FRUCTOSE ACTIVATES A STRESS RESPONSE SHARED BY METHYLGLYOXAL AND HYDROGEN PEROXIDE IN *STREPTOCOCCUS MUTANS*

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Abstract

Fructose catabolism by *Streptococcus mutans* is initiated by three PTS transporters yielding either fructose-1-phoshate (F-1-P) or fructose-6-phosphate (F-6-P). Deletion of one such F-1-P-generating PTS, *fruI*, has been shown to reduce the cariogenicity of *S. mutans* in rats fed a high-sucrose diet. Moreover, a recent study linked fructose metabolism in *S. mutans* to a reactive electrophile species (RES) methylglyoxal. Here, we conducted a comparative transcriptomic analysis of exponentially grown *S. mutans* shocked with 50 mM fructose, 50 mM glucose, 5 mM methylglyoxal, or 0.5 mM hydrogen peroxide (H_2O_2). The results revealed a striking overlap between the fructose and methylglyoxal transcriptomes, totaling 176 genes, 61 of which were also shared with the H_2O_2 transcriptome. This core of 61 genes encompassed many of the same pathways affected by exposure to low pH or zinc intoxication. Consistent with these findings, fructose negatively impacted metal homeostasis of a mutant deficient in zinc expulsion and the growth of a mutant of the major oxidative stress regulator SpxA1. We further demonstrated the induction of the superoxide dismutase (*sodA*) and the *fruRKI* operon by different levels of fructose. Finally, fructose metabolism lowered culture pH at a faster pace, allowed better survival under acidic and nutrient-depleted conditions, and enhanced the competitiveness of *S. mutans* against *Streptococcus sanguinis*, although a moderated level of F-1-P might further boost some of these benefits. In conclusion, fructose metabolism is integrated into the stress core of *S. mutans* and regulates critical functions required for survival in both the oral cavity and during systemic infections.

Importance. Fructose is a common monosaccharide in the biosphere, yet its overconsumption has been linked to various health problems in humans including insulin resistance, obesity, diabetes, and non-alcoholic liver diseases. These effects are in large part attributed to the unique biochemical characteristics and metabolic responses associated with the degradation of fructose. Yet, an understanding of the effects of fructose on the physiology of bacteria and its implications to the human microbiome is severely lacking. Here we performed a series of analyses on the gene regulation of a dental pathogen *Streptococcus mutans* by exposing it to fructose and other important stress agents. Further supported by growth, persistence, and competition assays, our findings revealed the ability of fructose to activate a set of cellular functions that may prove critical to the ability of the bacterium to persist and cause diseases both within and without of the oral cavity.

54 ## Introduction

55 Disruption in microbial homeostasis in human microbiomes, a condition termed
56 dysbiosis, has been associated with the development of many diseases that range from
57 GI tract disorders such as inflammatory bowel disease and pseudomembranous colitis
58 to oral infectious diseases such as dental caries, candidiasis, and periodontitis (1, 2).
59 Carbohydrate metabolism plays a vital role in oral health by contributing to the
60 biochemistry and ecology of oral microbiota. It is widely understood that
61 overconsumption of refined carbohydrates is a defining factor in the development of
62 dysbiosis through acid production and establishment of an acidogenic and aciduric
63 biofilm (3-6). Emerging evidence also supports the co-occurrence of dental caries and
64 periodontal diseases, pointing to potentially shared mechanisms in underlying etiology
65 (7-9). With the food industry shifting heavily toward the use of fructose and fructose-
66 releasing sugars in the past several decades and the concurrent pandemic of metabolic
67 syndrome, the impact of dietary fructose to oral and systemic health demands urgent
68 attention (10).

69 Fructose is a ubiquitous nutrient widely found in fruits, yet overconsumption of
70 fructose is often associated with adverse health outcomes in humans that include
71 insulin resistance, obesity, liver diseases, systemic inflammation, type-2 diabetes,
72 cogitative decline, and even cancer (11-15). According to the “fructose survival
73 hypothesis”, fructose triggers a conserved cellular response in mammalian cells that
74 manifests as mitochondrial oxidative stress and reduced cellular ATP levels,
75 accompanied by behavioral changes that includes hunger, thirst, foraging, and weight
76 gain (16). While this response could have been limited and beneficial to animals and our

77 human ancestors, the negative effects of fructose have been greatly exacerbated by its
78 widespread use in Western diet.
79 Streptococci are the most abundant commensal species colonizing oral surfaces, and
80 most of them rely on carbohydrate fermentation, via the Embden–Meyerhof–Parnas
81 (EMP) pathway of glycolysis, to produce energy and precursors for various biogenesis
82 processes (3). Multiple fructose-transporting mechanisms exist in streptococci, each
83 harboring a fructose: phosphotransferase (PTS) permease that generates either
84 fructose-1-phosphate (F-1-P) or fructose-6-phosphate (F-6-P), which is further
85 phosphorylated into F-1,6-bP by their respective phosphofructokinases (17). Studies so
86 far have indicated that F-1-P metabolism contributes to *in vitro* fitness, biofilm
87 development, and virulence for pathogenic species such as *Streptococcus pyogenes,*
88 *Streptococcus agalactiae,* and *Streptococcus gordonii* (18-22). For the cariogenic
89 pathobiont *Streptococcus mutans*, the primary fructose pathway is encoded by the
90 *fruRKI* operon, encoding a negative regulator FruR, a PTS permease (FruI) that yields
91 F-1-P, and a F-1-P kinase (FruK) (17). Deletion of *fruI* in *S. mutans* UA159 resulted in
92 lower levels of F-1-P when exposed to fructose (23) and reduced the ability of the
93 bacterium to colonize and induce dentin caries in rats fed a high-sucrose diet (24).
94 Based on genetic studies, expression of *fruRKI,* and its homologue *fruRBA* in related
95 streptococci, is inducible by F-1-P which acts allosterically on FruR (17, 18, 25). Another
96 fructose-PTS, encoded by *levDEFG,* exists in some of these oral streptococci, including
97 *S. mutans, Streptococcus sanguinis* and *S. gordonii*, which likely generates F-6-P and
98 requires a four-component system LevQRST for induction in response to extracellular
99 fructose (26). *S. mutans* harbors yet another fructose-PTS (*fruCD*) that is also predicted

100 to generate F-1-P, although its contribution to the overall fructose transport appears to
101 be minor (17). Overall, the F-1-P pathway encoded by *fruRBA/fruRKI* operon is
102 conserved in most Gram-positive bacteria encompassing *Bacillus, Enterococcus,*
103 *Lactococcus, Lactobacillus, Clostridium, Listeria,* and most *Streptococcus* species (27-
104 29). Moreover, *S. mutans, S. gordonii,* and *S. sanguinis* also harbor another fructose-
105 related operon, *sppRA.* SppA has been characterized as a hexose-phosphate
106 phosphohydrolase that specializes in dephosphorylating F-1-P, and F-6-P to a lesser
107 extent, and SppR is a negative auto-regulator whose activity is allosterically regulated
108 by F-1-P (23).
109 A recent study involving fructose centered on the molecular mechanisms required for
110 oral streptococci to degrade a group of highly reactive electrophile species (RES),
111 including methylglyoxal and glyoxal (30), that are created by all life as byproducts of
112 glycolysis and serve as important immune effectors (31, 32). It has been reported that
113 *S. mutans* is significantly more resistant to methylglyoxal than several commensal
114 streptococci including *S. gordonii, S. sanguinis,* and members of the mitis group (30). A
115 zinc-containing protein GloA2 (SMU.1112c), predicted to be a paralogue to the main
116 methylglyoxal-degrading enzyme glyoxalase A (LguL or GloA) based on its sequence
117 and crystal structure, was identified in all oral streptococci examined so far (30). The
118 *gloA2*-null mutants of both *S. mutans* and *S. sanguinis* displayed increased chaining
119 and autolysis upon treatment by fructose which resembled the effect of exposure to
120 methylglyoxal, including its reversal by treatment with glutathione (GSH) which is known
121 to scavenge methylglyoxal before degradation by LguL (32). Interestingly, the
122 expression of *lguL* was inducible by growth on fructose, and the fructose-sensitivity of

123 *gloA2* mutants was largely dependent on the integrity of the F-1-P-generating PTS, FruI
124 (30). Herein we further examined fructose-dependent gene regulation and physiology in
125 relation to streptococcal tolerance to important stressors including methylglyoxal and
126 hydrogen peroxide. Our findings revealed the integration of fructose-mediated response
127 with general stress response experienced during bacteria-host and inter-microbial
128 interactions.
129

130 ## Results

131 **Comparative RNA-seq analyses identified a stress core shared by methylglyoxal,**
132 **fructose, and H_2O_2.**

133 RNA-seq was conducted to compare bacterial gene regulation in response to
134 fructose and methylglyoxal, We have previously performed an RNA-seq analysis on *S.*
135 *mutans* cultured to the exponential phase with 0.5% fructose (33), however subsequent
136 studies suggested that fructose-mediated gene regulation may return to equilibrium
137 after the initial perturbance. As such, fructose was used at a higher concentration but for
138 only 30 min. Considering the similarity between glucose and fructose, the same UA159
139 cultures were treated with 50 mM glucose in the same manner and analyzed as an
140 additional control. We also obtained and reanalyzed the datasets of a similar RNA-seq
141 study performed on UA159 cultures that were prepared in a glucose-based medium and
142 treated with 0.5 mM H_2O_2 for 5 min (34) (see Fig. 1 for volcano plots, Table 1 for genes
143 discussed in text, and supplemental Tables S1&S2).

144 *Methylglyoxal.* Treatment of UA159 with 5 mM of methylglyoxal differentially affected
145 the expression of a total of 474 genes when compared to the mock-treated control

146 group, with 271 being up-regulated and 203 being down-regulated (Fig. 2).
147 Methylglyoxal is known to react with the reduced form of glutathione (GSH), which is
148 interconnected with other reductive potentials such as NADPH and ascorbate (35).
149 Treatment with methylglyoxal resulted primarily in several effects similar to oxidative
150 stress response (34): increased expression of genes involved in thiol homeostasis
151 including thioredoxins *trxAB*, GSH synthetase *gshAB* (36), glutathione reductase *gor,*
152 and glutathione S-transferase *gst*; increased expression of the iron-sulfur (Fe-S) cluster
153 biosynthesis system *sufCDSUB* (37), the ascorbate metabolic cluster (SMU.271 to
154 SMU.275) (38), the reactive oxygen species (ROS) scavenging genes *sodA*, *ahpCF*,
155 and *tpx*, and genes whose products are involved in metal homeostasis: copper exporter
156 operon *copYAZ*, and iron-sequestering gene *dpr*. Also upregulated were several
157 transcription regulators known to be involved in stress management: *relS*, *perR*,
158 *phoR/ycbL*, *scnKR*, *rcrRPQ*, SMU.1097c, *vicKX*, *comR*, *comDE*, and *lexA*. Likely as the
159 need to restore the GSH/GSSG balance, treatment of methylglyoxal upregulated
160 several putative aldo/keto reductases: SMU.382c and SMU.383c, SMU.677 to
161 SMU.681, SMU.1040c, and SMU.1602 residing immediately upstream of *lguL* which
162 encodes the major methylglyoxal-degrading enzyme LguL/GloA (both up by 3~4-fold). It
163 is worth noting that most of these aldo/keto reductases, other than *lguL*, are absent in
164 related commensal streptococci. Also related to improved reducing capacity was
165 upregulation of several putative ribonucleotide reductases (39, 40): SMU.667 to
166 SMU.669c, SMU.991, and SMU.2070 to SMU.2074.
167 Interestingly, methylglyoxal treatment reduced the expression of iron/manganese
168 transporter *sloAB* (not *sloC*) and cognate *sloR* regulator. It also reduced the expression

169 of ferrous ion transporter genes *feoAB*. These changes were consistent with the
170 purported ability of GSH to buffer intracellular metal ions (41), as a release of these ions
171 would likely increase the expression of metal exporters (*copA*) but reduce that of metal
172 importers (*sloABC* and *feoAB*). Another likely effect of methylglyoxal exposure is its
173 reaction with intracellular amino acids such as arginine and lysine. For this, we
174 observed increased expression of amino acid biosynthesis/transport operons, *argCJBD*
175 for arginine and *livFGMHK* for branched-chain amino acids. As a general response to
176 stressful stimuli, we observed elevated expression of genes required for DNA repair
177 (*exoA*, *end3*, *mutY*, and *uvrA*) and protein or peptide degradation (*clpC, clpE, pepO*,
178 and *pepP*). Conversely, there were also several stress management genes that showed
179 reduced expression: *mreCD*, *irvR*, *hrcA, grpE,* and *dnaK*. Most ribosomal proteins and
180 the translation initiation factor InfC also had lower mRNA levels, indicative of reduced
181 protein synthesis activity. There was likely a shift toward a more efficient, mixed-acid
182 fermentation as we saw greater expression by acetoin metabolic operon (*adhABCD-*
183 *lplA*), the related alpha-acetolactate metabolic genes *alsS* and *aldB*, NADH oxidase
184 (*nox*), and a pyruvate-formate lyase activating enzyme *pflA*. At the same time, the
185 genes encoding the two major enzymes of the PTS, *ptsH* and *ptsI*, along with the
186 transport and metabolic pathways for mannitol (EII^{Mtl}, SMU.1184c and SMU.1185),
187 cellobiose (EII^{Cel}, SMU.1596 to SMU.1600), sucrose (EII^{Scr}, SMU.1841 to SMU.1844),
188 and glucose (EII^{Man}, SMU.1877 to SMU.1879) all showed significantly reduced
189 expression.
190

191 *Comparative analysis of methylglyoxal regulon with H_2O_2-mediated stress response.*
192 During the analysis of the methylglyoxal-induced transcriptomic shift, it was immediately
193 apparent that many of these genes were also identified by a previous study on peroxide
194 stress response of *S. mutans* UA159 (34). A closer inspection of the peroxide stress
195 transcriptome identified a total of 115 genes (Supplemental Table S3) as being shared
196 by methylglyoxal and H_2O_2, indicative of the common bacterial mechanisms needed to
197 deal with these two stressors. These functions included ROS scavenging, thiol and
198 metal homeostasis, DNA repair and protein processing, and energy metabolism.
199 Important distinctions were also noted which encompassed 359 methylglyoxal-specific
200 genes that were missing in H_2O_2 regulon, and conversely 79 H_2O_2-responsive genes
201 absent in the methylglyoxal regulon (Supplemental Table S3). Briefly, absent in H_2O_2-
202 induced response included the reduced expression of the PTS genes, namely *ptsH,*
203 *ptsI*, and EII^{Mtl}, EII^{Cel}, EII^{Scr}, and EII^{Man} operons. Also absent were genes for ribosomal
204 proteins, *lrgAB-lytST*, *argCJBD* and agmatine deiminase operon (42), several putative
205 aldo/keto reductase clusters, potassium transporters *trkAB*, the *nrd* operon SMU.2070
206 to SMU.2074, *mreCD*, *hrcA-grpE-dnaK*, and transcription regulators including *mleR,*
207 *relS*, *phoR/ycbL*, *nagR*, *irvR, vicKX, comX,* and *ahrC*. Missing from the methylglyoxal
208 regulon were some of the H_2O_2-induced genes, including mutacin genes (*nlmABCD* and
209 *nlmTE*), *sloC*, *liaSR, levQRST, relA,* and the zinc import operon *adcBCR.* Interestingly,
210 while methylglyoxal induced expression of most of the competence regulators,
211 *comCDE, comR*, and *rcrR* (43), and the late-competence operon *comYA-YI* (44), H_2O_2
212 reduced expression of two important *nlm* operons, one of which (*nlmABCD*) requires an
213 active *comDE* circuit for expression (45).

214

215 *Significant overlap between fructose, methylglyoxal, and H_2O_2-mediated responses.*

216 Treatment of UA159 with 50 mM fructose differentially affected the expression of a total
217 of 449 genes, with 250 being up-regulated and 199 being down-regulated (Fig. 2 and
218 Table S2). As a control, similar treatment with glucose also upregulated 128 genes and
219 down-regulated 216 genes (Table S2). Significantly, there was a larger overlap between
220 the genes affected by fructose and methylglyoxal, totaling 176 genes, compared to the
221 116-gene overlap between glucose and methylglyoxal. Among the 176 genes shared by
222 methylglyoxal and fructose, 61 overlapped the H_2O_2 regulon; among the 116 genes
223 shared by methylglyoxal and glucose, only 24 overlapped with the H_2O_2 regulon
224 (Supplemental Table S4). Therefore, compared to glucose, significantly more genes
225 were shared by treatment of fructose with that of methylglyoxal and H_2O_2. Comparative
226 analysis identified a set of 44 genes that were similarly affected by fructose,
227 methylglyoxal, and H_2O_2, excluding 17 genes impacted by all four treatments.
228 Altogether, these 61 genes (Supplemental Table S4 tab "All three") encoded for some of
229 the aforementioned functions such as thiol and metal homeostasis (*trxAB, gst, gor,*
230 *sloA, dpr,* and *sufCDSUB*,), enzymes for ROS scavenging (*sodA, ahpCF,* and *tpx*) and
231 restoration of redox balance (*nox*, ascorbate operon, *gapN*, and multiple *nrd* genes),
232 glucan-binding protein *gbpC*, proteins implicated in general stress management
233 including *clpE, clpL*, *uvrA, mutY,* SMU.503c (46), *exoA, lexA* (47), *pepP,* and *pepO* (48,
234 49), and transcription regulators such as *scnKR*, *perR,* and *msmR.* Notably, many
235 genes shared by glucose and fructose, e.g., *sloA, sodA, gor, tpx, gst, trxAB*, and *lexA*,
236 showed significantly greater change due to the treatment by fructose than by glucose.

