importance of understanding the effect of mucosal environment on
43 phage-bacterium interactions.
44
45
46 **Introduction**

47 Many pathogenic bacteria use mucosal layers to invade metazoan hosts. Indeed, the mucosa
48 is an important chemotactic signal for many pathogenic bacteria [1,2], directing the bacteria
49 towards the metazoan host. Mucosal cues cause expressional changes in virulence-related
50 genes of many bacterial species [3]. For example, interaction with mucus and mucin
51 glycoproteins impacts bacterial phenotypic characteristics and increases virulence of many
52 pathogenic species [4–6], including *Acinetobacter baumannii* [7] and *Streptococcus mutans* [8].
53 Furthermore, some bacteria, such as pathogens and many intestinal anaerobes, can use mucosal
54 components directly as a nutrient source [5].
55 The mucosal surface consists of secreted mucin glycoproteins, that oligomerize to form a
56 complex viscous milieu allowing retention of antimicrobial molecules (e.g. immunoglobulins
57 A and G, lectins, antimicrobial peptides) and also phages. Over 20 families of mucin
58 glycoproteins exist, and they are expressed at a tissue-specific manner [1,9]. Mucin secretion is
59 primarily constitutive, leading to a dynamic gradient of mucins - high close to the epithelial
60 cell surface but lower in the outer surface of the mucosa.
61 The last 10 years of research have clearly shown that phages are major partners in the
62 mucosal microbiomes [10,11], thus affecting both the invading and local bacterial populations.
63 Understanding the ecology of pathogens and their phages in the mucosa is central for our
64 health, and for developing of phage therapy approaches against antibiotic resistant infections.
65 Yet, we know surprisingly little about the phage-bacterium interactions in the mucosal
66 environment. This has led to fundamental gaps and biases in our knowledge regarding the
67 antagonistic interactions between phages and bacteria as they happen in the mucosal milieu.
68 The oral environment is an important habitat for phage-bacterium interactions[12,13]. The
69 salivary microbial community has been shown to host 10^8 virus-like particles per ml (mostly
70 dsDNA phages) [14] whereas in the dental plaque the density is higher, 10^{10} virus-like particles
71 per gram [15]. Since previous research suggests mucosa may influence the virulence of bacteria
72 [4–6] and phages [6,16,17], and favours phage resistance mechanisms that maintain bacterial
73 virulence (e.g. CRISPR-Cas) [18], it is important to describe how the mucosal environment
74 shapes these interactions in *Streptococcus mutans*, causative agent of dental caries[18]
75 Here, we characterized the effect of mucosal environment on growth features and phage-
76 bacterium interactions in *S. mutans*. In its natural habitat, *S. mutans* forms biofilm and is in
77 continuous contact with oral mucosal surfaces. It has been previously demonstrated, that
78 several salivary components inhibit *S. mutans* biofilm formation, but the presence of sucrose

