
Article:
Damé-Teixeira, N, Deng, D and Do, T orcid.org/0000-0002-5668-2181 (2019) Streptococcus mutans transcriptome in the presence of sodium fluoride and sucrose. Archives of Oral Biology, 102. pp. 186-192. ISSN 0003-9969

https://doi.org/10.1016/j.archoralbio.2019.04.020

TITLE PAGE

Streptococcus mutans transcriptome in the presence of sodium fluoride and sucrose

Naile DAMÉ-TEIXEIRA[1], Dongmei DENG[2], Thuy DO[3]*

[1]Department of Dentistry, University of Brasilia, Brasilia, Distrito Federal, Brazil

[2] Department of Preventive Dentistry, Academic Center for Dentistry Amsterdam, University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[3]Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, West Yorkshire, United Kingdom

Running title

Streptococcus mutans, sucrose and fluoride

Declaration of interest: The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

***Corresponding author**

Thuy Do

Division of Oral Biology, School of Dentistry, Faculty of Medicine & Health, University of Leeds, Level 7 Wellcome Trust Brenner Building, St. James' University Hospital campus, Leeds, LS9 7TF, United Kingdom.

Email: n.t.do@leeds.ac.uk

ABSTRACT

OBJECTIVE: Considering the diverse mechanisms by which fluoride could affect oral bacteria, this study evaluated the effect of sodium fluoride on Streptococcus mutans transcriptome in the presence of sucrose.

METHODS: S. mutans UA159 was cultured in 3 different types of media: medium control[TY], sucrose control[TY_S], and sodium fluoride sucrose test[TY_S_NaF]. Triplicates of each group were sampled at exponential phase 3 hours after inoculation, total RNA was isolated, mRNA enriched and cDNA paired-end sequenced (Illumina Hi-Seq2500).

RESULTS: Genes related to S. mutans adhesion(gtfB and gtfC), acidogenicity and sugar transport were up-regulated in the presence of sucrose(TY_S) and sucrose/fluoride(TY_S_NaF), whereas gene dltA, D-alanine-activating enzyme, which is related to regulation of non-PTS sugar internalization was down-regulated. Up-regulation of the scrA gene and the PTS fructose-and mannose system, as well as functions such as those involved in stress and defence responses and peptidases; and down-regulation of lacACDG and pyruvate formate-lyase were observed in the TY_S_NaF group, as compared to TY_S group.

CONCLUSIONS: The presence of NaF has decreased the overall gene expression level in S. mutans. However, its major effect seems to be the inducing of expression of genes involved in some PEP:PTS systems and other metabolic transporters which imply specific cellular internalisation of sugars.

Keywords

Streptococcus mutans, gene expression, Sodium Fluoride, Sucrose

INTRODUCTION

Within the context of the ecological plaque hypothesis, Streptococcus mutans may be considered an important contributor in the pathogenesis of dental caries despite its low abundance in the oral microbiota of some individuals. It presents numerous virulence factors related to cariogenicity, such as adhesion, biofilm formation, aciduricity and acid production (Klein et al., 2010). It effectively utilizes dietary sucrose to synthesize exopolysaccharides through glucosyl-transferases and a fructosyl-transferase. It is also capable of utilizing various sugars, including mono and disaccharides and sugar-alcohols as well, converting them to glycolytic intermediates (Zeng & Burne, 2015). Therefore, S. mutans has been used as a model organism to understand the mechanisms involving cariogenic virulence factors as well as to evaluate oral antimicrobial treatments. The complex genetic machinery of S. mutans has been extensively investigated, which led to a better understanding of the functions related to caries initiation and caries progression, especially those related to adhesion (glucosyltransferases, gtfs), nutrient transport (PTS system, ABC transporters) and stress (clp) (Ajdić & Pham, 2007; Argimón & Caufield, 2011; Biswas, Drake, & Biswas, 2007; Deng, ten Cate, & Crielaard, 2007; Li, Shibata, Takeshita, & Yamashita, 2014; Moye, Zeng, & Burne, 2014).

S. mutans in dental biofilms has evolved multiple strategies to adapt to environmental challenges, such as the nutrient supply, oxygen concentration, pH, and the presence of competing bacteria and inhibiting substances like bacteriocins, host antimicrobial peptides, and chemical substances found in mouthwashes and toothpastes (Parahitiyawa et al., 2010). Fluoride is a common chemical substance in oral care products, which cariostatic effect is a result of its ability to modify the physiochemical properties of teeth. Fluoride's antimicrobial property (through the inhibition of enolase, arginine and urease systems, and the activity of pyrophosphatase and F-ATPase in S. mutans) and fluoride resistance mechanisms (through fluoride ion exporters) were described in a recent review (Liao et al., 2017). Generally less than 100 ppm fluoride could reduce 50% of the activity of F-ATPase enzyme in S. mutans although the exact mechanism of this inhibition remains unclear (Liao et al., 2017).