237 Also included in this group of genes was the CRISPR1-Cas system (SMU.1402c to
238 SMU.1406c) that was repressed by all four treatments, although the effect of H_2O_2 was
239 slightly below the 2-fold threshold. Conversely affected was the 10-gene CRISPR2-Cas
240 cluster (SMU.1753c to SMU.1764c) that produced increased levels of transcripts under
241 all four conditions, despite that the induction of several genes by methylglyoxal or
242 glucose was below the threshold. An earlier study characterizing these two systems in
243 *S. mutans* suggested that CRISPR-Cas overall was required for tolerance towards
244 membrane, thermal, pH, and oxidative stressors (50). The differential regulation of
245 these two systems in response to carbohydrates, methylglyoxal, and H_2O_2 warrants
246 further exploration. It is also worth noting that the relative impact of fructose to both
247 clusters was the highest among all four treatments.
248
249 *Similarity between responses to fructose and glucose*. It was not surprising to find a
250 substantial overlap between fructose- and glucose-responsive genes (total 165 genes,
251 80 genes when excluding methylglyoxal and H_2O_2; Supplemental Table S5), although
252 many (near 50%) encoded hypothetical proteins. Interestingly, the operon required for
253 malolactic fermentation (SMU.137-SMU.141) were most highly induced by either
254 treatment, with the mRNA levels increasing by 12~20-fold. Considering the inducibility
255 of this system by acidic pH (51, 52), a rapid drop in pH due to glycolytic activities likely
256 triggered this response. The agmatine deiminase (*aguA*) that has been shown to
257 contribute to acid tolerance through production of ammonia (42) was similarly
258 upregulated. Also induced by both carbohydrates were the majority of the genes/ORFs
259 included in the genomic island TnSmu1 (53), from SMU.196c to SMU.215c, showing an

260 increase in expression by 2~4-fold. Other notably upregulated genes included the
261 SMU.1602/*lguL* cluster, the zinc/cadmium-exporter *zccE* (54), the glucosyltransferase
262 *gtfD*, the fructosyltransferase *ftf*, a putative F-1-P kinase *pfk* (17), the lactose operon
263 (55), and the HPr kinase/phosphatase *hprK*. The fact that SMU.1602/*lguL* cluster was
264 induced by methylglyoxal, fructose, and glucose suggested that endogenous production
265 of methylglyoxal or related RES likely increased because of rapid influx of
266 carbohydrates. Like before, *zccE* expression was >2-fold higher when treated with
267 fructose compared to treatment by glucose. One cluster, SMU.1903c to SMU.1913c,
268 along with the orphan response regulator *gcrR* (56), showed reduced expression under
269 both treatments.
270
271 <u>*Genes affected only by fructose.*</u> A total of 168 genes were differentially affected by
272 fructose alone (Supplemental Table S5 tab "Fru only"). In addition to what have been
273 discussed thus far, treatment with 50 mM fructose altered the expression of several
274 pathways and additional genes required for carbohydrate metabolism. Namely, fructose
275 upregulated the operons of *sppRA*, *fruRKI*, *levDEFG*, and the sorbitol PTS, however
276 downregulated the nigerose PTS, the maltose PTS (*malT* or *ptsG*), *pfl,* the *msm* cluster
277 (57), *galK*, *gtfC,* the glycogen operon (*glg*) (58), an α-glucan metabolic cluster
278 (SMU.1564 to SMU.1571) (59), and importantly the fructose-bisphosphate aldolase
279 (*fbaA*, down 2-fold) which is responsible for cleaving F-1,6-bP into dihydroxyacetone
280 phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P). Expression of three
281 additional clusters was notably affected by fructose alone. First, SMU.1057 to
282 SMU.1063, encoding SatCDE, Ffh, YlxM, and two ABC transporters, were upregulated

283 by 2- to 3-fold. This cluster has been implicated in protein translocation and acid
284 tolerance in *S. mutans* (60). Second, operon (SMU.1527 to SMU.1534) which encodes
285 for the F_1F_0-ATPase complex were downregulated by 2- to 3-fold. It was reported that
286 the F_1F_0-ATPase, whose main function in *S. mutans* is expelling excess proton to the
287 environment, was upregulated in response to a shift from steady state at pH 7 to pH 5
288 as part of the acid tolerance response (ATR) (61-63). Despite the likelihood of an acidic
289 stress under the treatment of both glucose and fructose, there was no significant impact
290 by glucose to this operon, and the treatment of fructose resulted in a lower rather than
291 higher expression of all 8 genes encoding the F_1F_0-ATPase. Last, SMU.1734 to
292 SMU.1745c, encoding for the pathway for fatty acid biosynthesis and similarly involved
293 in ATR, showed 2- to 3-fold reduction in expression when treated with 50 mM fructose.
294 While this effect was largely consistent with previous findings made in *S. mutans* under
295 a glucose shock at 200 mM (63), here again 50 mM glucose failed to induce a similar
296 response. Considering the importance of both pathways in maintaining membrane
297 integrity and overall fitness, these findings could provide explanations to previously
298 reported fructose-specific phenotypes such as autolysis and increased biofilm
299 development (23, 30).
300
301 **Fructose impacts metal homeostasis and Spx-dependent oxidative stress**
302 **response.**
303 A likely effect of methylglyoxal on bacterial physiology was the reduction of
304 intracellular GSH/GSSG ratio and potential impact on metal homeostasis. Previously it
305 was demonstrated that a zinc/cadmium exporter ZccE was responsible for the high zinc

306 tolerance of *S. mutans*, and its expression required a cognate regulator ZccR (54).
307 Deletion of either *zccE* or *zccR* resulted in a peroxide sensitivity under high-zinc
308 conditions due to a disturbed zinc/manganese ratio which could be alleviated by
309 manganese supplementation. When we tested the growth phenotype of a *zccR* null
310 mutant in TV medium (64), which contains ~19 μM of zinc according to ICP-OES
311 analysis (54), the mutant nonetheless grew normally when supported by glucose as the
312 sole carbohydrate source (Fig. 3A). When fructose was substituted for glucose
313 however, Δ*zccR* showed a significant reduction compared to the WT UA159 in both
314 growth rate and the final optical density (Fig. 3B). Like before, addition of 25 μM $MnSO_4$
315 to the TV-fructose medium significantly alleviated the growth deficiency of Δ*zccR*.
316 Importantly, after we introduced a *fruI* deletion into the *zccR* background, the double
317 mutant grew at levels comparable to the WT UA159 on either glucose or fructose,
318 without manganese supplementation (Fig. 3B). As *fruI* deletion was previously shown to
319 reduce intracellular F-1-P levels (23), these findings strongly supported the theory that
320 excess F-1-P levels negatively impacted metal homeostasis in *S. mutans*.

321 Since fructose-mediated transcriptome included a cohort of oxidative stress genes
322 primarily regulated by SpxA1 (65), we repeated this growth assay by including a
323 deletion mutant of *spxA1*. To our surprise, Δ*spxA1* grew at a faster rate in TV-fructose
324 than in TV-glucose (Fig. 4A). Considering the relatively high zinc content in TV medium,
325 we next switched to a chemically defined FMC medium that contained very little zinc but
326 sufficient manganese (around 110 μM). In FMC medium, Δ*spxA1* grew relatively well on
327 glucose but could not grow on fructose (Fig. 4B). When we introduced the same *fruI*
328 deletion into the Δ*spxA1* background, the growth deficiency on fructose was greatly

329 alleviated (Fig. 4C). At the same time, only modest benefit was observed on fructose
330 when a *levD* deletion was introduced into Δ*spxA1.* Considered with the transcriptomic
331 data, the medium-specific fructose sensitivity of Δ*spxA1* added further support to the
332 notion that F-1-P affects oxidative stress response and metal homeostasis by
333 interacting with Spx-mediated regulation.

334 To further test this hypothesis, a P*sodA::gfp* promoter-reporter fusion was established
335 in the WT UA159 background to assess the response of Spx-mediated regulation to the
336 presence of fructose; the superoxide dismutase (*sodA*) requires Spx proteins for optimal
337 expression (34). As shown in Fig. 5, the *sodA* promoter was activated by the presence
338 of fructose in a dose-dependent manner, from levels as low as 0.5 mM, yet it only
339 produced a modest and slightly delayed response to the presence of 0.5 mM of H_2O_2.
340 While 0.5 mM H_2O_2 significantly impeded the growth of *S. mutans* [Fig. S1 and (66)], as
341 much as 10 mM fructose showed little to now effect. To ascertain the nature of fructose-
342 derived signal in this effect, *fruI* and *levD* deletions were each introduced into UA159
343 containing the P*sodA::gfp* fusion. Consistent with above observations, deletion of *fruI*
344 but not *levD* resulted in the loss of fructose-dependent activation of the *sodA* promoter
345 activity. As expected, deletion of *spxA1* also abolished the expression of *sodA* promoter
346 (34). These results not only confirmed the role of F-1-P in influencing Spx regulation,
347 but given the magnitude of impact, suggested that fructose may serve as a primary
348 signal for bacterial response to environmental changes.

349 F-1-P is the likely allosteric inducer for activating the *fruRKI* operon. To assess the
350 levels of fructose needed to induce the expression, we utilized a strain containing a
351 promoter-reporter fusion P*fruR::cat* (20). Bacteria were cultured in glucose-based FMC

352 medium and then exposed to different amounts of fructose for 30 min. CAT activities
353 from UA159/P*fruR::cat* suggested that at levels as low as 20 μM, fructose can
354 significantly induce the expression of the *fruRKI* operon (Table S6).
355

356 **F-1-P contributes to fitness during starvation, acidic pH, and competition with**
357 **peroxigenic streptococci.**

358 Our previous studies have suggested that fructose, when used in excess under
359 laboratory conditions, may induce autolysis and cell death. Paradoxically, considering
360 the activation of much of the stress response core by fructose, we could anticipate
361 improved persistence under stressful conditions. To test this hypothesis, we subjected
362 *S. mutans* to a series of physiological tests by exposing it to fructose. First, strain
363 UA159 was cultivated in FMC with 20 mM glucose or fructose, and their CFU was
364 monitored while these cultures were continuously incubated without medium
365 refreshment for 11 days. While fructose produced lower CFU than glucose at day 1 of
366 the incubation as reported previously (23), it maintained the viable CFU of UA159 by at
367 least 1 log higher than on glucose throughout the rest of the experiment (**Fig. 6A**).
368 When 1 mM fructose was used together with 19 mM glucose, the fructose-associated
369 effects were reduced and limited to the first two days of the incubation.

370 As a further test of acid tolerance under the fructose condition, we cultured UA159 for
371 20 h in a TV medium (FMC contains 10 mM phosphate buffer thus not ideal) supported
372 with different amounts of glucose or fructose and measured the resting pH of these
373 cultures. The results (Table 2) showed comparable growth yield but significantly lower
374 resting pH in TV-fructose cultures than in TV-glucose cultures. For comparison, we

375 conducted the same experiment on a health-associated commensal, *S. sanguinis* (SSA)
376 strain SK36. To our surprise, SK36 failed to produce substantial growth on fructose,
377 especially for higher concentrations (100 or 200 mM). Similar findings were made when
378 this experiment was repeated using an additional *S. sanguinis* isolate BCC23 (67).
379 Further tests on several low-passage, clinical isolates of additional commensal
380 streptococci (68) showed similar sensitivity in most but not all species tested (Table 2).
381 We then performed a pH drop assay to further test the notion that fructose could lower
382 the culture pH better than glucose. When a suspension of UA159 cells from an
383 exponential phase, BHI culture was fed 50 mM of fructose without buffering, it lowered
384 the environmental pH at a notably faster rate, which ended at a slightly lower point, than
385 it did on 50 mM glucose (Fig. 6B). It appeared that *S. mutans* catabolized fructose more
386 rapidly than glucose and was more acid-tolerant under our test conditions, while *S.*
387 *sanguinis* may be less capable of tolerating high levels of fructose.

388 Next, *S. mutans* was subjected to a competition assay in a mixed-species planktonic
389 culture with the peroxigenic commensal *S. sanguinis*. Cultures from the exponential
390 phase were mixed at approximately 1:1 ratio between two species and diluted into the
391 FMC medium supplemented with 20 mM of glucose or fructose. After an overnight of
392 incubation, CFUs for each species were enumerated by plating on selective agar plates
393 (Fig. S2 includes CFU from mixed-species cultures and CFU from single-species
394 controls). The resultant competitive indices (Fig. 7) indicated a greater competitiveness
395 for *S. mutans* UA159 in the presence of fructose than glucose, especially when used at
396 higher concentrations. Interestingly, deletion of *fruI* in UA159 further enhanced the
397 competitiveness of *S. mutans* against *S. sanguinis* SK36 when supported with fructose.

398 Since Δ*fruI* has a defect in fructose transport and grows more slowly than the WT on
399 fructose (17), these results suggested that a certain optimal level of F-1-P could benefit
400 *S. mutans* when competing against commensal species capable of producing inhibitory
401 levels of H_2O_2.

402

403 ## Discussion

404 It is frequently observed in stress biology that different stressors result in overlapping
405 responses in bacteria (69). As an oral bacterium, *S. mutans* is frequently subjected to
406 environmental insults that include acidic pH, oxygen and H_2O_2, hyperosmolarity, thermal
407 fluctuation, metal ions, and nutrient starvation (70). In this study, we identified
408 methylglyoxal and fructose as two potential stress signals capable of inducing
409 streptococcal stress responses (Fig. 8). A stress core comprised of 61 genes were
410 shared by the regulons of fructose, methylglyoxal, and H_2O_2. This commonality in gene
411 regulation is in large part due to the overlapping effects exerted by these treatments.
412 For example, sugar shock likely leads to acidic stress, hyperosmolarity, and increased
413 RES production. Treatments by both RES and ROS are expected to perturb intracellular
414 redox pools that could in turn influence bacterial metal and thiol homeostasis. Exposure
415 to extreme pH, RES, and ROS can all lead to denaturation of amino acids, proteins,
416 lipids, and nucleic acids. Each of these outcomes likely activates a central regulatory
417 system to restore essential nutrients or repair damage. Not surprisingly, 40 out of the 61
418 core stress genes (Table S4) have been previously identified in *S. mutans* by an acid
419 adaptation study in response to either a glucose shock (200 mM) or a shift from neutral
420 to acidic pH (63). Our results suggested a substantially greater ability for *S. mutans* to

421 lower environmental pH when fructose compared to glucose was used as the
422 supporting carbohydrate, especially at higher concentrations. This ability to tolerate
423 fructose appears to be limited or absent in many commensal oral streptococci we have
424 tested so far. Conversely, only 17 out of the 61 stress-related genes were affected by
425 our treatment of 50 mM glucose, likely due to the difference in sugar content and culture
426 conditions between these two studies (continuous culturing was used as opposed to
427 batch cultures herein). Furthermore, several pathways triggered by hyperosmolarity in
428 previously studies (71-73), including genes *lguL, sodA, nox*, the *opcA* cluster
429 (SMU.2116 to SMU.2119), the *opu/ffh/sat* cluster, and potassium uptake mechanism
430 *trkAB*, were also identified as part of the fructose/methylglyoxal/H_2O_2 response core.
431 Theories established in mostly Gram-negative bacteria suggest that methylglyoxal
432 bypass, a function that converts DHAP into pyruvate via the activities of a methylglyoxal
433 synthase (*mgsA*), two glyoxalases (*gloAB*), and a D-lactate dehydrogenase (D-*ldh*),
434 serves an important function in modulating glycolysis under sugar excess and
435 phosphate limitation (32). Genes *mgsA* and D-*ldh* are absent in most lactic acid bacteria
436 (LAB) genomes (30). However, methylglyoxal and related RES can be produced from
437 DHAP spontaneously or from a variety of other mechanisms (74). The conservation of
438 glyoxalases in streptococci (75) and the transcriptomic overlap among fructose,
439 glucose, and methylglyoxal support the notion that methylglyoxal metabolism is an
440 integral part of bacterial physiology, and the sources of methylglyoxal could be both
441 endogenous and exogenous. This conclusion is consistent with an emerging theory in
442 bacterial pathophysiology termed the aldehyde hypothesis (76), where a group of
443 reactive metabolic intermediates (RES) from the host and pathogens may serve as

444 potential antimicrobial effectors. This theory has supports from studies on LAB
445 pathogens that engage host immunity, including *S. pyogenes* and *S. agalactiae*, which
446 required glyoxalases for fitness and virulence (31, 75). For oral streptococci, the effects
447 of fructose on methylglyoxal metabolism could involve several mechanisms. First,
448 multiple RES compounds can be generated during fructose metabolism, given the
449 presence of three phosphorylation products, F-1-P, F-6-P, and F-1,6-bP. Glucose
450 metabolism generates only F-6-P and F-1,6-bP. DHAP is a direct product of cleavage of
451 F-1,6-bP; cleavage of F-1-P by an aldolase produces glyceraldehyde (GA) and DHAP;
452 and similarly, cleavage of F-6-P yields G3P and dihydroxyacetone (DHA). These
453 metabolites are either themselves RES, e.g., GA, or in the case of DHAP and DHA, can
454 generate methylglyoxal due to a reaction that is spontaneous or enzymatic in nature
455 (MgsA). Second, fructose degradation may be regulated differently than glucose
456 degradation, which could affect the accumulation or degradation of metabolic
457 intermediates that are precursors of RES. Rapid phosphorylation of fructose by
458 fructokinase and the resultant drawdown of ATP is a major factor in fructose-dependent
459 metabolic impact in mammalian cells (16). Although much remains uncharacterized
460 regarding fructose catabolism in bacteria, here we showed a notably faster rate of
461 glycolysis by *S. mutans* on fructose than glucose. A major difference between
462 mammalian cells and bacteria is the fact carbohydrates are phosphorylated by PTS at
463 the expense of PEP instead of ATP. Here fructose-specific transcriptome included
464 upregulation of two likely F-1-P kinases (*pfk* and *fruK*) and downregulation of the F-1,6-
465 bP aldolase *fbaA*. Significance of these changes in gene regulation must be interpreted
466 comprehensively with biochemical evidence. Lastly, we have identified a LguL paralog,

467 GloA2, whose function remains uncharacterized but appears to be specific to F-1-P and
468 involves phenotypes resembling methylglyoxal exposure (30). Homologues of *gloA2* are
469 widely conserved in both Gram-positive and Gram-negative bacteria. Consistent with
470 these reasonings is the finding during our transcriptomic analysis that many common
471 genes shared by fructose and glucose produced a greater amplitude of change under
472 fructose treatment. We infer from these results that fructose exerted a strong impact to
473 bacterial physiology due to its unique metabolic processes, in part by influencing
474 methylglyoxal accumulation and degradation.