79 can reverse this effect [19]. Yet, so far there has been no direct demonstration of the impact of
80 mucins on the interactions between *S. mutans* and its phages This aspect is central for
81 understanding the phage-bacterium interactions in the mucosa, and for developing successful
82 phage therapy approaches against caries. Here, we characterized the impact of mucosal
83 environment on *S. mutans* growth characteristics in physiologically relevant mucin
84 concentrations [20,21]. We found that while mucin supplementation decreases *S. mutans* biofilm
85 formation, it increases bacterial survival in starvation. More importantly, mucin has a central
86 role for *S. mutans* susceptibility to phage infections, and, consequently, phage resistance.
87
88 **Materials and methods**
89
90 Host and phage strains
91 *Streptococcus mutans* OMZ381 and its phage M102 were obtained from Félix d'Hérelle
92 Reference Center for Bacterial Viruses (www.phage.ulaval.ca) and stored in -80°C. In the
93 experiments, the bacteria were cultured in TSB or BHI at 37°C. Phage stocks were prepared
94 by harvesting the soft-agar of a confluent double-agar plate, to which four ml of media were
95 added, followed by centrifugation (11000g) and filtration. Phage titration was made by plaque
96 forming unit counts using the double-agar method.
97
98 Bioinformatic analysis
99 Phage M102 [22] genome is publicly available (RefGenome: NC_012884). We used HHpred [23,24]
100 to analyse M102 ORFS and to find hits associated to Ig-like domains and carbohydrate binding.
101
102 Phage binding to mucin
103 To evaluate phage M102 binding to mucin, BHI (Brain Heart Infusion Broth, Sigma) agar
104 plates were prepared with or without 1% mucin in its composition. Purified porcine mucin
105 (Sigma, catalog no. M1778) was used as source. The phage was diluted to a concentration of
106 $2.5x10^2$ pfu (plaque forming units) ml^{-1} in liquid BHI media and five milliliters of the dilution
107 were added to the plates containing mucin or not. The plates were kept under agitation for 30
108 minutes, then the liquid was removed by careful pipetting and three milliliters of BHI soft agar
109 containing the *S. mutans* host was added to each plate. Plates were incubated overnight at 37°C
110 and plaques enumerated. The experiment was done in triplicates.
111
112 Influence of mucin on *S. mutans* growth and biofilm formation
113 Influence of mucin supplementation on *S. mutans* was studied first in 1x (i.e. undiluted) TSB
114 (Tryptic Soy Broth, Sigma) supplemented with 0.5% yeast extract and 0.5% K_2HPO_4). Fresh
115 bacterial culture was inoculated into 5 ml of TSB supplemented with 0, 0.05 or 0.1 % mucin
116 in triplicates, and cultured overnight in 37°C under constant shaking (200 rpm). OD (at 595
117 nm) of the cultures was measured in 6 technical replicates per tube. Data were analysed using
118 Kruskal-Wallis non-parametric ANOVA in SPSS.
119
120
121 In a second experiment we explored the ability of *S. mutans* to grow with mucin without any
122 additional energy source. Bacteria were inoculated in 5 ml of sterile H_2O without mucin and
123 with 0.2% and 0.5% mucin supplementation in triplicates. These cultures were grown in 37°C
124 under constant shaking (200 rpm). Bacterial growth was recorded by estimating cfu (colony
125 forming units) ml^{-1} via applying 2 µl drops of the ten-fold dilutions of culture on BHI plates.
126 Data were analysed with Kruskal-Wallis non-parametric ANOVA in SPSS.
127

Finally, we explored how different nutrient conditions and mucin concentrations influence *S. mutans* biofilm formation. In this experiment, overnight-grown *S. mutans* was inoculated 1:10 000 into sterile H_2O, 0.1xBHI (i.e. ten times diluted BHI) and 1x BHI, supplemented with 0%, 0.2% or 0.5% mucin. Each treatment was done in six replicates in 150 µl volume, on a sterile 96-well plate, and incubated for 2 days at 37°C without shaking. To determine the biofilm formation, all medium was removed from the wells. Plates were washed three times with MilliQ water and stained 45 min with 0.1% crystal violet. After staining, the plates were washed again three times with MilliQ water, dried, and de-stained with ethanol for 15 minutes. OD (at 595 nm) of 100 µl samples were measured to analyse biofilm formation (Multiskan FC, Thermo Scientific, China). Statistical differences between mucin concentrations and the control were analysed using T-test in GraphPad Prism 9.

Influence of mucin on phage-bacterium interaction

First, we explored the effect of mucin supplementation and culture nutrient concentration on *S. mutans*-phage interaction in TSB medium. Overnight cultures were diluted to 1:10 000 and inoculated to 1x and 0.1xTSB supplemented with 0.5% mucin in triplicates (control cultures were done without mucin), and incubated in 37°C for 4h, after which phage M102 was added (10^5 PFU ml^{-1}). Samplings of 100 µl were made 20h after the infection, mixed with 10 µl of chloroform, and titrated. Another sampling was made 48h after the start of the experiment and also titrated. Kruskal-Wallis non-parametric ANOVA was used to analyse the results.