475 In addition to influencing methylglyoxal metabolism, F-1-P homeostasis appears to be
476 an essential physiological signal in most streptococci. The *fruRKI/fruRBA* operon
477 required for catabolizing fructose via the F-1-P pathway is highly conserved in most
478 Gram-positive bacteria. Several oral streptococci also harbor the *sppRA* operon for
479 moderating the F-1-P levels (23). We previously showed that a *fruK* deficient strain
480 accumulated significant levels of F-1-P even at the absence of fructose and was highly
481 defective in growth on fructose, suggesting an endogenous source of F-1-P and the
482 need to keep its level within an optimal range (23). Notably, the *sppRA* promoter
483 required higher levels of fructose (25 to 50 mM) for induction (23), suggesting that
484 *sppRA* are expressed only when F-1-P is above a certain threshold to avoid futile
485 cycling between F-1-P and fructose. We have determined herein that 0.5 mM fructose is
486 capable of activating Spx-mediated regulation and as little as 20 μM of fructose
487 efficiently induced the expression of the *fruRKI* operon. We also observed in *S.*
488 *sanguinis* the induction of *fruRBA* expression by similar levels of fructose or 25% human
489 sera (Table S6). It is worth noting that for diabetic or hyperglycemic individuals, blood

490 fructose levels are often significantly elevated as a function of the polyol pathway (16),
491 capable of activating the *fruRKI/fruRBA* pathway (77). These findings allow us to posit
492 that fructose regulates streptococcal function at both high (mM) and low (μM) levels,
493 with the former contributing to fitness during the feast-and-famine cycle of the oral cavity
494 and the latter in human circulatory system. While we only showed that fructose can
495 benefit *S. mutans* at mM concentrations, we have good reasons to speculate that a
496 similar benefit applies under μM levels of fructose. Several studies have suggested that
497 *fruRKI/fruRBA* genes are required for streptococcal virulence during extraoral infections
498 (19, 22, 78), although the mechanisms remain uncharacterized. We further posit that
499 fructose may trigger a surveillance mechanism against host-derived ROS and RES
500 molecules, which primes bacterial stress response and additional fructose-specific
501 pathways (Fig 8). Aside from FruR, SppR, LevQRST, and LacR that were previously
502 shown to be responsive to fructose, herein we identified several transcription regulators
503 whose expression appeared uniquely responsive to fructose treatment, including *spxA2,*
504 *glnR, hdrR,* and a two-component system *spaRK*. Targeting these regulators and F-1-P-
505 specific metabolism, i.e., GloA2, could help us unravel the molecular mechanisms
506 required for cells to optimize stress management in response to fructose. With many
507 oral streptococci existing as dual-niche organisms and the widespread use of fructose in
508 food, this research has implications in both oral microbial homeostasis and systemic
509 health.
510 Lastly, it is worth reiterating that a fructose-PTS mutant (*fruI*) of *S. mutans* displayed
511 reduced colonization and cariogenicity in an earlier rat study under high-sucrose
512 condition (24). This study revealed an important connection between fructose and

513 sucrose in *S. mutans* physiology, as sucrose metabolism releases significant quantities
514 of fructose both intracellularly and extracellularly, the latter of which can be
515 reinternalized by the PTS (33, 79, 80). It is probable that fructose liberated from sucrose
516 activated bacterial stress response which enhanced the ability of *S. mutans* to colonize
517 and induce caries in rats fed with sucrose. Our findings in fructose-mediated physiology
518 and its potential contribution to sucrose cariogenicity illustrate that fructose has an
519 underappreciated role in caries development and biofilm dysbiosis that warrants in-
520 depth investigation.
521
522 *Concluding remarks*. By establishing a stress regulon shared by fructose,
523 methylglyoxal, and H_2O_2, our study revealed a novel function of fructose in bacterial
524 physiology as both a stressor and potentially an important environmental signal for oral
525 streptococci. Gene regulations associated with an F-1-P homeostasis contributed to
526 bacterial fitness under stressful conditions, especially for the cariogenic pathobiont. The
527 overlapping nature of multiple stress responses also suggests that fructose metabolism
528 may affect the overall resilience against other stressors that include acidic pH, ROS,
529 RES, toxic metals, starvation, and hyperosmolarity; conditions to be expected in both
530 oral cavity and the blood stream. Considering the widespread conservation of some of
531 these genetic pathways, most of the fructose-mediated effects are likely applicable to
532 other lactic acid bacteria which have high relevance in both oral and systemic health.
533

534 ## Materials and Methods

535 **Bacterial strains and culture conditions.** Brain heart infusion (BHI) medium (Difco
536 Laboratories, Detroit, MI) was used to maintain *S. mutans* and other streptococcal
537 strains (Table 3). Liquid BHI was used to prepare for starter cultures for most of the
538 experiments. Antibiotics were used, only when necessary, at the following
539 concentrations: kanamycin (Km) 1 mg/mL, erythromycin (Em) 10 μg/mL, and
540 spectinomycin (Sp) 1 mg/mL. For cultures requiring specific carbohydrates, a semi-
541 defined Tryptone-Vitamin (TV) medium (64) or a synthetic medium FMC (81) was used,
542 each supplemented with specified carbohydrates. Other than growth curves, all cultures
543 were incubated without agitation at 37˚C in an aerobic atmosphere supplemented with
544 5% of CO_2.
545 For growth curves, bacterial cultures were diluted 100-fold into a TV or FMC medium
546 containing specified carbohydrates, overlaid with 70 μL mineral oil, and maintained at
547 37˚C in a Bioscreen C lab system (Labsystems Oy, Helsinki, Finland) that recorded the
548 optical density of the cultures at 600 nm (OD_{600}) every hour. A brief lateral shaking was
549 included before each reading to disperse aggregates. To monitor the expression levels
550 of the *sodA* promoter over time using a P*sodA::gfp* reporter fusion, bacterial cultures
551 from the exponential phase were similarly diluted into an FMC medium containing
552 specified amounts of carbohydrates and H_2O_2 and overlaid with mineral oil. For 20 h,
553 the OD_{600} and the relative fluorescence units (RFU) of the cultures were recorded hourly
554 using a Synergy 2 Multi-Mode reader (BioTek, Winooski, VT) and the RFU results were
555 normalized against the OD_{600} of the culture (82).
556

557 **Construction of genetic mutants.** Genetic mutants of *S. mutans* were constructed
558 through allelic exchange strategy and natural transformation facilitated by the use of
559 competence-stimulating peptide (30). Recombinant DNA fragments used in this study
560 were generated by PCR amplification and ligated together with antibiotic cassettes via
561 Gibson Assembly (23). Primers used in DNA amplification and RT-qPCR are listed in
562 Table S7. All mutants used in this study have been validated by PCR followed by
563 Sanger sequencing. Promoter: reporter fusions were constructed using the plasmid
564 pMC340B by inserting a promoter of interest in front of a promoterless *gfp* (green
565 fluorescent protein) or *cat* (chloramphenicol acetyltransferase) gene, followed by
566 integration at a distal site (between *mtlA* and *phnA*) of the chromosome unrelated to
567 functions studied here (20).
568
569 **RNA deep sequencing and transcriptomic analysis.** To compare bacterial gene
570 regulation in response to fructose metabolism and exposure to methylglyoxal, *S.*
571 *mutans* strain UA159 was cultivated to early exponential phase (OD_{600} = 0.4) in a
572 chemically defined medium FMC (81) supplemented with 20 mM glucose. Bacteria were
573 harvested by centrifugation (3,800× *g*, room temperature, 10 min), resuspended in fresh
574 FMC containing 50 mM fructose, 50 mM glucose, or 5 mM methylglyoxal. An equal
575 portion of the culture was kept in the original FMC-glucose medium as control. All
576 samples were returned to incubation for 30 min before being harvested for RNA
577 extraction.
578 RNA extraction and deep sequencing were carried out by following a previously
579 published protocol detailed elsewhere (83). Briefly, cells were disrupted using a

580 beadbeater in the presence of equal volume of acidic phenol:chloroform. After
581 centrifugation, the cell lysate was processed using the RNeasy minikit (Qiagen,
582 Germantown, MD) and an RNase-free DNase I solution (Qiagen) for extraction of total
583 RNA. The RNA samples were shipped on dry ice to SeqCenter (Pittsburgh, PA) for
584 quality check, cDNA preparation, and deep sequencing, yielding up to 12 million paired
585 end reads (2 × 51 bp) per sample. The analysis of the RNA-seq data was performed in
586 software R version 3.5.2 Eggshell Igloo as described previously (83). The compositional
587 matrix of the expression data was normalized with the voom (84) function from the R
588 package limma (85). The statistical analysis of the expression data was conducted
589 using the package edgeR (version 3.24.3). A false-discovery rate (FDR) of 0.05 and a
590 fold-of-change of 2.0 were used as the cutoff values for identification of genes with
591 differential expression. Findings from this analysis were validated by reverse
592 transcriptase quantitative PCR (RT-qPCR) (30) carried out on a few randomly selected
593 genes (data not shown). The sequencing data from this study have been deposited at
594 NCBI Gene Expression Omnibus (GEO) under the accession numbers GSE279080.
595 For comparative analysis under different treatments, we obtained the RNA-seq
596 datasets (GEO, GSE98526) of an analysis previously performed on strain UA159 that
597 was treated with 0.5 mM H_2O_2 for 5 min (34). Comparison between transcriptomes was
598 carried out in R statistical language using the package edgeR as done previously (86).
599

600 **Long-term persistence assay.** For comparison of the ability to persist under low-pH
601 and starvation conditions, the cultures of *S. mutans* were each diluted 1000-fold into an
602 FMC medium supplemented with 20 mM glucose, 20 mM fructose, or 19 mM glucose

603 and 1 mM fructose combined. The cultures were incubated at 37°C in an ambient
604 atmosphere supplemented with 5% CO_2 for a duration of 11 days. After two days of
605 incubation, a 30-second sonication at 100% power (FB120 water bath sonicator, Fisher
606 Scientific) was applied to all cultures to disperse bacterial chains and aggregates, then
607 a 100-μL aliquot of each culture was removed. The cultures were returned to incubation
608 without any other treatments, and the 100-μL aliquots were each diluted decimally in
609 sterile PBS and plated on BHI agar. After 2 days of incubation, the CFU on each plate
610 were enumerated. Plating was repeated at specified time points thereafter till end of the
611 experiment.
612
613 **Mixed-species competition in planktonic cultures.** For interspecies competition in
614 liquid cultures, differentially marked strains of *S. mutans* (UA159-Km and Δ*fruI::Km*) (87)
615 and *S. sanguinis* (MMZ1945, Em resistant) (88) were each cultured in BHI to the
616 exponential phase (OD_{600} = 0.5). An inoculum of approximately 10^6 CFU/ml of *S.*
617 *mutans* together with similar numbers of *S. sanguinis* were added to FMC
618 supplemented with 20 mM glucose or fructose, then cultured for 20 h in a 5%-CO_2
619 environment at 37°C. At both the start and the end of the incubation, cultures were
620 sonicated for 15 sec, decimally diluted and plated on respective antibiotic plates to
621 enumerate CFU of both species. The competitive index was calculated by following this
622 formula: [SMU(t_{end})/SSA(t_{end})] / [SMU(t_{start})/SSA(t_{start})], with values >1 indicating SMU (*S.*
623 *mutans*) being more competitive than SSA (*S. sanguinis*), and vice versa.
624

625 **pH drop and chloramphenicol acetyltransferase (CAT) assay.** Glycolytic profiling via
626 pH drop (89) and CAT assay (90) were conducted according to previously published
627 protocols detailed elsewhere.
628
629 **Statistics.** Statistical analysis of data was carried out using the software of Prism
630 (GraphPad of Dotmatics, San Diego, CA)
631

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636

637 ## Figure Legends

638 **Fig. 1 Volcano plots illustrating transcriptomes of four treatments.** *S. mutans*
639 UA159 was grown to the exponential phase in a synthetic FMC medium containing 20
640 mM glucose. The samples were treated for 30 min with 50 mM fructose, 50 mM
641 glucose, or 5 mM MG before harvesting for mRNA sequencing. Differential expression
642 analyses were performed against untreated cells using *edgR*, with cutoffs for
643 foldchange >2 and FDR <0.05. Upregulated genes are shown in red, and
644 downregulated ones in blue. Results of treatment by H_2O_2 (0.5 mM, 5 min) were from an
645 independent study (34).
646

647 **Fig. 2 Transcriptomic overlaps among treatments of MG, fructose and H_2O_2.**

648

649 **Fig. 3 Fructose impacts metal homeostasis in Δ*zccR* deficient in zinc expulsion.**
650 *S. mutans* strains UA159 and its mutant derivatives were diluted from exponential
651 phase cultures into TV supplemented with 20 mM glucose (A) or fructose (B). Mn^{2+} was
652 added at 25 μM ($MnSO_4$). Growth was monitored using a Synergy 2 plate reader
653 maintained at 37°C. Results are average of three biological replicates, each tested in
654 technical duplicates. Error bars denote standard deviations.

655

656 **Fig. 4 Fructose impacts growth of *spxA1* in a medium-specific manner.** *S. mutans*
657 strains UA159 and various mutant derivatives were each diluted from exponential phase
658 cultures into TV (A) or FMC (B, C) supplemented with 20 mM glucose (Glc) or fructose
659 (Fru). Growth was monitored using a Synergy 2 plate reader maintained at 37°C.
660 Results are average of three biological replicates, each tested in technical duplicates.
661 Error bars denote standard deviations.

662

663 **Fig. 5 Induction of the *sodA* promoter by fructose.** (A) Cultures of UA159 harboring
664 a P*sodA::gfp* fusion were diluted into FMC containing specified concentrations (in mM)
665 of glucose (G), fructose (F), or H_2O_2. The cultures were monitored in a Synergy 2 plate
666 reader for optical densities (OD_{600}) and green fluorescence for 20 hours. The relative
667 fluorescence units (RFU) of each culture were recorded as a measure of *sodA* promoter
668 activity, after normalization against OD_{600}. (B) The same experiment was performed on
669 various mutants derived from UA159/P*sodA::gfp,* using FMC containing 20 mM glucose

670 (G20) or fructose (F20). Results are the average from three biological replicates,
671 conducted in technical duplicates.
672
673 **Fig. 6 Fructose promotes long-term survival (A) and acidogenicity (B) of *S.***
674 ***mutans*.** (A) Cultures of *S. mutans* UA159 (n =4) were diluted into FMC containing 20
675 mM of glucose (G20) or fructose (F20), or a combination of 19 mM glucose and 1 mM
676 fructose (G19/F1), followed by incubation without medium refreshment for 11 days. At
677 specified time points, an aliquot of culture was removed for CFU enumeration. (B) pH
678 drop assay. UA159 was cultured in BHI to exponential phase and resuspended in a
679 solution of 50 mM KCl and 1 mM $MgCl_2$, with OD_{600} adjusted to 5.0. The pH was
680 adjusted to 7.2 slowly with 0.1 N KOH to consume intracellular carbohydrate storage.
681 Immediately after addition of 50 mM glucose or fructose, the pH of the suspension was
682 monitored continuously on a stirring plate for the next 60 min. Each experiment was
683 repeated three times, with representative results shown. After conversion into proton
684 concentrations, area under the curve (AUC, see inset) of each sample was calculated
685 and used to assess statistical significance using Student's *t*-test (, $P < 0.05$).
686
687 **Fig. 7 Fructose enhances competitiveness of *S. mutans*.** Cultures of *S. mutans* wild
688 type UA159 or its isogenic *fruI* deletion mutant was mixed at 1:1 ratio with a differentially
689 marked *S. sanguinis* strain, then diluted at 1:100 into FMC containing glucose or
690 fructose, each used at 20 mM or 200 mM. After 24 h of incubation, the cultures were
691 sonicated, decimally diluted and plated on selective agar plates for CFU enumeration.

692 CFUs from both the start and end of the incubation were used to calculate competitive
693 index of each species.
694
695 **Fig. 8 Fructose metabolism triggers stress response in streptococci.** Fructose is
696 internalized by oral streptococci primarily through a F-1-P-generating PTS transporter,
697 FruI, but also through two additional PTS permeases, including EII^{Lev} (LevDEFG) that
698 generates F-6-P. A hexose-phosphate phosphohydrolase SppA helps to maintain the
699 homeostasis of intracellular F-1-P. Compared to F-6-P and the glucose pathway, F-1-P
700 is likely catabolized more rapidly via glycolysis, during which RES such as MG is
701 accumulated and impacts cytoplasmic redox balance represented by the ratios of
702 GSH/GSSG and $NADPH/NADP^+$. Also affected are the pools of intracellular metal ions.
703 Sensing these and other not-yet-characterized physiological changes and specific
704 metabolites, the bacterium reprograms a suite of stress responsive genes. The
705 outcomes of exposure to fructose include not only elevated activities in processing RES,
706 through the functions of LguL/GloB and related protein GloA2, but importantly enhanced
707 tolerance to multiple environmental stressors including ROS, RES, acidic pH, toxic
708 metals, and nutrient depletion.

709 **Reference**

710 1. Baker JL, Mark Welch JL, Kauffman KM, McLean JS, He X. 2024. The oral
711 microbiome: diversity, biogeography and human health. Nat Rev Microbiol 22:89-
712 104.
713 2. Hsu CL, Schnabl B. 2023. The gut-liver axis and gut microbiota in health and
714 liver disease. Nat Rev Microbiol 21:719-733.
715 3. Willenborg J, Goethe R. 2016. Metabolic traits of pathogenic streptococci. FEBS
716 Lett 590:3905-3919.
717 4. Bowen WH, Burne RA, Wu H, Koo H. 2018. Oral Biofilms: pathogens, matrix, and
718 polymicrobial interactions in microenvironments. Trends Microbiol 26:229-242.
719 5. Tanner ACR, Kressirer CA, Rothmiller S, Johansson I, Chalmers NI. 2018. The
720 Caries Microbiome: Implications for Reversing Dysbiosis. Adv Dent Res 29:78-
721 85.
722 6. Spatafora G, Li Y, He X, Cowan A, Tanner ACR. 2024. The Evolving Microbiome
723 of Dental Caries. Microorganisms 12.
724 7. Stangvaltaite-Mouhat L, Skudutyte-Rysstad R, Ko H, Stankeviciene I,
725 Aleksejuniene J, Puriene A. 2024. Co-occurrence of dental caries and
726 periodontitis: multilevel modelling approach. BMC Oral Health 24:149.
727 8. Strauss F-J, Espinoza I, Stähli A, Baeza M, Cortés R, Morales A, Gamonal J.
728 2019. Dental caries is associated with severe periodontitis in Chilean adults: a
729 cross-sectional study. BMC Oral Health 19:278.

730 9. Romandini P, Marruganti C, Romandini WG, Sanz M, Grandini S, Romandini M.
731 2024. Are periodontitis and dental caries associated? A systematic review with
732 meta-analyses. Journal of Clinical Periodontology 51:145-157.
733 10. Tappy L, Lê KA. 2010. Metabolic effects of fructose and the worldwide increase
734 in obesity. Physiol Rev 90:23-46.
735 11. Azevedo-Martins AK, Santos MP, Abayomi J, Ferreira NJR, Evangelista FS.
736 2024. The Impact of Excessive Fructose Intake on Adipose Tissue and the
737 Development of Childhood Obesity. Nutrients 16.
738 12. Gillespie KM, White MJ, Kemps E, Moore H, Dymond A, Bartlett SE. 2023. The
739 Impact of Free and Added Sugars on Cognitive Function: A Systematic Review
740 and Meta-Analysis. Nutrients 16.
741 13. Siddiqui SH, Rossi NF. 2024. Acute Intake of Fructose Increases Arterial
742 Pressure in Humans: A Meta-Analysis and Systematic Review. Nutrients 16.
743 14. Staltner R, Burger K, Baumann A, Bergheim I. 2023. Fructose: a modulator of
744 intestinal barrier function and hepatic health? Eur J Nutr 62:3113-3124.
745 15. Ting KKY. 2024. Fructose-induced metabolic reprogramming of cancer cells.
746 Front Immunol 15:1375461.
747 16. Johnson RJ, Lanaspa MA, Sanchez-Lozada LG, Tolan D, Nakagawa T, Ishimoto
748 T, Andres-Hernando A, Rodriguez-Iturbe B, Stenvinkel P. 2023. The fructose
749 survival hypothesis for obesity. Philos Trans R Soc Lond B Biol Sci
750 378:20220230.
751 17. Wen ZT, Browngardt C, Burne RA. 2001. Characterization of two operons that
752 encode components of fructose-specific enzyme II of the

753 sugar:phosphotransferase system of *Streptococcus mutans*. FEMS Microbiol Lett
754 205:337-42.
755 18. Loo CY, Mitrakul K, Voss IB, Hughes CV, Ganeshkumar N. 2003. Involvement of
756 an inducible fructose phosphotransferase operon in *Streptococcus gordonii*
757 biofilm formation. J Bacteriol 185:6241-54.
758 19. Cao Q, Dong Y, Guo C, Ji S, Nie M, Liu G, Wan X, Lu C, Liu Y. 2023. *luxS*
759 contributes to intramacrophage survival of *Streptococcus agalactiae* by positively
760 affecting the expression of *fruRKI* operon. Vet Res 54:83.
761 20. Zeng L, Chakraborty B, Farivar T, Burne RA. 2017. Coordinated regulation of the
762 EII^{Man} and *fruRKI* operons of *Streptococcus mutans* by global and fructose-
763 specific pathways. Appl Environ Microbiol 83:e01403-17.
764 21. Jesionowski AM, Mansfield JM, Brittan JL, Jenkinson HF, Vickerman MM. 2016.
765 Transcriptome analysis of *Streptococcus gordonii* Challis DL1 indicates a role for
766 the biofilm-associated *fruRBA* operon in response to *Candida albicans*. Mol Oral
767 Microbiol 31:314-328.
768 22. Valdes KM, Sundar GS, Vega LA, Belew AT, Islam E, Binet R, El-Sayed NM, Le
769 Breton Y, McIver KS. 2016. The *fruRBA* operon is necessary for group A
770 streptococcal growth in fructose and for resistance to neutrophil killing during
771 growth in whole human blood. Infect Immun 84:1016-31.
772 23. Zeng L, Burne RA. 2019. Essential roles of the *sppRA* fructose-phosphate
773 phosphohydrolase operon in carbohydrate metabolism and virulence expression
774 by *Streptococcus mutans*. J Bacteriol 201:e00586-18.

775 24. Tanzer JM, Thompson A, Wen ZT, Burne RA. 2006. *Streptococcus mutans*:
776 fructose transport, xylitol resistance, and virulence. J Dent Res 85:369-73.
777 25. Gauthier L, Mayrand D, Vadeboncoeur C. 1984. Isolation of a novel protein
778 involved in the transport of fructose by an inducible phosphoenolpyruvate
779 fructose phosphotransferase system in *Streptococcus mutans*. J Bacteriol
780 160:755-63.
781 26. Zeng L, Wen ZT, Burne RA. 2006. A novel signal transduction system and
782 feedback loop regulate fructan hydrolase gene expression in *Streptococcus*
783 *mutans*. Mol Microbiol 62:187-200.
784 27. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, Primeaux
785 C, Tian R, Kenton S, Jia H, Lin S, Qian Y, Li S, Zhu H, Najar F, Lai H, White J,
786 Roe BA, Ferretti JJ. 2002. Genome sequence of *Streptococcus mutans* UA159, a
787 cariogenic dental pathogen. Proc Natl Acad Sci U S A 99:14434-9.
788 28. Xu P, Alves JM, Kitten T, Brown A, Chen Z, Ozaki LS, Manque P, Ge X, Serrano
789 MG, Puiu D, Hendricks S, Wang Y, Chaplin MD, Akan D, Paik S, Peterson DL,
790 Macrina FL, Buck GA. 2007. Genome of the opportunistic pathogen
791 *Streptococcus sanguinis*. J Bacteriol 189:3166-3175.
792 29. Makarewicz O, Lucas M, Brandt C, Herrmann L, Albersmeier A, Rückert C, Blom
793 J, Goesmann A, van der Linden M, Kalinowski J, Pletz MW. 2017. Whole
794 Genome Sequencing of 39 Invasive *Streptococcus pneumoniae* Sequence Type
795 199 Isolates Revealed Switches from Serotype 19A to 15B. PLOS ONE
796 12:e0169370.

797 30. Zeng L, Noeparvar P, Burne RA, Glezer BS. 2024. Genetic characterization of
798 glyoxalase pathway in oral streptococci and its contribution to interbacterial
799 competition. J Oral Microbiol 16:2322241.
800 31. Zhang MM, Ong CL, Walker MJ, McEwan AG. 2016. Defence against
801 methylglyoxal in Group A *Streptococcus*: a role for Glyoxylase I in bacterial
802 virulence and survival in neutrophils? Pathog Dis 74.
803 32. Cooper RA. 1984. Metabolism of methylglyoxal in microorganisms. Annu Rev
804 Microbiol 38:49-68.
805 33. Zeng L, Burne RA. 2016. Sucrose- and fructose-specific effects on the
806 transcriptome of *Streptococcus mutans*, as determined by RNA sequencing. Appl
807 Environ Microbiol 82:146-56.
808 34. Kajfasz JK, Ganguly T, Hardin EL, Abranches J, Lemos JA. 2017. Transcriptome
809 responses of *Streptococcus mutans* to peroxide stress: identification of novel
810 antioxidant pathways regulated by Spx. Sci Rep 7:16018.
811 35. Noctor G, Foyer CH. 1998. ASCORBATE AND GLUTATHIONE: Keeping Active
812 Oxygen Under Control. Annu Rev Plant Physiol Plant Mol Biol 49:249-279.
813 36. Zheng X, Zhang K, Zhou X, Liu C, Li M, Li Y, Wang R, Li Y, Li J, Shi W, Xu X.
814 2013. Involvement of *gshAB* in the interspecies competition within oral biofilm. J
815 Dent Res 92:819-24.
816 37. Ellepola K, Huang X, Riley RP, Bitoun JP, Wen ZT. 2021. *Streptococcus mutans*
817 lacking *sufCDSUB* Is viable, but displays major defects in growth, stress
818 tolerance responses and biofilm formation. Front Microbiol 12:671533.

819 38. Wu X, Hou J, Chen X, Chen X, Zhao W. 2016. Identification and functional
820 analysis of the L-ascorbate-specific enzyme II complex of the
821 phosphotransferase system in *Streptococcus mutans*. BMC Microbiol 16:51.
822 39. Makhlynets O, Boal AK, Rhodes DV, Kitten T, Rosenzweig AC, Stubbe J. 2014.
823 *Streptococcus sanguinis* class Ib ribonucleotide reductase: high activity with both
824 iron and manganese cofactors and structural insights. J Biol Chem 289:6259-72.
825 40. Rhodes DV, Crump KE, Makhlynets O, Snyder M, Ge X, Xu P, Stubbe J, Kitten T.
826 2014. Genetic characterization and role in virulence of the ribonucleotide
827 reductases of Streptococcus sanguinis. J Biol Chem 289:6273-87.
828 41. Stewart LJ, Ong CY, Zhang MM, Brouwer S, McIntyre L, Davies MR, Walker MJ,
829 McEwan AG, Waldron KJ, Djoko KY. 2020. Role of Glutathione in Buffering
830 Excess Intracellular Copper in *Streptococcus pyogenes*. mBio 11.
831 42. Griswold AR, Chen YY, Burne RA. 2004. Analysis of an agmatine deiminase
832 gene cluster in *Streptococcus mutans* UA159. J Bacteriol 186:1902-4.
833 43. Seaton K, Ahn S-J, Burne RA. 2014. Regulation of competence and gene
834 expression in *Streptococcus mutans* by the RcrR transcriptional regulator. Mol
835 Oral Microbiol 30:147-59.
836 44. Merritt J, Qi F, Shi W. 2005. A unique nine-gene *comY* operon in *Streptococcus*
837 *mutans*. Microbiology 151:157-66.
838 45. Merritt J, Qi F. 2012. The mutacins of *Streptococcus mutans*: regulation and
839 ecology. Mol Oral Microbiol 27:57-69.
840 46. Banu LD, Conrads G, Rehrauer H, Hussain H, Allan E, van der Ploeg JR. 2010.
841 The *Streptococcus mutans* serine/threonine kinase, PknB, regulates competence

842 development, bacteriocin production, and cell wall metabolism. Infect Immun
843 78:2209-20.
844 47. Leung V, Ajdic D, Koyanagi S, Levesque CM. 2015. The formation of
845 *Streptococcus mutans* persisters induced by the quorum-sensing peptide
846 pheromone is affected by the LexA regulator. J Bacteriol 197:1083-94.
847 48. Alves LA, Ganguly T, Harth-Chu EN, Kajfasz J, Lemos JA, Abranches J, Mattos-
848 Graner RO. 2020. PepO is a target of the two-component systems VicRK and
849 CovR required for systemic virulence of *Streptococcus mutans*. Virulence 11:521-
850 536.
851 49. Underhill SAM, Shields RC, Burne RA, Hagen SJ. 2019. Carbohydrate and PepO
852 control bimodality in competence development by *Streptococcus mutans*. Mol
853 Microbiol 112:1388-1402.
854 50. Serbanescu MA, Cordova M, Krastel K, Flick R, Beloglazova N, Latos A, Yakunin
855 AF, Senadheera DB, Cvitkovitch DG. 2015. Role of the *Streptococcus mutans*
856 CRISPR-cas systems in immunity and cell physiology. Journal of Bacteriology
857 197:749-761.
858 51. Lemme A, Sztajer H, Wagner-Döbler I. 2010. Characterization of *mleR*, a positive
859 regulator of malolactic fermentation and part of the acid tolerance response in
860 *Streptococcus mutans*. BMC Microbiol 10:58.
861 52. Sheng J, Marquis RE. 2007. Malolactic fermentation by *Streptococcus mutans*.
862 FEMS Microbiol Lett 272:196-201.

863 53. King S, Quick A, King K, Walker AR, Shields RC. 2022. Activation of TnSmu1, an
864 integrative and conjugative element, by an ImmR-like transcriptional regulator in
865 *Streptococcus mutans*. Microbiology (Reading) 168.
866 54. Ganguly T, Peterson AM, Burkholder M, Kajfasz JK, Abranches J, Lemos JA.
867 2022. ZccE is a novel P-type ATPase that protects *Streptococcus mutans* against
868 zinc intoxication. PLoS Pathog 18:e1010477.
869 55. Zeng L, Das S, Burne RA. 2010. Utilization of lactose and galactose by
870 *Streptococcus mutans*: transport, toxicity, and carbon catabolite repression. J
871 Bacteriol 192:2434-44.
872 56. Sato Y, Yamamoto Y, Kizaki H. 2000. Construction of region-specific partial
873 duplication mutants (merodiploid mutants) to identify the regulatory gene for the
874 glucan-binding protein C gene *in vivo in Streptococcus mutans*. FEMS Microbiol
875 Lett 186:187-91.
876 57. Tao L, Sutcliffe IC, Russell RR, Ferretti JJ. 1993. Transport of sugars, including
877 sucrose, by the *msm* transport system of *Streptococcus mutans*. J Dent Res
878 72:1386-90.
879 58. Costa Oliveira BE, Ricomini Filho AP, Burne RA, Zeng L. 2021. The Route of
880 Sucrose Utilization by Streptococcus mutans Affects Intracellular Polysaccharide
881 Metabolism. Front Microbiol 12:636684.
882 59. Sato Y, Okamoto-Shibayama K, Azuma T. 2013. The *malQ* gene is essential for
883 starch metabolism in *Streptococcus mutans*. J Oral Microbiol 5:21285.

884 60. Kremer BH, van der Kraan M, Crowley PJ, Hamilton IR, Brady LJ, Bleiweis AS.
885 2001. Characterization of the *sat* operon in *Streptococcus mutans*: evidence for a
886 role of Ffh in acid tolerance. J Bacteriol 183:2543-52.
887 61. Gong Y, Tian XL, Sutherland T, Sisson G, Mai J, Ling J, Li YH. 2009. Global
888 transcriptional analysis of acid-inducible genes in *Streptococcus mutans*: multiple
889 two-component systems involved in acid adaptation. Microbiology 155:3322-32.
890 62. Baker JL, Faustoferri RC, Quivey RG, Jr. 2017. Acid-adaptive mechanisms of
891 *Streptococcus mutans*-the more we know, the more we don't. Mol Oral Microbiol
892 32:107-117.
893 63. Baker JL, Abranches J, Faustoferri RC, Hubbard CJ, Lemos JA, Courtney MA,
894 Quivey R, Jr. 2015. Transcriptional profile of glucose-shocked and acid-adapted
895 strains of *Streptococcus mutans*. Mol Oral Microbiol doi:10.1111/omi.12110.
896 64. Burne RA, Wen ZT, Chen YY, Penders JE. 1999. Regulation of expression of the
897 fructan hydrolase gene of *Streptococcus mutans* GS-5 by induction and carbon
898 catabolite repression. J Bacteriol 181:2863-71.
899 65. Kajfasz JK, Rivera-Ramos I, Scott-Anne K, Gregoire S, Abranches J, Lemos JA.
900 2015. Transcription of Oxidative Stress Genes Is Directly Activated by SpxA1
901 and, to a Lesser Extent, by SpxA2 in *Streptococcus mutans*. J Bacteriol
902 197:2160-2170.
903 66. Kreth J, Merritt J, Shi W, Qi F. 2005. Competition and coexistence between
904 *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. J
905 Bacteriol 187:7193-7203.