To observe the effect of mucin and phage on bacterial growth and biofilm formation, 2% porcine mucin was added to BHI to reach final concentration of 0.2%, and inoculated with 10^4 CFU ml^{-1} of overnight-grown *S. mutans*. Control cultures were done in BHI without mucin. Half of the cultures were also (simultaneously) inoculated with bacteriophage M102 at MOI of 1. From each treatment, 150 µl culture was distributed into a sterile 96-well plate in six replicates, and incubated for 2 days at 37°C without shaking. After incubation, bacterial and phage numbers were sampled from 3 wells per treatment by standard plating methods. Then, the remaining biofilm from all six replicate wells was determined by crystal violet staining as explained above. The data were analysed with non-parametric ANOVA.

To understand the effect of mucosal environment on phage resistance, we co-cultured *S. mutans* with M102 phage in 0% and 0.2% mucin supplementation (in BHI, 37°C, 200 rpm) in triplicate 5 ml cultures. Samples were plated after 72 hours and bacterial colony forming units were recorded. Phage titers at the end of the experiment were determined with the original *S. mutans* host. Up to 10 bacterial colonies were picked from each replicate and purified by three rounds of colony-picking and plating. Purified colonies were then inoculated into fresh BHI, grown at 37°C with shaking (200 rpm), and preserved into -80°C freezer stocks with 10% glycerol. Phage resistance of the isolates was determined with a plaque assay using the ancestral M102 phage.

RESULTS

Phage M102 adhesion to mucin-containing plates

A brief survey of the M102 genome (NC_012884) revealed that it has a putative carbohydrate-binding domain in ORF13 (HHPred [25]: Lactobacillus phage J-1 carbohydrate binding module, Probability: 99.95%, E-value: 8.8e-26) as a tail component, which may mediate binding to mucin, or to the bacterial host. We evaluated the ability of M102 to bind *in vitro* to purified mucin. Such approach has been applied before to evaluate mucin-binding capacity of different

178 phages [6,10]. Despite a trend in having slightly more phages in mucin containing plates (on
179 average 1.48 more phages on mucin plates), there is no significant difference between the tested
180 conditions (Figure 1, unpaired t test, p= 0.1310).
181
182 **Influence of mucin on *S. mutans* growth and biofilm formation**
183 We measured the effect of mucin supplementation on *S. mutans* growth in 5 ml triplicate
184 cultures in TSB alone or supplemented with mucin. Mucin supplementation significantly
185 influenced bacterial growth (Kruskal-Wallis non-parametric ANOVA, test statistic=47.167, df
186 =2, p<0.01), measured as OD of the cultures. In pairwise comparisons all treatments
187 significantly differed from each other (p-values Bonferroni-corrected) (Figure 2A).
188
189 When the bacteria were inoculated in pure water, there was no bacterial growth observed
190 without mucin supplementation, whereas 0.2% and 0.5% mucin resulted in numbers of 7.2×10^5
191 and 1.1×10^5 CFU ml^{-1}, respectively (Figure 2B). There was no statistical difference between
192 these two treatments.
193
194 Although it was evident that mucin supplementation benefits *S. mutans* growth, this was not
195 reflected in biofilm formation (Figure 3). Mucin presence in water cultures did not lead to
196 biofilm formation, and the same was seen when diluted (0.1x) medium was used. In complete
197 (1x) medium, biofilm was detected in all conditions, with or without mucin. However, t-tests
198 revealed that mucin addition significantly decreased biofilm formation in 0.1% BHI (p<0.001
199 0% vs 0.2% mucin and 0% vs 0.5%) and in the 1x BHI (p<0.001 for 0% vs 0.2% mucin and
200 0% vs 0.5%) . It may be that the presence of nutrients (culture media) favours biofilm formation
201 while mucin could serve as an inducer of the planktonic state.
202
203 **Influence of mucin on phage-bacterium interaction**
204 First, we explored the effect of mucin supplementation on *S. mutans*-phage interaction in TSB
205 medium. Twenty hours after phage inoculation there was a significant increase in phage titer
206 when 0.1 TSB was supplemented with 0.5% mucin (Kruskall Wallis 1-way ANOVA p=0.0094,
207 bonferroni-corrected p-value for pairwise comparisons 0.027) (Figure 4). After 48h, phage
208 titers were similar in all treatments (data not shown).
209
210 To understand whether the improved phage growth was because of mucin presence or increased
211 bacterial population in the mucin condition, we cultured *S. mutans* in presence of phage M102
212 and mucin for 48h in 1x BHI. In the non-infected cultures bacteria titers did not differ between
213 control and mucin conditions. However, bacterial numbers significantly differed between
214 treatments (Kurskal-Wallis 1-way ANOVA; test statistic=9.359, df=3, p=0.025) in the
215 presence of the phage (Figure 5). Notably, phage significantly reduced bacterial numbers only
216 when mucin was present. Phage titration of the treatment indicated, that phage replicated only
217 in presence of 0.2% mucin (titer 10^7 pfu ml^{-1} vs 10^3 pfu ml^{-1} in treatment without mucin
218 addition). Phage presence also significantly affected biofilm formation, but only when mucin
219 was present (Figure 5B).
220
221
222 **Effect of mucin on phage-bacterium interaction and phage resistance**
223 To understand the effect of mucosal environment on phage resistance, we co-cultured *S. mutans*
224 with phage M102 in 0% and 0.2% mucin supplementation (in BHI, 37°C, 200 rpm) in triplicate
225 5 ml cultures. Compared to the previous experiment, we used a longer incubation time to allow
226 phage resistance to evolve. After 72 hours, bacterial colony forming units were recorded and
227 phage titers determined with the original *S. mutans* host (Figure 6). Ten bacterial colonies per