906 67. Culp DJ, Hull W, Bremgartner MJ, Atherly TA, Christian KN, Killeen M, Dupuis
907 MR, Schultz AC, Chakraborty B, Lee K, Wang DS, Afzal V, Chen T, Burne RA.
908 2020. *In vivo* colonization with candidate oral probiotics attenuates colonization
909 and virulence of *Streptococcus mutans*. Appl Environ Microbiol 87.
910 68. Velsko IM, Chakraborty B, Nascimento MM, Burne RA, Richards VP. 2018.
911 Species designations belie phenotypic and genotypic heterogeneity in oral
912 streptococci. mSystems 3:e00158-18.
913 69. Papadimitriou K, Alegria A, Bron PA, de Angelis M, Gobbetti M, Kleerebezem M,
914 Lemos JA, Linares DM, Ross P, Stanton C, Turroni F, van Sinderen D, Varmanen
915 P, Ventura M, Zuniga M, Tsakalidou E, Kok J. 2016. Stress physiology of lactic
916 acid bacteria. Microbiol Mol Biol Rev 80:837-90.
917 70. Abranches J, Zeng L, Kajfasz JK, Palmer SR, Chakraborty B, Wen ZT, Richards
918 VP, Brady LJ, Lemos JA. 2018. Biology of oral streptococci. *In* Fischetti VA,
919 Novick RP, Ferretti J, Portnoy DA, Braunstein M, Rood JI (ed), Gram-positive
920 pathogens doi:doi:10.1128/microbiolspec.GPP3-0042-2018. Microbiol Spectrum.
921 71. Liu C, Niu Y, Zhou X, Zhang K, Cheng L, Li M, Li Y, Wang R, Yang Y, Xu X. 2013.
922 Hyperosmotic response of *Streptococcus mutans*: from microscopic physiology
923 to transcriptomic profile. BMC Microbiology 13:275.
924 72. Abranches J, Lemos JA, Burne RA. 2006. Osmotic stress responses of
925 *Streptococcus mutans* UA159. FEMS Microbiol Lett 255:240-6.
926 73. Kunii M, Arimoto T, Hasegawa T, Kuwata H, Igarashi T. 2014. Role of protease
927 maturation lipoprotein in osmoadaptation of *Streptococcus mutans*. FEMS
928 Microbiol Lett 356:45-52.

929 74. Lee C, Park C. 2017. Bacterial responses to glyoxal and methylglyoxal: reactive
930 electrophilic species. Int J Mol Sci 18.
931 75. Akbari MS, Joyce LR, Spencer BL, McIver KS, Doran KS. 2024. Identification of
932 Glyoxalase A in Group B *Streptococcus* and its contribution to methylglyoxal
933 tolerance and virulence. bioRxiv
934 doi:10.1101/2024.07.30.605887:2024.07.30.605887.
935 76. Darwin KH, Stanley SA. 2022. The aldehyde hypothesis: metabolic intermediates
936 as antimicrobial effectors. Open Biology 12:220010.
937 77. Kawasaki T, Akanuma H, Yamanouchi T. 2002. Increased fructose concentrations
938 in blood and urine in patients with diabetes. Diabetes Care 25:353-7.
939 78. Li HK, Zhi X, Vieira A, Whitwell HJ, Schricker A, Jauneikaite E, Li H, Yosef A,
940 Andrew I, Game L, Turner CE, Lamagni T, Coelho J, Sriskandan S. 2023.
941 Characterization of emergent toxigenic M1(UK) Streptococcus pyogenes and
942 associated sublineages. Microb Genom 9.
943 79. Zeng L, Burne RA. 2013. Comprehensive mutational analysis of sucrose-
944 metabolizing pathways in *Streptococcus mutans* reveals novel roles for the
945 sucrose phosphotransferase system permease. J Bacteriol 195:833-43.
946 80. Tanzer JM, Chassy BM, Krichevsky MI. 1971. Sucrose metabolism by
947 *Streptococcus mutans*, SL-I. Biochim Biophys Acta 261:379-87.
948 81. Terleckyj B, Willett NP, Shockman GD. 1975. Growth of several cariogenic strains
949 of oral streptococci in a chemically defined medium. Infect Immun 11:649-55.

950 82. Zeng L, Chen L, Burne RA. 2018. Preferred hexoses influence long-term memory
951 in and induction of lactose catabolism by *Streptococcus mutans*. Appl Environ
952 Microbiol 84:e00864-18.
953 83. Chen L, Walker AR, Burne RA, Zeng L. 2020. Amino sugars reshape interactions
954 between *Streptococcus mutans* and *Streptococcus gordonii*. Appl Environ
955 Microbiol 87:e01459-20.
956 84. Law CW, Chen Y, Shi W, Smyth GK. 2014. voom: Precision weights unlock linear
957 model analysis tools for RNA-seq read counts. Genome Biol 15:R29.
958 85. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. 2015. limma
959 powers differential expression analyses for RNA-sequencing and microarray
960 studies. Nucleic Acids Res 43:e47.
961 86. Ranjit DK, Moye ZD, Rocha FG, Ottenberg G, Nichols FC, Kim HM, Walker AR,
962 Gibson FC, 3rd, Davey ME. 2022. Characterization of a Bacterial Kinase That
963 Phosphorylates Dihydrosphingosine to Form dhS1P. Microbiol Spectr
964 10:e0000222.
965 87. Chen L, Chakraborty B, Zou J, Burne RA, Zeng L. 2019. Amino sugars modify
966 antagonistic interactions between commensal oral streptococci and
967 *Streptococcus mutans*. Appl Environ Microbiol 85:e00370-19.
968 88. Zeng L, Walker AR, Burne RA, Taylor ZA. 2022. Glucose phosphotransferase
969 system modulates pyruvate metabolism, bacterial fitness, and microbial ecology
970 in oral streptococci. J Bacteriol 205:e0035222.

971 89. Moye ZD, Zeng L, Burne RA. 2014. Modification of gene expression and
972 virulence traits in *Streptococcus mutans* in response to carbohydrate availability.
973 Appl Environ Microbiol 80:972-985.
974 90. Zeng L, Burne RA. 2008. Multiple sugar: phosphotransferase system permeases
975 participate in catabolite modification of gene expression in *Streptococcus*
976 *mutans*. Mol Microbiol 70:197-208.
977 91. Huang X, Palmer SR, Ahn SJ, Richards VP, Williams ML, Nascimento MM, Burne
978 RA. 2016. A highly arginolytic *Streptococcus* species that potently antagonizes
979 *Streptococcus mutans*. Appl Environ Microbiol 82:2187-201.
980
981

982 **Table 1.** Genes differentially expressed after treatments with methylglyoxal (MG),
983 fructose (Fru), and glucose (Glc) relative to untreated controls. Results of H_2O_2
984 treatment was obtained from a previous study (34). The cutoff for significance is
985 $|Log_2(foldchange)| > 1$ and FDR <0.05. Genes are organized based on functional
986 categories. See supplemental material for details.

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
Thiol homeostasis/oxidative stress						
140	*gshR*	glutathione reductase		3.53	4.09	
141	*SMU_141*	conserved hypothetical protein	0.63	3.78	4.08	
267	*gshAB*	glutamate--cysteine ligase (gamma ECS)	1.14	1.29	1.00	
382	*SMU_382c*	possible oxidoreductase	3.67			
383	*SMU_383c*	undetermined reductase or epimerase	3.73			
463	*trxB*	thioredoxin reductase (NADPH)	1.90	1.31		1.75
629	*sodA*	superoxide dismutase	3.00	2.89	1.02	2.22
667	*nrdB*	ribonucleotide reductase, small subunit	3.50	1.31	0.66	1.54
668	*nrdA*	ribonucleotide reductase, large subunit	3.07	1.09		1.37
669	*nrdH*	glutaredoxin	2.63	0.83	-0.63	1.48
676	*gapN*	NADP-dependent G3P dehydrogenase	1.21	1.20	0.93	1.40
677	*rmeA*	transcriptional regulator, MerR family	2.46	1.01		
678	*ycgG*	aldo/keto oxidoreductase	3.15	1.21	0.96	
679	*ycgG*	aldo/keto oxidoreductase	3.24	1.49	1.12	
680	*SMU_680*	carboxymuconolactone decarboxylase family protein	3.20	1.22	0.71	
681	*SMU_681*	conserved hypothetical protein	3.14	1.14		
728	*SMU_728*	oxidoreductase		1.03		
764	*ahpC*	alkyl hydroperoxide reductase subunit C	3.76	2.18		2.33
765	*ahpF*	alkyl hydroperoxide reductase subunit F	4.22	2.30		2.24
837	*ycgG*	aldo/keto oxidoreductase			1.39	
838	*gor*	glutathione reductase	3.44	1.51	1.17	2.14
924	*tpx*	thiol peroxidase	3.01	2.48	1.06	2.72
991	*SMU_991*	possible ribonucleotide reductase	1.62	1.26		1.93
1040	*ydfG*	oxidoreductase, short-chain dehydrogenase/reductase	1.34			
1117	*nox*	H_2O-forming NADH Oxidase	1.81	1.42	0.97	1.94
1296	*gst*	glutathione S-transferase	2.70	2.43	1.46	1.79
1473	*SMU_1473c*	oxidoreductase, short-chain dehydrogenase/reductase family	1.21	1.05		
1602	*frp*	NAD(P)H-flavin oxidoreductase	1.78	1.19	1.16	
1603	*lguL*	lactoylglutathione lyase	2.14	1.78	1.70	
1869	*trxA*	thioredoxin	1.63	2.28	1.34	2.25
2070	*SMU_2070*	conserved hypothetical protein	3.21			

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
2071	*nrdG*	ribonucleoside-triphosphate reductase activating protein	3.66			
2072	*SMU_2072c*	possible acetyltransferase, GNAT family	3.70			-1.22
2073	*SMU_2073c*	conserved hypothetical protein	3.59	-1.09	-1.61	
2074	*nrdD*	anaerobic ribonucleoside-triphosphate reductase	3.52	-1.40		
Metal homeostasis						
182	*sloA*	ABC transporter for iron/manganese	-1.87	-2.89	-1.51	-1.01
183	*sloB*	manganese ABC transporter permease element	-0.92	-1.20		-0.96
184	*sloC*	ABC transporter element, iron/manganese binding protein			-1.34	-1.14
185	*SMU_185*	hypothetical protein		-0.60	-1.51	-1.56
186	*sloR*	metal-dependent transcriptional regulator	-0.77			-1.01
247	*sufC*	ABC transporter, ATP-binding protein	2.24	0.98	0.72	1.89
248	*sufD*	ABC transporter permease	2.56	1.22	1.09	1.82
249	*sufS*	class-V aminotransferase, NifS protein homolog,	2.83	1.31	0.96	1.80
250	*sufU*	nitrogen fixation-like protein, NifU	2.91	1.01		1.90
251	*sufB*	ABC transporter permease	2.76	1.08	0.77	1.70
424	*copY*	negative transcriptional regulator, CopY	3.82	0.84	1.23	1.24
426	*copA*	copper-transporting ATPase	3.72	0.64	0.77	1.18
427	*copZ*	copper chaperone	3.47	0.82		1.09
540	*dpr*	peroxide resistance protein/iron binding protein	2.96	1.87		1.99
569	*feoA*	ferrous ion transport protein A	-2.44			-1.17
570	*feoB*	ferrous ion transport protein B	-1.65	-0.66	0.77	-1.00
1561	*trkB*	potassium uptake protein B	2.20	1.64		
1562	*trkA*	potassium uptake protein A	2.32	1.53		
1993	*adcB*	zinc ABC transporter, permease				1.08
1994	*adcC*	zinc ABC transport, ATP-binding protein				1.10
1995	*adcR*	zinc transport transcriptional repressor				1.04
2057	*zccE*	zinc/cadmium-efflux ATPase	-0.42	2.44	1.17	
DNA and protein repair						
80	*hrcA*	heat-inducible transcription repressor	-2.47			
81	*grpE*	co-chaperone protein GrpE	-2.12			
82	*dnaK*	chaperone protein, DnaK	-1.33			
561	*mutT*	mutator protein, Nudix hydrolase	2.46			
562	*clpE*	ATP-dependent protease	2.86	1.44		1.42
809	*uvrB*	excinuclease ABC subunit B			1.26	
956	*clpL*	ATP-dependent Clp protease	1.77	1.13		1.11
1649	*exoA*	exodeoxyribonuclease III	1.68	1.19	1.10	1.93
1650	*end3*	endonuclease III	1.82			1.56
1850	*pepP*	XAA-Pro aminopeptidase	1.78	1.16	0.89	1.45
1851	*uvrA*	excinuclease ABC subunit A	1.34	1.03		1.70
1865	*mutY*	A/G-specific adenine glycosylase	2.41	1.24	0.66	1.66

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
2029	*clpC*	ATP-dependent Clp protease	1.11			
2036	*pepO*	endopeptidase O	1.61	1.18	1.07	1.42
2085	*recA*	recombinase A		1.18		
Transcription regulators						
61	*comR*	transcriptional regulator	1.01			1.55
363	*glnR*	glutamine synthetase repressor		-2.29		
381	*plcR*	transcriptional regulator	1.45			
486	*liaS*	two-component sensor histidine kinase				-1.00
487	*liaR*	two-component response regulator				-1.01
576	*lytT*	response regulator	-1.55	-1.58		
577	*lytS*	sensor histidine kinase	-2.26	-1.51		
593	*perR*	ferric uptake regulator protein	1.06	1.08		1.03
660	*spaK*	histidine kinase		-1.05		
661	*spaR*	transcriptional regulator	1.72			
928	*relS*	sensor histidine kinase	1.00			
1037	*phoR*	histidine kinase	2.12			
1038	*ycbL*	response regulator	1.79			
1065	*nagR*	transcriptional regulator, GntR family	-1.42			
1097	*SMU_1097c*	transcriptional regulator	1.37		-1.42	
1226	*SMU_1226c*	histidine protein kinase	1.03			
1287	*pmrA*	transcriptional regulator	-1.03		-1.48	1.12
1398	*irvR*	phage associated repressor protein	-1.60			
1515	*vicX*	*gtfB/C* regulator	1.28			
1516	*vicK*	two-component sensor histidine kinase	1.17			
1814	*scnK*	histidine kinase, ScnK homolog	2.50	1.37		1.58
1815	*scnR*	response regulator, ScnR homolog	2.26	1.37	0.59	1.72
1854	*hdrR*	high density responsive regulator		1.35		
1915	*comC*	competence stimulating peptide	2.15			
1916	*comD*	histidine kinase	2.95	1.01		1.24
1917	*comE*	response regulator	2.78			1.31
1924	*gcrR*	response regulator for glucan-binding protein		-1.77	-1.09	
1963	*levQ*	sugar-binding periplasmic protein		-1.02	-1.23	-1.07
1964	*levR*	two-component response regulator			-1.07	-1.20
1965	*levS*	histidine kinase				-1.12
1966	*levT*	ABC transport ribose-binding protein, periplasmic				-1.19
2027	*lexA*	transcriptional regulator/repressor	3.03	3.08	1.09	1.29
2084	*spxA2*	oxidative stress regulator		1.30		
Osmoprotection						
1057	*satE*	acid tolerance protein		1.05		
1058	*satD*	acid tolerance protein		1.26		
1060	*ffh*	signal recognition particle		1.13		
1061	*ylxM*	DNA-binding protein		1.22		

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
1062	*busAB*	glycine-betaine binding ABC transporter permease	1.09	1.57		
1063	*opuAA*	amino acid ABC transporter, ATP-binding protein		1.68		
2116	*opuCa*	ABC transporter, ATP-binding protein		1.05		
2117	*opuCb*	ABC transporter permease		1.12		
2118	*opuCc*	ABC transporter, substrate-binding protein		1.13		
2119	*opuCd*	ABC transport permease		1.07		
F1F0 ATPase						
1527	*atpA*	ATPase epsilon subunit		-1.06		
1528	*atpB*	ATPase beta subunit		-1.13		
1529	*atpC*	ATPase gamma subunit		-1.31		
1530	*atpD*	ATPase alpha subunit		-1.20		
1531	*atpE*	ATPase delta subunit		-1.61	-1.25	
1532	*atpF*	ATPase b subunit		-1.35		
1533	*atpG*	ATPase a subunit		-1.40		
1534	*atpH*	ATPase c subunit		-1.26		
Fatty acid biosythesis						
1735	*accD*	acetyl-CoA carboxylase beta subunit	0.72	-1.15		
1736	*accC*	acetyl-CoA carboxylase biotin carboxylase subunit	0.61	-1.29		
1737	*fabZ*	(3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase	0.77	-1.38		
1738	*accB*	biotin carboxyl carrier protein of acetyl-CoA carboxylase	0.80	-1.46		
1739	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase	0.69	-1.51		
1740	*fabG*	3-oxoacyl-acyl-carrier-protein reductase		-1.78		
1741	*fabD*	[acyl-carrier-protein] S-malonyltransferase		-1.74		
1742	*fabK*	trans-2-enoyl-ACP reductase II		-1.56		
1743	*acp*	acyl carrier protein		-1.55	-1.56	
1744	*fabH*	3-oxoacyl-[acyl-carrier-protein] synthase III	-0.76	-1.39		
1745	*fabT*	transcriptional regulator, MarR family	-1.11	-1.06	-0.66	
Other stress/competence-related proteins						
20	*mreC*	cell shape-determining protein MreC	-2.19	-1.36	-1.45	
21	*mreD*	cell shape-determining protein MreD	-1.95	-2.02	-0.59	
150	*nlmA*	non-lantibiotic mutacin IV A				-1.30
151	*nlmB*	non-lantibiotic mutacin IV B				-1.13
152	*SMU_152*	immunity protein for NlmAB			-1.88	-1.64
153	*SMU_153*	hypothetical protein				-1.75
284	*nlmT*	transport and processing of non-lantibiotic mutacins		-1.24	-1.38	-1.05
285	*nlmE*	transport and processing of non-lantibiotic mutacins		-1.13	-1.16	-1.16