228 replicate were pure cultured from treatments evolving with the phage (and 3 colonies per
229 replicate from phage-free conditions) and their phage resistance determined by plaque assays.
230 Under mucin supplementation and phage exposure, 26 of 30 colonies showed a reduced
231 susceptibility to M102 after 72 h. All colonies isolated from the control conditions remained
232 sentisitive to phage infection.
233
234 Bacterial densities did not statistically differ between experimental conditions but, again, phage
235 replication was detected only in 0.2% mucin (average 1.05 x 10^7 PFU mL^{-1}). In replicates
236 without mucin no plaques were detected. Since also the phage-only control was negative for
237 phage plaques, the phage may not have survived the 3-day culture conditions, or the titer was
238 under the detection limit.
239
240 **Discussion**
241
242 *S.mutans* causes tooth decay i.e. dental caries, a common condition which is hard to treat. The
243 challenges of the increasing antibiotic resistance in general and the susceptibility of the aging
244 population to infections require developing approaches that target bacterial pathogens also in
245 the oral context. This has led to a growing interest towards phage therapy. Phages against *S.*
246 *mutans* have already been shown to be potential preventive agents against caries *in vitro* and
247 *in vivo* models, highlighting its relevance as a non-invasive treatment [26]. Therefore, we need to
248 understand how phages infect bacteria and how bacteria resist phage infections in the mucosal
249 environment.
250
251 To our knowledge, there has not been prior studies addressing *S. mutans* phage infections in
252 presence of mucins. In general, phage-bacterium interactions are most often explored in
253 standard defined growth media, where the possible signals from the vertebrate host targeted by
254 the bacteria are absent. However, in real life, the interactions between pathogenic bacteria and
255 phages often occur on the mucosal surfaces of vertebrate hosts. Our results show that mucosal
256 environment (achieved by mucin supplementation of growth media) significantly alters *S.*
257 *mutans* growth, phage sensitivity and phage resistance. These results underline the importance
258 of understanding the effect of mucosa on phage-bacterium interactions.
259
260 Our results indicate that *S. mutans* can benefit from mucin as an additional energy source, as
261 mucin supplementation increases bacterial replication at least if added in TSB (Figure 2), where
262 additional nutrients are available for growth. This is in line with results obtained previously[7],
263 where addition of mucin and glucose enhanced *S. mutans* growth in chemically defined
264 medium. Furthermore, presence of mucins increases *S. mutans* survival [17]. This may explain
265 the higher bacterial numbers in mucin-supplemented H_2O observed in our study in contrast to
266 none in replicates without mucin (Figure 2b). Yet, it is clear that the interactions between *S.*
267 *mutans* and mucins are more complex *in vivo*. For example, the microbial diversity found in
268 the oral environment can influence *S. mutans* behaviour and phenotype. Mucins have been
269 shown to influence microbial interactions and support the coexistence of *S. mutans* and its
270 competitors [27].
271
272 Interestingly, mucin decreased *S. mutans* biofilm formation, which was more dependent on the
273 concentration of nutrients. A previous study using human salivary mucins suggested that mucin
274 decreases *S. mutans* surface attachment by inducing planktonic growth form [27]. This may be
275 linked directly to sensitivity to phage, as bacteria growing in the planktonic phase are not
276 protected by biofilm, allowing phage infections. Furthermore, presence of oxygen has been
277 shown to decrease biofilm formation in *S. mutans* [19], which may be one of the reasons why the