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
474	*luxS*	S-ribosylhomocysteine lyase (S-ribosylhomocysteinase)	-1.34			
503	*SMU_503c*	hypothetical protein	-1.86	-1.69	-1.58	-1.11
921	*rcrR*	transcriptional regulator	1.35		-1.31	1.21
922	*rcrP*	ABC transporter, ATPase component	1.75			1.07
923	*rcrQ*	AB transporter, ATPase component	1.87			1.08
1046	*relQ*	GTP pyrophosphokinase	-1.08	1.03		
574	*lrgB*	effector of murein hydrolase	-2.42	-2.46	-1.11	
575	*lrgA*	murein hydrolase regulator	-2.68	-3.00	-1.63	
1700	*cidB*	LrgB-like protein; possible murein hydrolase regulator	1.93	1.39		1.31
1701	*cidA*	conserved hypothetical protein	1.90	1.05		1.29
1702	*pgpB*	uncharacterized phosphatase	1.73	1.82		1.59
1703	*SMU_1703c*	conserved hypothetical protein	1.41	1.78		1.75
1980	*SMU_1980c*	conserved hypothetical protein	3.14	2.29	1.08	
1981	*comGF*	competence protein G	3.42	2.29		
1982	*SMU_1982c*	conserved hypothetical protein	2.76	1.96		
1983	*comYD*	late competence protein	2.20			
1984	*comYC*	late competence protein	2.50	1.26		
1985	*comYB*	late competence protein	2.19			
1987	*comYA*	late competence protein	2.28			
1988	*SMU_1988c*	probable DNA binding protein	2.80	1.43		2.19
1997	*comX*	competence-specific sigma factor	-1.57		-1.46	
2044	*relA*	GTP pyrophosphokinase				1.22
Carbohydrate metabolism						
99	*fbaA*	fructose-bisphosphate aldolase		-1.25		
100	*nigB*	nigerose PTS system, IIB component		-1.52		
105	*nigR*	nigerose operon transcriptional repressor		-1.15		
113	*pfk*	fructose-1-phosphate kinase		1.39	1.13	
114	*fruC*	PTS system, fructose-specific IIBC component			1.08	
119	*adh*	alcohol dehydrogenase class III	1.02	1.74	1.76	
127	*adhA*	acetoin dehydrogenase E1 component alpha subunit	2.76			1.72
128	*adhB*	acetoin dehydrogenase E1 component beta subunit	3.33			1.88
129	*adhC*	dihydrolipoamide S-acetyltransferase	3.50			1.91
130	*adhD*	dihydrolipoamide dehydrogenase	3.30	1.27		1.96
131	*lplA*	lipoate-protein ligase	3.43	1.39		1.88
135	*mleR*	transcriptional regulator	-1.05			
137	*mleS*	malolactic enzyme	0.62	4.00	4.36	
138	*mleP*	malate permease/auxin efflux carrier		3.78	4.08	
139	*oxdC*	oxalate decarboxylase		3.80	4.33	
148	*adhE*	alcohol-acetaldehyde dehydrogenase	-1.11		1.26	
270	*sgaT*	ribulose monophosphate PTS pathway enzyme IIC			1.36	

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
271	*ptxB*	L-ascorbate-specific PTS enzyme IIB component	1.88	1.00	0.96	1.19
272	*ptxA*	L-ascorbate-specific PTS enzyme IIA component	2.13	1.04	1.03	0.90
273	*rmpD*	hexulose-6-phosphate synthase	2.30	1.11	0.87	1.01
274	*rmpE*	hexulose-6-phosphate isomerase	2.48	1.17	1.01	1.16
275	*rmpF*	L-ribulose 5-phosphate 4-epimerase	2.59	1.66	1.06	1.11
276	*SMU_276c*	hypothetical protein	2.37		-2.62	
311	*srlA*	sorbitol PTS system enzyme IIC2		1.02		
312	*srlE*	sorbitol PTS system enzyme IIBC		1.36		
313	*srlB*	sorbitol PTS system enzyme IIA		1.68		
402	*pfl*	pyruvate formate-lyase		-1.49		
507	*sppR*	transcriptional regulator, DeoR family		4.56		
508	*sppA*	hexose-phosphate phosphohydrolase		4.56		
674	*ptsH*	PTS EI	-2.25			
675	*ptsI*	PTS HPr	-1.14			
754	*hprK*	HPr (serine) kinase/phosphatase		1.34	1.15	
870	*fruR*	transcriptional repressor of the fructose operon		1.68		
871	*fruK*	fructose-1-phosphate kinase		1.99		
872	*fruI*	fructose-specific PTS system enzyme IIBC component		1.87		
876	*msmR*	MSM operon regulatory protein	-1.93	-1.26		-1.28
877	*agaL*	alpha-galactosidase	-1.46	-1.73		-1.36
878	*msmE*	ABC transporter, sugar-binding protein		-1.79		-1.25
879	*msmF*	ABC transporter, sugar permease protein		-1.95		
880	*msmG*	ABC transporter permease		-1.78		
881	*gtfA*	sucrose phosphorylase		-1.64		
882	*msmK*	ABC transporter ATP-binding protein		-1.54		
883	*dexB*	glucan 1,6-alpha-glucosidase		-1.40		
886	*galK*	galactokinase		-1.46		
910	*gtfD*	glucosyltransferase-S		1.24	1.82	
1004	*gtfB*	glucosyltransferase-I			1.82	
1005	*gtfC*	glucosyltransferase-SI		-1.10		
1184	*mtlR*	transcriptional regulator	-1.05			
1185	*mtlA*	mannitol PTS EII	-1.73			
1396	*gbpC*	glucan-binding protein C	2.39	1.32	1.27	1.28
1451	*aldB*	alpha-acetolactate decarboxylase	2.86			1.66
1452	*alsS*	alpha-acetolactate synthase	2.33			1.54
1489	*lacX*	aldose 1-epimerase		1.13		
1492	*lacF*	PTS system, lactose-specific IIA component		1.85	1.92	
1493	*lacD*	tagatose-1,6-bisphosphate aldolase		1.39		
1494	*lacC*	tagatose-6-phosphate kinase		1.59	0.65	
1495	*lacB*	galactose-6-phosphate isomerase	-0.78	1.59	0.98	

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
1496	*lacA*	galactose-6-phosphate isomerase		1.71	1.18	
1498	*lacR*	lactose phosphotransferase system repressor		3.11		
1537	*glgD*	glycogen biosynthesis protein		-1.06		
1564	*glg*	glycogen phosphorylase		-1.24		
1565	*malM*	4-alpha-glucanotransferase		-1.02		
1566	*malR*	maltose operon transcriptional repressor	1.53			1.57
1568	*malE*	maltose / maltodextrin-binding protein	-1.55	-2.32		
1569	*malF*	maltodextrin ABC transport system permease		-2.00		
1570	*malG*	maltose / maltodextrin ABC transport system (permease)		-2.06		
1571	*msmK*	ABC-type transport system ATP-binding protein (maltose)		-1.89		
1596	*celD*	cellobiose PTS system IIC	-1.59	-2.92	-1.02	
1597	*celX*	conserved hypothetical protein	-2.21	-3.06	-2.52	
1598	*celC*	cellobiose PTS system IIA component	-2.43	-3.00	-1.11	
1599	*celR*	cellobiose transcriptional regulator	-2.80	-3.62	-1.71	
1600	*celB*	cellobiose PTS system IIB	-3.15	-2.59	-1.18	
1664	*acoB*	acetoin utilization protein, acetoin dehydrogenase	1.66	0.76		
1692	*pflA*	pyruvate-formate lyase activating enzyme	1.36			1.81
1841	*scrA*	sucrose PTS system EIIABC components	-1.99	-2.13		
1843	*scrB*	sucrose-6-phosphate hydrolase	-1.65			
1844	*scrR*	sucrose operon repressor	-1.07	-1.24		
1867	*adhB*	alcohol dehydrogenase	1.75	2.12	1.97	1.85
1877	*manL*	mannose PTS system component IIAB	-2.50	-2.79	-0.85	
1878	*manM*	mannose PTS system component IIC	-1.76	-2.34		
1879	*manN*	mannose PTS system component IID	-1.24	-2.25	-0.92	
1956	*levX*	conserved hypothetical protein		2.07		
1957	*levG*	fructose-specific Enzyme IID component		2.16		
1958	*levF*	fructose-specific Enzyme IIC component		2.15		
1960	*levE*	fructose-specific Enzyme IIB component		1.58		
1961	*levD*	fructose-specific Enzyme IIA component		1.77		
2028	*ftf*	fructosyltransferase		1.48	1.72	
2047	*malT*	maltose PTS enzyme II		-1.71		
Amino acid metabolism						
261	*aguR*	transcriptional regulator	1.42	1.65		
262	*argF*	ornithine carbamoyltransferase	1.48	2.21	1.16	
263	*aguD*	amino acid permease /putrescine antiporter	1.49	2.13	1.41	
264	*aguA*	agmatine deiminase		1.74	1.48	

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
265	*aguC*	carbamate kinase		1.18		
563	*argF*	ornithine carbamoyltransferase		1.00		
663	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase	3.25	2.41	1.17	
664	*argJ*	ornithine acetyltransferase / N-acetylglutamate synthase	3.25	2.42	1.94	
665	*argB*	acetylglutamate kinase	3.32	2.24	1.68	
666	*argD*	N-acetylornithine aminotransferase	2.94	1.54	1.78	1.09
1665	*livF*	branched chain amino acid ABC transporter, ATP-binding protein	1.57	0.73		1.05
1666	*livG*	branched chain amino acid ABC transporter, ATP-binding protein	1.54	0.71	0.14	1.16
1667	*livM*	branched chain amino acid ABC transporter, permease	1.35			1.29
1668	*livH*	branched chain amino acid ABC transporter, permease	1.36			1.24
1669	*livK*	branched-chain amino acid ABC transporter, substrate-binding protein	1.06			1.34
2097	*ahrC*	arginine transcriptional repressor	-1.22			
Translation						
169	*rplM*	50S ribosomal protein L13	-1.22	-1.18		
170	*rpsI*	30S ribosomal protein S9		-1.32		
697	*infC*	translation initiation factor IF-3	-1.06			
818	*rpsU*	30S ribosomal protein S21		-1.38		
957	*rplJ*	50S ribosomal protein L10	-1.80	-1.89		
960	*rpl*	50S ribosomal protein L7/L12	-1.33	-1.77		
1127	*rpsT*	30S ribosomal protein S20	-1.03			
1200	*rpsA*	30S ribosomal protein S1	-1.21			
1288	*rplS*	50S ribosomal protein L19	-1.06			
1627	*rplK*	50S ribosomal protein L11	-1.11			
1860	*rpsF*	30S ribosomal protein S6	-1.16			
2000	*rplQ*	50S ribosomal protein L17	-1.66			
2002	*rpsK*	30S ribosomal protein S11	-1.27			
2003	*rpsM*	30S ribosomal protein S13, N-terminal fragment	-1.06			
2003a	*rl36*	50S ribosomal protein L36	-1.05		-1.22	
2024	*rplD*	50S ribosomal protein L4, N-terminal fragment		-1.01		
2025	*rplC*	50S ribosomal protein L3		-1.06		
2104a	*rl32*	50S ribosomal protein L32	-1.03			
2135	*rpsD*	30S ribosomal protein S4		-1.08	-1.03	
TnSmu1						
196	*SMU_196c*	immunogenic secreted protein (transfer protein)		1.35	1.60	
197	*SMU_197c*	hypothetical protein		1.09	1.35	
199	*SMU_199c*	hypothetical protein		1.20	1.14	
200	*SMU_200c*	hypothetical protein		1.98		
201	*SMU_201c*	conserved hypothetical protein		1.12		

SMU Number	Gene Name	Gene Function	MG	Fru	Glc	H_2O_2
202	*SMU_202c*	conserved hypothetical protein		1.80	1.55	
204	*SMU_204c*	hypothetical protein		1.80	1.57	
205	*SMU_205c*	conserved hypothetical protein		1.91	1.28	
206	*SMU_206c*	hypothetical protein		1.48	1.27	
207	*SMU_207c*	transcriptional regulator			1.53	
208	*SMU_208c*	conserved hypothetical protein, FtsK/SpoIIIE family		1.01	1.46	
209	*SMU_209c*	hypothetical protein		1.31	1.56	
210	*SMU_210c*	hypothetical protein		1.15	1.33	
211	*SMU_211c*	hypothetical protein		1.31	2.27	
212	*SMU_212c*	hypothetical protein		1.20	1.50	
213	*SMU_213c*	hypothetical protein		1.52	1.90	
214	*SMU_214c*	hypothetical protein		1.27		
215	*SMU_215c*	hypothetical protein		1.72	1.09	
216	*SMU_216c*	hypothetical protein		1.37		
217	*SMU_217c*	conserved hypothetical protein		1.34		
218	*SMU_218*	transcriptional regulator			-1.27	
CRISPR-Cas						
1402	*csn2*	conserved hypothetical protein	-1.28	-2.56	-1.95	
1403	*cas2*	conserved hypothetical protein	-1.49	-2.32	-0.85	
1404	*cas1*	conserved hypothetical protein	-1.30	-2.24	-1.22	
1405	*cas9*	conserved hypothetical protein	-1.50	-2.31	-1.74	
1406	*SMU_1406c*	possible oxidoreductase	-1.52	-1.15		
1753	*cas2*	conserved hypothetical protein		1.59		1.14
1754	*cas1*	conserved hypothetical protein		1.91	1.09	1.29
1755	*cas1*	conserved hypothetical protein		1.70		1.21
1757	*cas1*	conserved hypothetical protein		1.77		1.04
1758	*cas4*	conserved hypothetical protein		1.78		1.36
1760	*csd2*	conserved hypothetical protein	1.03	2.02		1.51
1761	*cas8c*	conserved hypothetical protein	1.48	1.79		1.63
1762	*csd1*	conserved hypothetical protein	1.43	1.88		1.87
1763	*cas5*	conserved hypothetical protein	1.47	1.94		1.88
1764	*cas3*	conserved hypothetical protein	1.22	1.90		2.09

987
988

989 **Table 2**. pH and final OD_{600} of 20-h TV cultures.
990

Strains	Sugars	Resting pH			Final OD_{600}		
		20 mM	100 mM	200 mM	20 mM	100 mM	200 mM
SMU_UA159	Glucose	4.92 (0.06)	4.93 (0.03)	4.93 (0.02)	0.83 (0.01)	0.72 (0.01)	0.57 (0.01)
	Fructose	4.76 (0.04)**	4.82 (0.06)*	4.81 (0.01)***	0.82 (0.01)	0.73 (0.01)	0.64 (0.02)**
SSA_SK36	Glucose	6.51 (0.06)	6.15 (0.02)	5.70 (0.07)	0.34 (0.05)	0.41 (0.02)	0.45 (0.01)
	Fructose	6.55 (0.02)	ND	ND	0.27 (0.01)*	0.03 (0.01)****	0.00 (0.002)****
SSA_BCC23	Glucose	6.09 (0.12)	6.01 (0.07)	5.93 (0.07)	0.43 (0.04)	0.37 (0.02)	0.31 (0.01)
	Fructose	5.76 (0.15)*	ND	ND	0.49 (0.02)	0.20 (0.04)**	0.02 (0.005)****
SDE_BCC10	Glucose	5.48 (0.27)	5.08 (0.06)	5.49 (0.19)	0.63 (0.14)	0.69 (0.004)	0.43 (0.04)
	Fructose	ND	ND	ND	0.28 (0.01)**	0.14 (0.02)****	0 (0)****
SOR_BCC11	Glucose	5.72 (0.06)	5.65 (0.05)	5.72 (0.07)	0.56 (0.01)	0.52 (0.04)	0.40 (0.01)
	Fructose	5.09 (0.02)****	5.56 (0.05)	ND	0.66 (0.01)****	0.55 (0.01)	0.01 (0.01)****
SPA_BCC15	Glucose	6.53 (0.07)	6.45 (0.08)	6.38 (0.12)	0.12 (0.03)	0.11 (0.05)	0.09 (0.04)
	Fructose	ND	ND	ND	0.02 (0.02)**	0 (0)**	0 (0)**
SMI_BCC2	Glucose	4.75 (0.03)	4.73 (0.03)	4.77 (0.03)	0.74 (0.01)	0.65 (0.01)	0.56 (0.01)
	Fructose	4.87 (0.04)**	4.87 (0.05)**	4.87 (0.05)*	0.61 (0.01)****	0.54 (0.01)****	0.47 (0.01)****
SGO_BCC9	Glucose	4.95 (0.06)	4.90 (0.05)	4.91 (0.03)	0.81 (0.02)	0.66 (0.02)	0.54 (0.01)
	Fructose	4.87 (0.07)	5.03 (0.05)**	5.97 (0.02)***	0.70 (0.01)****	0.68 (0.01)	0.46 (0.05)*
A12_BCC21	Glucose	5.62 (0.08)	5.37 (0.07)	5.48 (0.11)	0.65 (0.01)	0.56 (0.005)	0.46 (0.02)
	Fructose	5.16 (0.04)***	5.36 (0.12)	ND	0.64 (0.01)	0.54 (0.02)	0.28 (0.10)*
SCR_BCC41	Glucose	6.20 (0.17)	5.93 (0.17)	5.85 (0.05)	0.50 (0.07)	0.47 (0.07)	0.42 (0.02)
	Fructose	5.69 (0.07)**	5.95 (0.08)	ND	0.51 (0.02)	0.41 (0.03)	0.10 (0.003)****
SIN_BCA19	Glucose	5.28 (0.08)	5.12 (0.04)	5.18 (0.02)	0.66 (0.01)	0.58 (0.01)	0.48 (0.01)
	Fructose	5.11 (0.09)*	5.01 (0.01)**	5.14 (0.02)*	0.61 (0.01)**	0.55 (0.005)**	0.48 (0.003)

991

992 Results show average (standard deviation) of four independent cultures. Statistical significance
993 was determined relative to glucose cultures of the same concentration using Student's *t*-test. *,
994 *P* <0.05; **, *P* <0.01; ***, *P* <0.001; ****, *P* <0.0001. ND, not determined due to lack of
995 substantial growth (in red). SMU, *S. mutans*; SSA, *S. sanguinis*; SDE, *S. dentisani*; SOR, *S.*
996 *oralis*; SPA, *S. parasanguinis*; SMI, *S. mitis*; SGO, *S. gordonii;* A12*, S. sp. A12;* SCR*, S.*
997 *cristatus;* SIN*, S. intermedius* (68, 91).