278 biofilm formation was low in general in our experiments, which were made in aerobic
279 conditions. The influence of mucin under anaerobic conditions is a factor to be considered in
280 future research.
281
282 Our results show that mucins significantly influence *S. mutans* phage survival and replication,
283 exemplifying the role of mucosal surfaces on the phage-bacterium interaction and potentially
284 phage therapy approaches. Studies comparing the density of free and metazoan-associated
285 phages have found that phages tend to concentrate on mucosal surfaces [10]. For example, 10^8
286 virus-like particles per ml can be found in the human gut microbiota, but the density is higher
287 in the gut mucosa (10^9 phages per ml)[28]. This can be explained by the affinity of some phages
288 towards mucins: some tailed dsDNA phages have (structural) protein domains that mediate
289 their adherence to mucin glycoproteins found in the mucosal surfaces. A bioinformatic analysis
290 of 246 dsDNA tailed-phage genomes suggested that roughly 25% have proteins with Ig-like
291 folds, all related to the viral structure [29]. More recently, also other domains [30, 31] have been
292 found and suggested to facilitate phage binding to mucosa. Phage M102 [22] has a putative
293 carbohydrate-binding domain in ORF13 which might mediate the phage-mucus interaction, but
294 the role of this domain remains unknown so far. Nevertheless, although M102 mucin binding
295 *in vitro* was not significant, the ratio between plaque count in mucin divided by plaque count
296 in control was 1.48. Since it has been shown that phage-mucin interactions are transient but
297 constant [20], even a slightly positive ratio allied to the numerous interactions between the phage
298 and mucins may still play a role in maintaining phages in the mucosa. Furthermore, while the
299 phage titers in mucin-free cultures were below detection limit, it is also possible that presence
300 of mucins stabilize phage particles, influencing their survival in the mucosal environment.
301
302 While phage-host interactions in *S. mutans* have been previously explored [26,32,33], there is a
303 lack of knowledge on how the mucosal environment influences bacterial susceptibility to
304 phage. We observed that mucin has a major role for phage susceptibility: mucin
305 supplementation caused a 4-log increase in phage titer of *S. mutans*. While the exact
306 mechanisms for this phenomenon are unknown, it is possible that phages benefit from higher
307 bacterial replication or that they exploit their changed metabolic state. It is also possible that
308 the upregulation of bacterial virulence factors (related to cell wall structures, secretion,
309 metabolism or motility) in the mucosal environment increases the availability of phage
310 receptors. This could explain the previously observed increased virulence of pathogens in the
311 mucosal environment [4–6]. In our experiments, it seems evident that the choice of medium
312 influences the impact of mucin on bacterial growth and phage sensitivity. In TSB, which is a
313 less rich medium than BHI, phage replication was improved only in the diluted medium
314 (Figure 4) whereas in BHI phage replication (and a drop in bacterial population density) was
315 observed only under mucin supplementation. Yet, in BHI, mucin supplementation did not cause
316 any differences in bacterial numbers in absence of phage (Figure 5). This suggests that mucin-
317 induced expressional changes are more likely to cause sensitivity to phage infection.
318 Consequently, phage resistance was elicited only under mucin supplementation when the
319 bacteria were co-culture with phage for 72h. This is seen as a comparable bacterial population
320 size in phage-treated and control culture in Figure 6a. While phage resistance in *S. mutans* has
321 been observed in previous studies without mucin [32,33], it remains to be studied how the mucosal
322 environment influences the evolution of resistance in *S. mutans*, e.g. CRISPR-Cas [18].
323
324 The influence of mucins on bacterial phage sensitivity has also been studied previously e.g.
325 with *Salmonella enterica* [34], and many other bacterial species [35]. However, in some cases
326 mucins may also impair phage infectivity [6,17]. Such phages are probably more efficient in