998 **Table 3**. Bacterial strains used in this study (excluding clinical isolates used in Table 2).
999

Strains	Relevant characteristics[a]	Source or reference
S. mutans UA159	*S. mutans* wild type, *perR*$^+$	ATCC 700610
MMZ1654	UA159 *fruI::Km*	From UA159
MMZ2120	UA159 *fruI::Em*	From UA159
Δ*zccR*	UA159 *zccR::Sp*	(54)
MMZ2156	UA159 *zccR::Sp fruI::Km*	From Δ*zccR*
Δ*spxA1*	UA159 *spxA1::Sp*	(34)
MMZ2161	UA159 *spxA1::Sp fruI::Em*	From Δ*spxA1*
MMZ2162	UA159 *spxA1::Sp levD::Em*	From Δ*spxA1*
Δ*spxA2*	UA159 *spxA2::Em*	(34)
MMZ2158	UA159 *sodA::gfp* fusion (*Km*)	From UA159
MMZ2160	UA159 *spxA1::Sp sodA::gfp* fusion	From Δ*spxA1*
MMZ2159	UA159 *fruI::Em sodA::gfp* fusion	From MMZ2120
MMZ2173	UA159 *levD::Em sodA::gfp* fusion	From MMZ2158
MMZ799	UA159 *fruR::cat* fusion (*Km*)	(20)
UA159-Km	UA159 *gtfA::Km*	(87)
S. sanguinis SK36	*S. sanguinis* wild type	Kitten laboratory
MMZ1945	SK36 *gtfP::Em*	(88)

1000 [a] *Km, Em,* and *Sp*: resistance against kanamycin, erythromycin, and spectinomycin,
1001 respectively.
1002

Table S6. Expression of *fruRBA* in *S. sanguinis* SK36 (by RT-qPCR) and activities of the *fruR* promoter in UA159 (by CAT assay).

	SK36 relative mRNA levels			UA159 P*fruR::cat* sp. act.
	fruR	*fruB*	*fruA*	
control	1.00 ± 0.07	1.00 ± 0.10	1.00 ± 0.06	186.18 ± 13.87
20 μM fructose	9.70 ± 1.16	9.54 ± 3.15	7.98 ± 4.93	230.71 ± 7.22
50 mM fructose	10.67 ± 1.74	10.29 ± 1.92	8.83 ± 3.22	271.29 ± 3.59
25% human sera	1.27 ± 0.19	1.71 ± 0.54	2.04 ± 0.31	ND

1003 Bacteria were cultured in FMC-glucose till exponential phase (OD_{600} = 0.4) and then treated for 30
1004 min with fructose or 25% pooled human sera. Gene expression was measured by RT-qPCR or CAT
1005 assays.

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1007 **Table S7** Primers used in this study.

Primers	Sequence	Purpose
fruI-1	GGC GGT TTT ACT GGT AGG TT	Δ*fruI*
fruI-2GA	GCC ATT TAT TAT TTC CTT CCT CTT TTA GCC TGC AAG TCA AGC AAC A	Δ*fruI*
fruI-3GA	ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCT GTT GGA GCA GTG ATT GCT	Δ*fruI*
fruI-4	AGC TGC TTG GAG ATA ATC TTT ACC T	Δ*fruI*
SodpromF	CCGGAATTCGATGTAGCTTTGC	*sodA::gfp*
SodpromR	CGCGGATCCTTCCTCTTTTC	*sodA::gfp*
ahpF-S	GAA ACC ATG GGT GGT CAA GT	RT-qPCR
ahpF-AS	GAG CCA TTA ATT GGG GTC CT	RT-qPCR
copY-S	ACC AGT CAG CGT CAA GGA AG	RT-qPCR
copY-AS	ACA AAT GCG CGA GAA AAC TT	RT-qPCR
SMU.667-S	TGA CTT GGA TGA CTG GCG TA	RT-qPCR
SMU.667-AS	TGG GAT TGC ATG GTG TCT AA	RT-qPCR
feoA-S	ACG GTT GAA ACA TTG CCT TT	RT-qPCR
feoA-AS	CGG CGC TTA GCT TCA TTA AC	RT-qPCR
SMUsloC-S	AAG GAA AAG TCG GGT GTC TG	RT-qPCR
SMU_sloC-AS	TTT AGC GGC AAT TGG GAT AC	RT-qPCR
gbpC-S	AGC TCA AAA GGC AGC TTA CG	RT-qPCR
gbpC-AS	TCCTTGGGCTTTTTCAACAC	RT-qPCR
scnK-S	CAAACGGCTGATTTCACAAG	RT-qPCR
scnK-AS	AAAGTGCGTTCCAATCTGCT	RT-qPCR
SMU.924-S	TCAGTTGATTTGCCATTTGC	RT-qPCR
SMU.924-AS	CCATAAGCCTTTCCAAACGA	RT-qPCR
SMU_phoR-S	AAACACGGCAAAAATCAAGC	RT-qPCR
SMU_phoR-AS	TTGTCAGCTGATCCAAATGC	RT-qPCR
SMU_vicK-S	TTTGGCACAGGAGAAAAACC	RT-qPCR
SMU_vicK-AS	TAATCTTTCCGCTGCGATCT	RT-qPCR
SMU.679-S	TTG GTG CTT ATC GTG CTC TG	RT-qPCR
SMU.679-AS	TGG AAT AAC CGA CAC CGT TT	RT-qPCR
SMU.1602-S	GTC GCG GTT TAA AGG ACA AA	RT-qPCR
SMU.1602-AS	TAG CGC CAA ATC GTT TTC TT	RT-qPCR
gshAB-S	GAGGCCCTCCGCTATTATTC	RT-qPCR
gshAB-AS	TTGTGACTGGCTGCTGTTTC	RT-qPCR
SMU.1603-S	GAA GAC GAC CCC GAC TAT GA	RT-qPCR
SMU.1603-AS	TCG CTT CAA GGT CAT CAA CA	RT-qPCR

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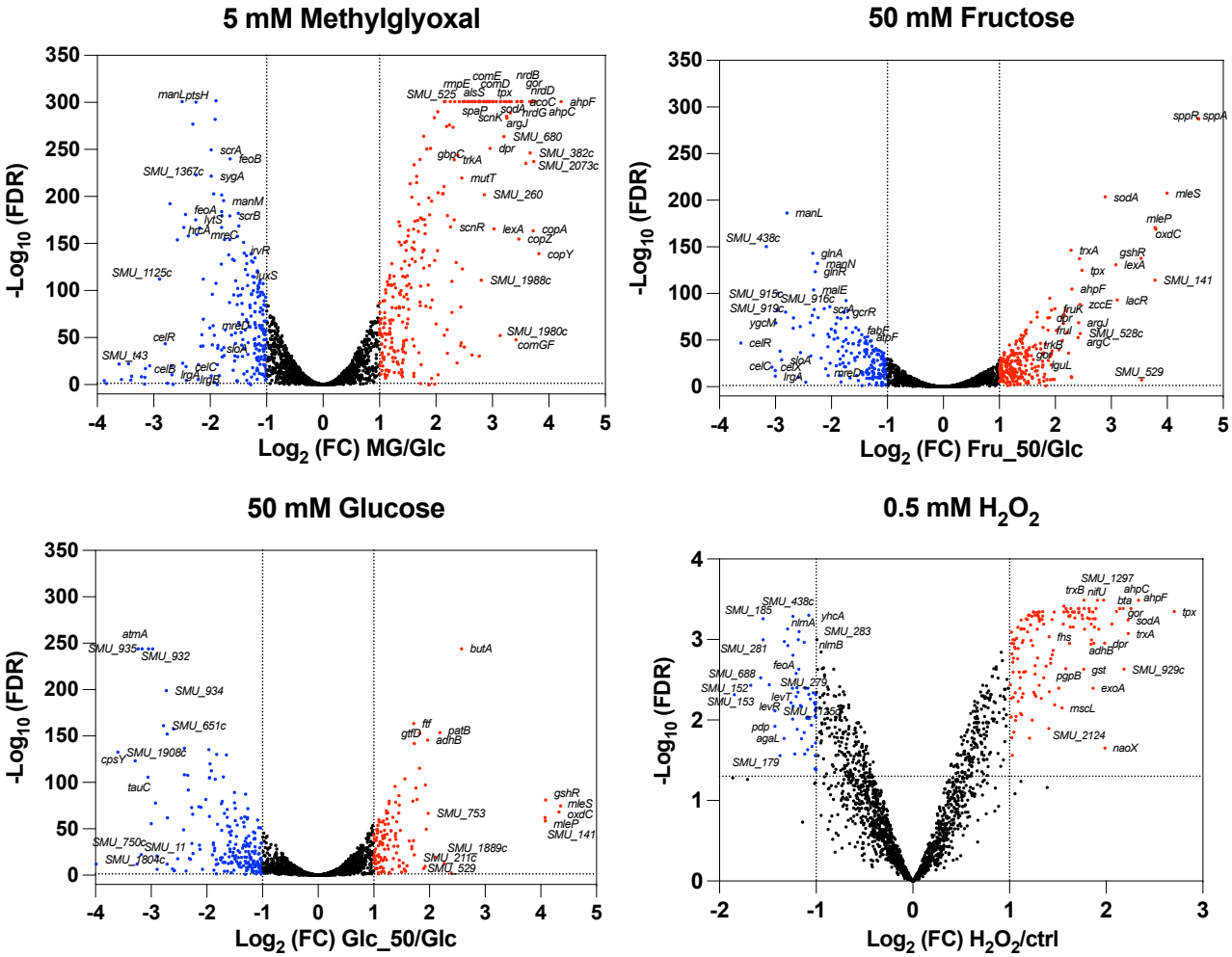

Fig. 1 Volcano plots illustrating transcriptomes of four treatments. *S. mutans* UA159 was grown to the exponential phase in a synthetic FMC medium containing 20 mM glucose. The samples were treated for 30 min with 50 mM fructose, 50 mM glucose, or 5 mM MG before harvesting for mRNA sequencing. Differential expression analyses were performed against untreated cells using *edgR*, with cutoffs for foldchange >2 and FDR <0.05. Upregulated genes are shown in red, and downregulated ones in blue. Results of treatment by H_2O_2 (0.5 mM, 5 min) were from an independent study (34).

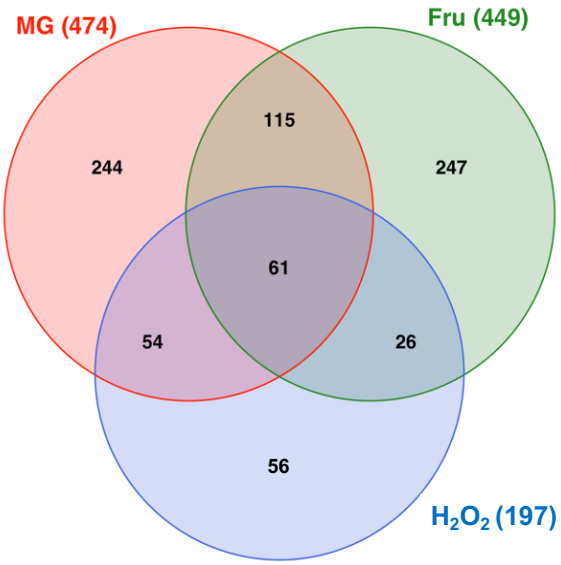

Fig. 2 Transcriptomic overlaps among treatments of MG, fructose and H_2O_2.

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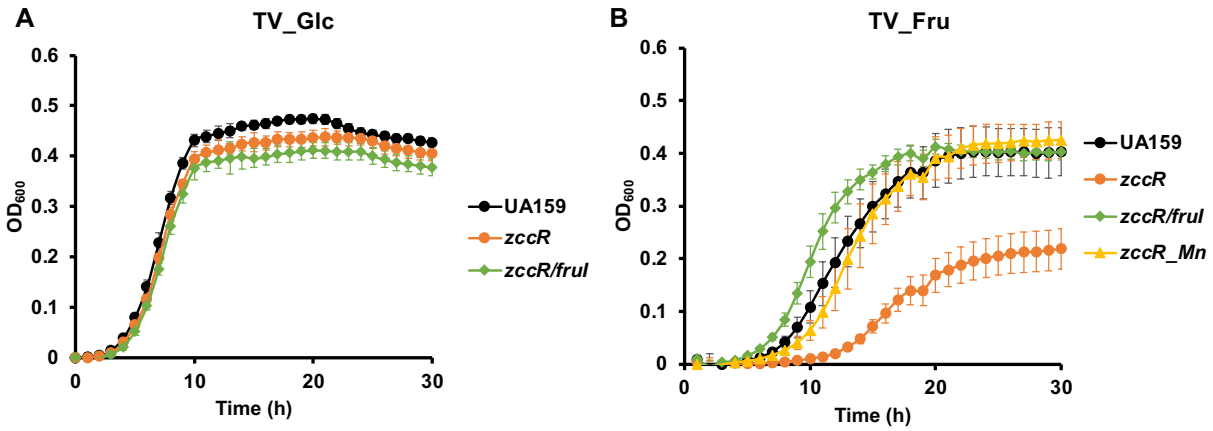

Fig. 3 Fructose impacts metal homeostasis in Δ*zccR* deficient in zinc expulsion. *S. mutans* strains UA159 and its mutant derivatives were diluted from exponential phase cultures into TV supplemented with 20 mM glucose (A) or fructose (B). Mn was added at 25 μM (MnSO4). Growth was monitored using a Synergy 2 plate reader maintained at 37°C. Results are average of three biological replicates, each tested in technical duplicates. Error bars denote standard deviations.

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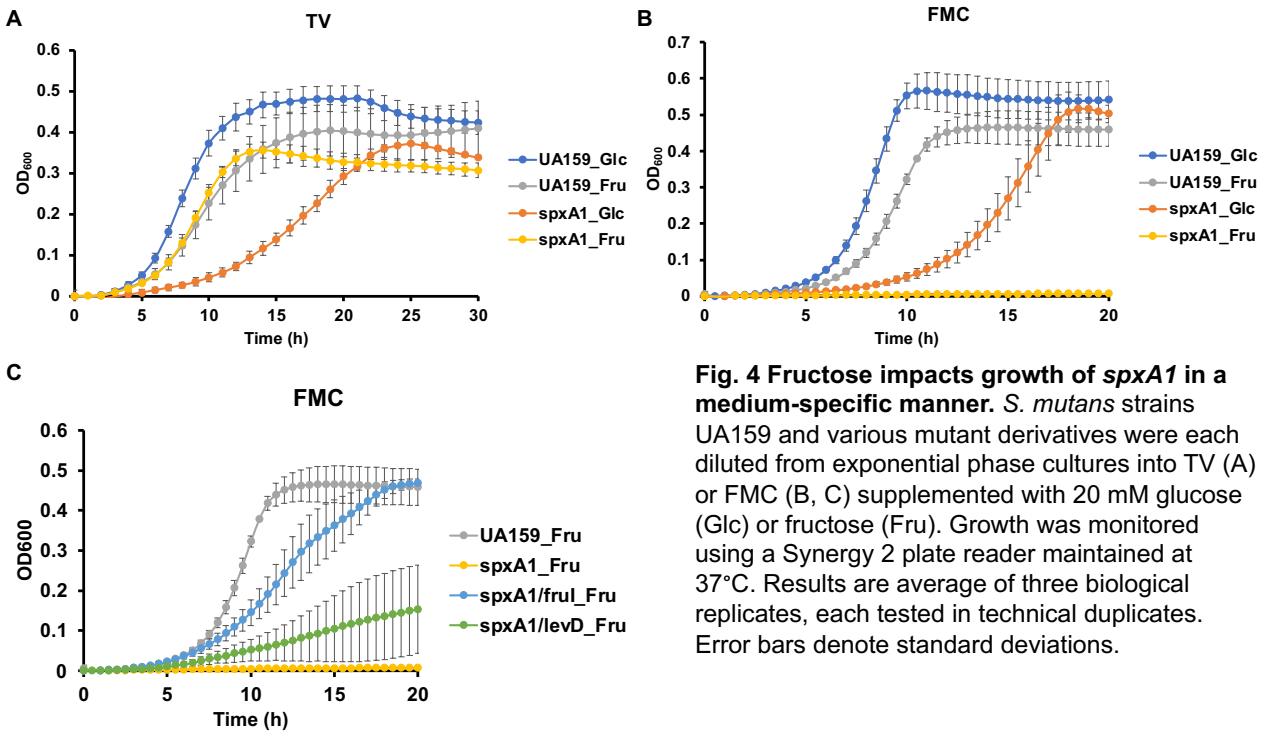

Fig. 4 Fructose impacts growth of *spxA1* in a medium-specific manner. *S. mutans* strains UA159 and various mutant derivatives were each diluted from exponential phase cultures into TV (A) or FMC (B, C) supplemented with 20 mM glucose (Glc) or fructose (Fru). Growth was monitored using a Synergy 2 plate reader maintained at 37°C. Results are average of three biological replicates, each tested in technical duplicates. Error bars denote standard deviations.