327 targeting pathogens outside the mucosa. Differences in the capacity to adhere to mucus (or to
328 replicate in mucosal environment) could be considered as one additional phenotypic feature of
329 phage-bacterium interaction, that could be exploited in building phage cocktails for therapy
330 purposes. A combination of phages with different strategies (mucin-binding or not) may
331 produce efficient treatment and prevention tools that target pathogens both from the infection
332 site and during transmission.
333
334 The mucin concentrations used in these experiments are relevant and comparable to what is
335 observed in the body, although mucin species and concentrations vary depending on body site
336 and and in response to physical conditions, disease and age [36–39]. For example, in the gastric
337 environment the concentration of the mucins is ~5% [21], but the density of the mucosal layer is
338 not consistent, as the mucin concentration is highest near the cell surface and less dense at the
339 outer layer. The oral mucosal environment contains several different mucins; secreted salivary
340 mucins MUC5B , MUC7 and MUC19, and membrane-associated mucins MUC1 and MUC4
341 [40]. The salivary mucin levels are generally higher in patients with dental karies, e.g. with
342 MUC5B concentration varying from 0.06 to 2.34 ng mL^{-1} [38]. While the exact mucin species
343 composition of the porcine mucin used in our experiments is not known, it mainly consists of
344 MUC2. It thus seems that *S. mutans* may not have a specific affinity towards certain mucins as
345 observed in some other bacteria [40]. Furthermore, it is important to note that porcine mucin has
346 been shown previously to be relevant for phage-bacterium studies in also other species,
347 eliciting a similar increase in phage replication[6].
348
349 Combined with previous research, these results highlight the effect of mucosal environment on
350 phage-bacterium interactions, and emphasizes the ecological relevance of microbial
351 interactions in the eukaryotic mucosa, with implications for the outcome and prevention of
352 diseases.
353
354 **Conflict of Interest**
355 G.M.D.F.A., L.-R.S. and University of Jyväskylä have patented the commercial use of mucin
356 in a patent titled “Improved methods and culture media for production, quantification and
357 isolation of bacteriophages" (FI20185086, PCT/FI2019/050073).
358
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361
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475
476 **FIGURES**
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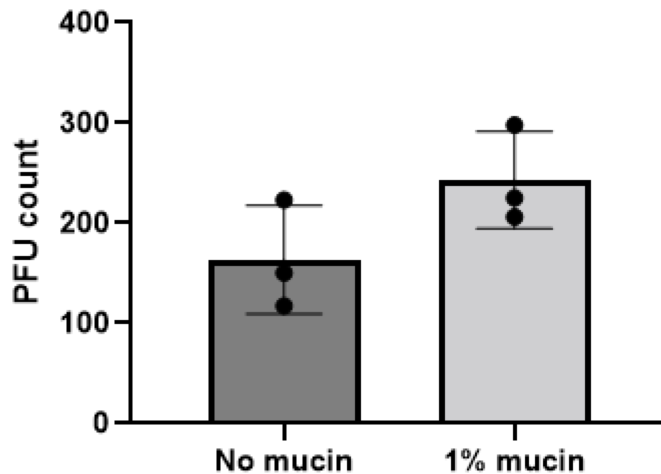

478
479 Figure 1. Streptococcus mutans phage M102 adhesion to mucin. Each data point represents an
480 individual replicate that consists of phage plaques count in an agar plate. The mean and
481 standard deviation of each condition is indicated in the graph.
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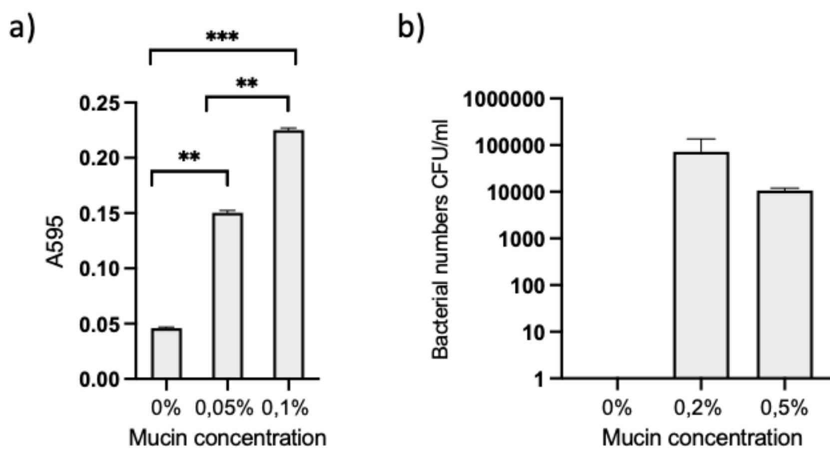

486
487 Figure 2. Effect of mucin supplementation on Streptococcus mutans growth. a) Mean optical
488 density (+/- Standard error) of bacteria grown in TSB supplemented with 0%, 0,05% and 0,1%
489 ucin, and b) mean colony forming units per ml (+/- S.E.) in bacteria grown in sterile water
490 supplemented with 0%, 0,2% and 0,5% mucin. Asterisks indicate statistical significance (p-
491 values: ***< 0.001, **=0.001-0.01.*=0.01-0.05)
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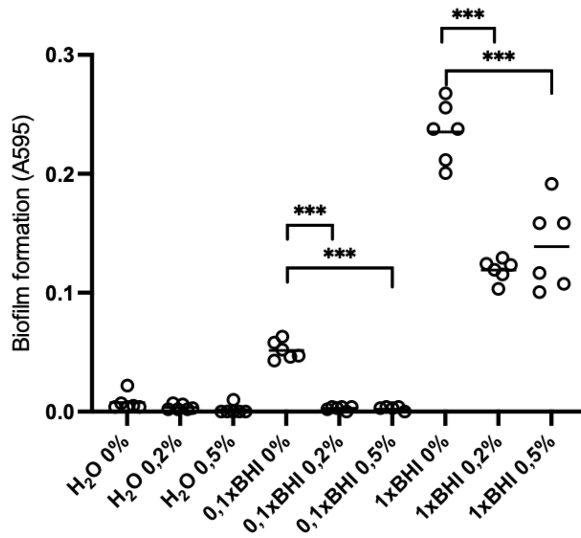

493
494 Figure 3. Effect of nutrients and mucin concentration on *Streptococcus mutans* biofilm
495 formation. Bacteria were cultured without nutrients (H_2O), in 0.1X (ten times diluted) and 1X
496 (normal) BHI under 0%, 0.2% and 0.5% mucin supplementation for 48h. Asterisk indicates
497 statistical significance (***= p-value <0.001)
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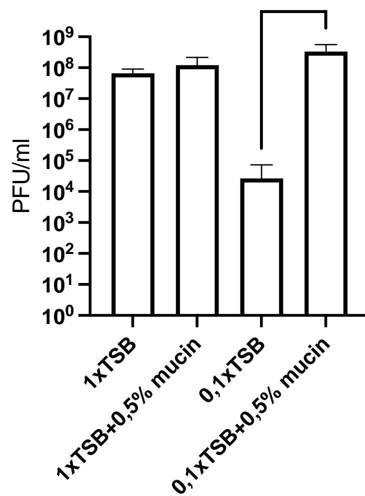