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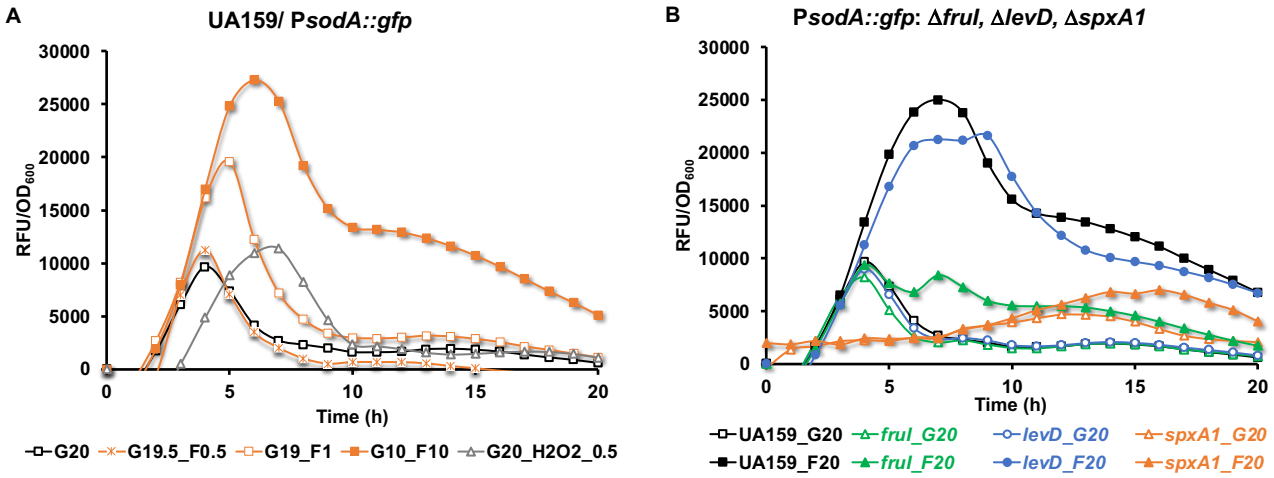

Fig. 5 Induction of the *sodA* promoter by fructose. (A) Cultures of UA159 harboring a P*sodA::gfp* fusion were diluted into FMC containing specified concentrations (in mM) of glucose (G), fructose (F), or H2O2. The cultures were monitored in a Synergy 2 plate reader for optical densities (OD600) and green fluorescence for 20 hours . The relative fluorescence units (RFU) of each culture were recorded as a measure of *sodA* promoter activity, after normalization against OD600. (B) The same experiment was performed on various mutants derived from UA159/P*sodA::gfp,* using FMC containing 20 mM glucose (G20) or fructose (F20). Results are the average from three biological replicates, conducted in technical duplicates.

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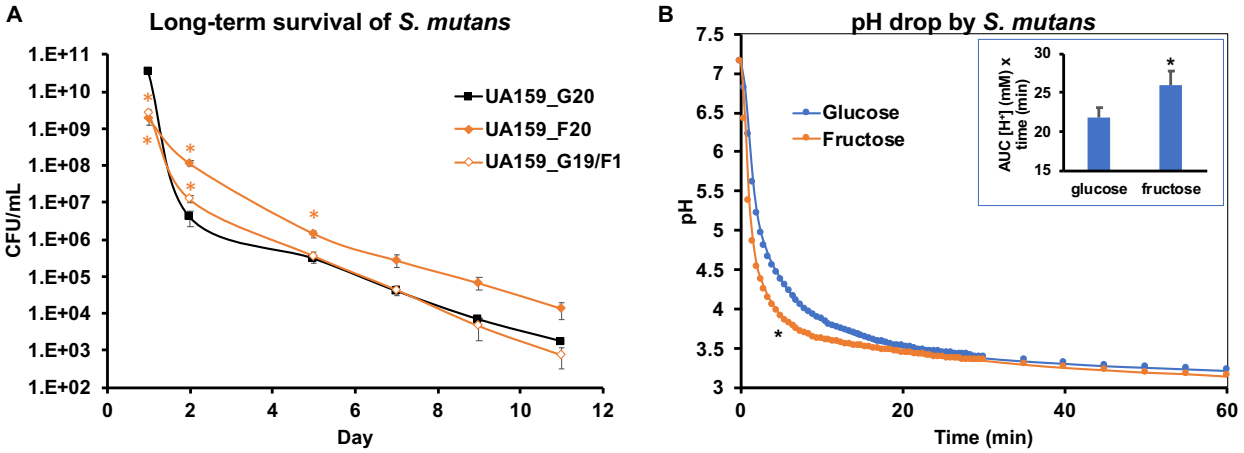

Fig. 6 Fructose promotes long-term survival (A) and acidogenicity (B) of *S. mutans*. (A) Cultures of *S. mutans* UA159 (n =4) were diluted into FMC containing 20 mM of glucose (G20) or fructose (F20), or a combination of 19 mM glucose and 1 mM fructose (G19/F1), followed by incubation without medium refreshment for 11 days. At specified time points, an aliquot of culture was removed for CFU enumeration. (B) pH drop assay. UA159 was cultured in BHI to exponential phase and resuspended in a solution of 50 mM KCl and 1 mM $MgCl_2$, with OD600 adjusted to 5.0. The pH was adjusted to 7.2 slowly with 0.1 N KOH to consume intracellular carbohydrate storage. Immediately after addition of 50 mM glucose or fructose, the pH of the suspension was monitored continuously on a stirring plate for the next 60 min. Each experiment was repeated three times, with representative results shown. After conversion into proton concentrations, area under the curve (AUC, see inset) of each sample was calculated and used to assess statistical significance using Student's *t*-test (, P <0.05).

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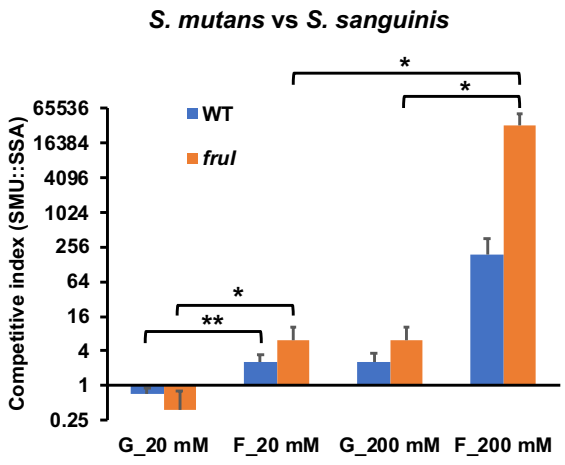

Fig. 7 Fructose enhances competitiveness of *S. mutans*. Cultures of *S. mutans* wild type UA159 and its isogenic *frul* deletion mutant were each mixed at 1:1 ratio with a differentially marked *S. sanguinis* strain, then diluted at 1:100 into FMC containing glucose or fructose, each used at 20 mM or 200 mM. After 24 h of incubation, the cultures were sonicated, decimally diluted and plated on selective agar plates for CFU enumeration. CFUs from both the start and end of the incubation were used to calculate competitive index of each species.

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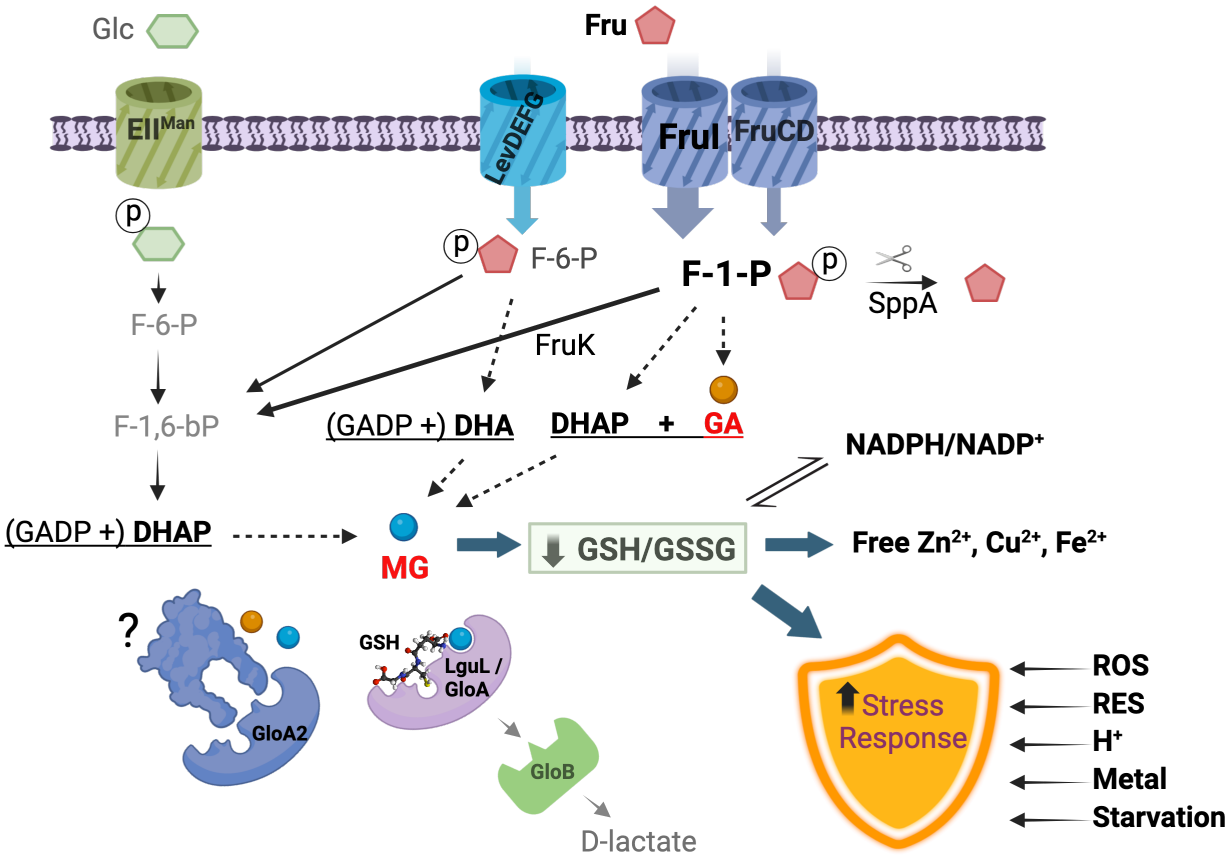

Fig. 8 Fructose metabolism triggers stress response in streptococci. Fructose is internalized by oral streptococci primarily through a F-1-P-generating PTS transporter, FruI, but also through two additional PTS permeases, including EII^{Lev} (LevDEFG) that generates F-6-P. A hexose-phosphate phosphohydrolase SppA helps to maintain the homeostasis of intracellular F-1-P. Compared to F-6-P and the glucose pathway, F-1-P is likely catabolized more rapidly via glycolysis, during which RES such as MG is accumulated and impacts cytoplasmic redox balance represented by the ratios of GSH/GSSG and $NADPH/NADP^+$. Also affected are the pools of intracellular metal ions. Sensing these and other not-yet-characterized physiological changes and specific metabolites, the bacterium reprograms a suite of stress responsive genes. The outcomes of exposure to fructose include not only elevated activities in processing RES, through the functions of LguL/GloB and related protein GloA2, but importantly enhanced tolerance to multiple environmental stressors including ROS, RES, acidic pH, toxic metals, and nutrient depletion.

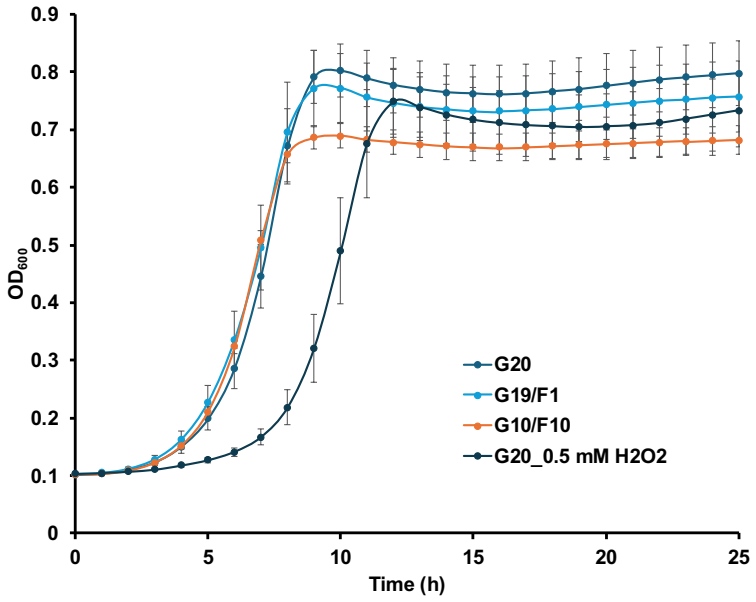

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Fig. S1 Growth of UA159/P*sodA::gfp*. BHI cultures (n = 3) of the bacterium were diluted into FMC containing 20 mM glucose (G20), 19 mM glucose plus 1 mM fructose (G19/F1), 10 mM glucose plus 10 mM fructose (G10/F10), or 20 mM glucose plus 0.5 mM H_2O_2. Optical density (OD_{600}) of these cultures was monitored for 25 h.

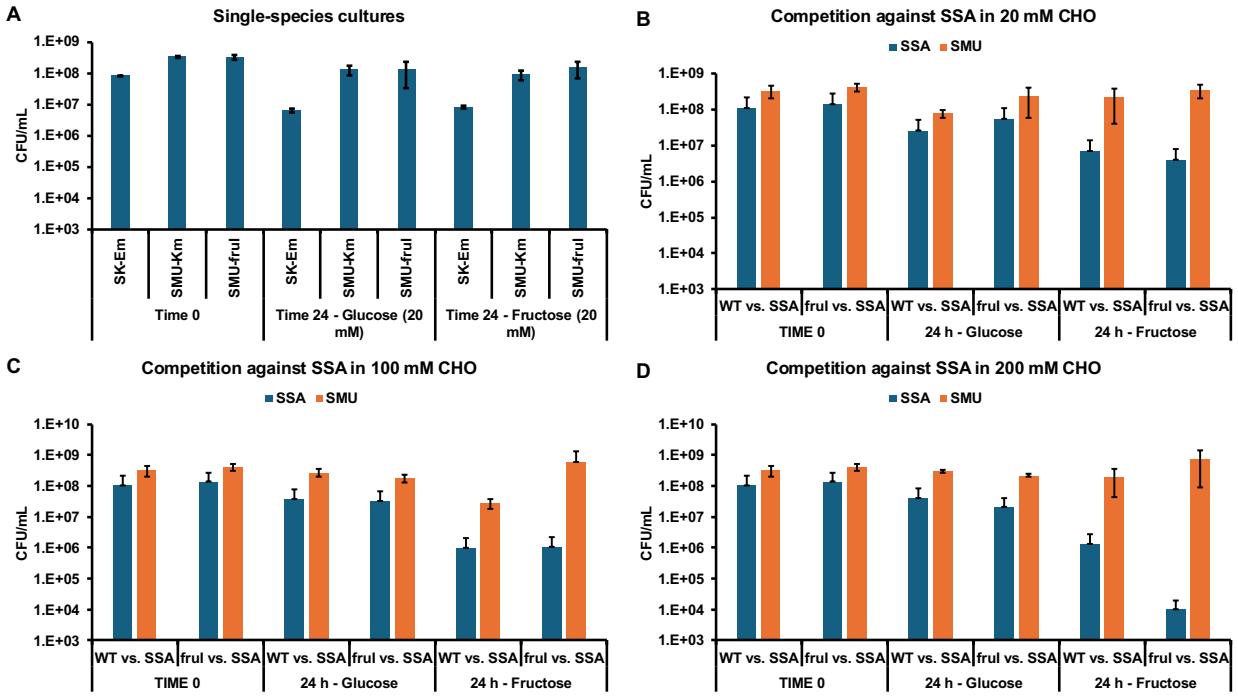

Fig. S2 CFU recovered during competitions between *S. mutans* (SMU) and *S. sanguinis* (SSA). Exponential-phase cultures in BHI (n = 4) from both species were mixed at 1:1 (v:v) ratio, or used singly, and diluted into a FMC medium supplemented with specified amounts of carbohydrates, followed by 24 h of incubation in a 5%-CO_2 atmosphere. CFU enumeration was carried out to assess bacterial viability. (A) controls run with only single species in 20 mM carbohydrates. (B, C, D) mixed-species cultures enumerated at time 0 and 24 h of competition. See Fig. 7 for the final competitive indices.