502
503 Figure 4. Effect of mucin supplementation on M102 phage titers in TSB (1X: normal TSB,
504 0.1X: 10 times diluted TSB). Asterisk indicates statistical significance (* = p-value <0.05)
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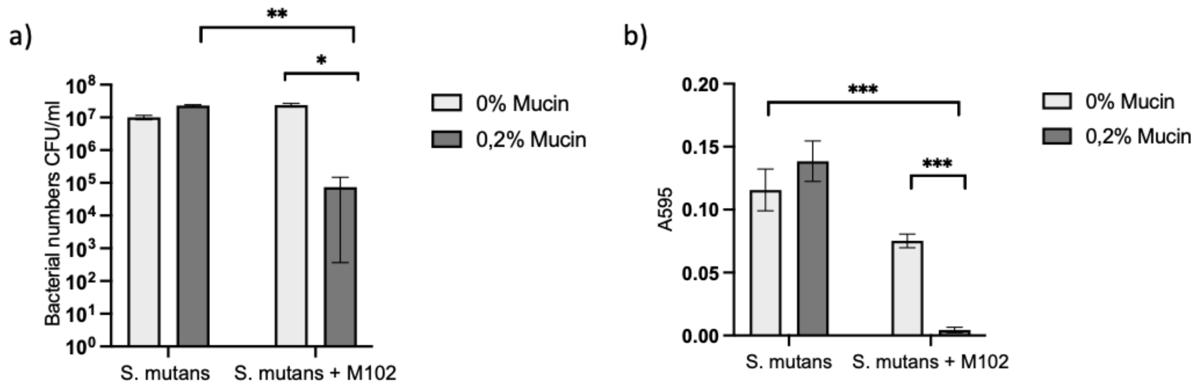

508
509 Figure 5. S. mutans growth and biofilm formation when cultured with 0.2% mucin
510 supplementation and phage M102 for 48 hours. A) Mean bacterial numbers +/- SE, b) bacterial
511 biofilm formation (mean OD+/- SE). Asterisks indicate statistical significance between
512 treatments (p-values: ***< 0.001, **=0.001-0.01.*=0.01-0.05)
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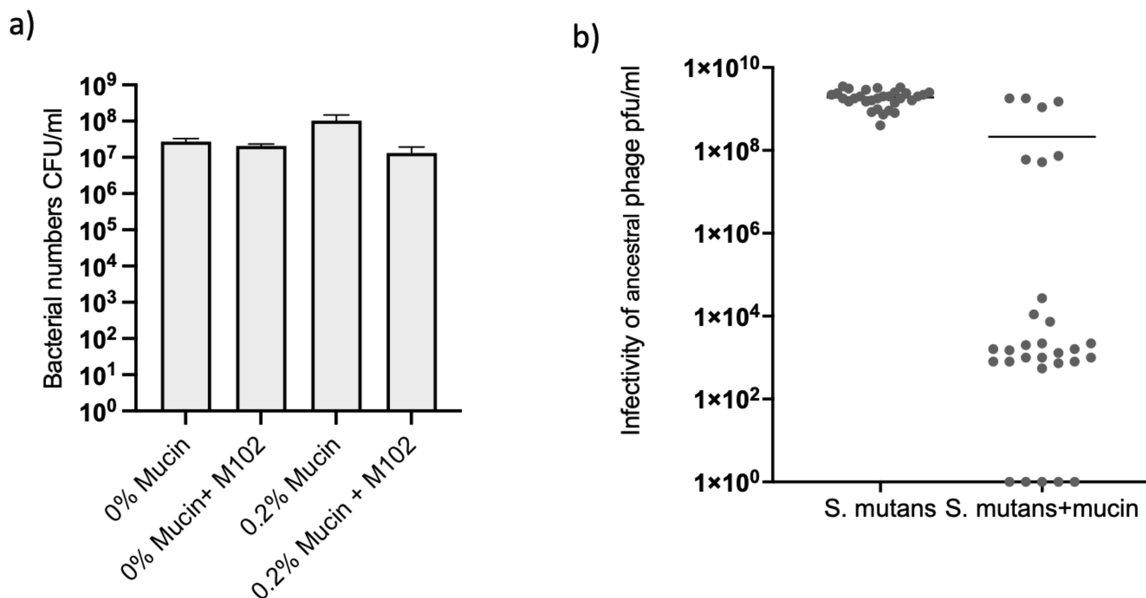

519
520 Figure 6. Phage resistance experiment in mucosal environment. Phage and bacteria were co-
521 cultured for 72h to allow phage resistance to evolve. A) Bacterial cell numbers with phage
522 M102 and 0,2% mucin supplementation. B) phage infectivity (pfu/ml by plaque assay) of
523 isolates originating from phage exposure with and without mucin supplementation.
524