Dental biofilms are exposed to large amounts of dietary carbohydrates. The corresponding subsequent metabolic pathways may alter the biochemical composition as well as the antimicrobial resistance

capability of biofilms (Ccahuana-Vásquez & Cury, 2010). For example, sucrose can be taken up by S. mutans through a high-affinity, high-capacity sugar-phosphotransferase system (PTS) (Zeng & Burne, 2015). Once internalized, it can be broken down into glucose and fructose for glucan or fructan synthesis: glucosyltransferases use glucose (available after breakdown of sucrose) into glucans, while fructosyltransferase (Ftf) catalyzes the incorporation of the available fructose into fructans which may be used as extracellular stores of carbohydrate within the biofilm matrix. In the presence of sucrose, the expression of the genes related to the pathways abovementioned are increased (Zeng & Burne, 2015). Fluoride is effective in the presence of sucrose (at high cariogenic condition) (Cury, do Amaral, Tenuta, Del Bel Cury, & Tabchoury, 2010). Toothpastes with >1000ppm/F have shown significant reduction in caries-lesion progression in vitro at sucrose frequencies increasing from two to eight times per day (Cury et al., 2010). One potential mechanism is that fluoride could inhibit the enolase (EC 4.2.1.11) within the glycolysis pathway in S. mutans and enhance cell death (Guha-Chowdhury, Clark, & Sissons, 1997). However, it is possible that in the presence of sucrose, fluoride affects more cell functions than glycolysis pathway alone.

Using a RNA-sequencing analysis approach, a recent study showed that the phosphoenolpyruvate-phosphotransferase transport system (PEP:PTS system), a system for sugar internalization by S. mutans, is upregulated in the presence of sucrose (Zeng & Burne, 2015). The genome of S. mutans UA159 contains 14 PTS with a variety of enzyme II domain and specificity for different sugars (Ajdić et al., 2002). Bender et al. proposed that the function of glucose uptake of PTS is sensitive to fluoride (Bender, Thibodeau, & Marquis, 1985). It is likely the inhibitory effect of fluoride is enhanced through targeting the glucose/sugar uptake systems in the presence of sucrose.

Considering the diverse mechanisms that could be affected in oral bacteria in the presence of fluoride, in this study we aimed to evaluate the effect of sodium fluoride on Streptococcus mutans UA159 transcriptome in the presence of sucrose, using a high-throughput sequencing of mRNA (Do, Sheehy, Mulli, Hughes, & Beighton, 2015; Zeng et al., 2013).

MATERIALS & METHODS

Growth conditions and fluoride treatment

S. mutans UA159 [SM] was grown in a medium containing 1.4% tryptone and 0.8% yeast extract (TY) (Oxoid Ltd.) and incubated overnight at 37°C in a CO_2 incubator. Cultures were then pelleted (3,200rpm for 3min) and resuspended in TY (OD_{600} adjusted to 1.0). The TY mixture was diluted in different media to obtain an OD_{600} of 0.2 (final volume=10mL). SM was inoculated into the following 3 sets of experiments:

- medium control group = TY medium, no sucrose [TY]
- sucrose control group= TY medium + 10mM sucrose [TY_S]
- sodium fluoride test group = TY medium + 10mM sucrose + 100ppm NaF [TY_S_NaF]

The experiments were performed in biological triplicate (designated as a, b and c). All samples were incubated in a CO_2 incubator and pellets were collected at exponential phase after 3 hours, and immediately placed in an RNAprotect reagent (Qiagen inc.). The mid-log phase was previously tested (Suppl. Figure 1), as well as the dose response to NaF concentrations, ranging from 50ppm/NaF to 500ppm/NaF. The concentration of 100ppm/NaF was the highest concentration where there was visible growth. The pH of each sample was measured and was similar between groups: TY = 6.42±0.03; TY_S = 6.09±0.03; and TY_S_NaF = 6.36±0.03.

RNA-seq and data analysis

The total RNA was extracted from all the samples using the UltraClean® Microbial RNA Isolation (Mo-bio, San Diego, USA) with on-column DNase digestion (Qiagen, Inc). The total RNA was depleted of ribosomal RNA using the Ribo-Zero™ Meta-Bacteria Kit (Epicentre, Illumina). Further processing of the samples involved library preparation from the enriched mRNA following the Illumina®TruSeq™ library prep protocols (Illumina Inc., San Diego, CA). Paired-end sequencing was then performed on the Illumina HiSeq2500 (Illumina Inc.) sequencer to obtain 2 x 100bp sequence reads (Sequence read archive SRA - accession number PRJNA506386).

Sequence data were trimmed to remove low quality reads, using sickle (https://github.com/najoshi/sickle). The annotated genome of S. mutans UA159 was downloaded from the NCBI database and used as the reference genome for short read mapping, using the CLC Genomics workbench (version 9). The total number of mapped reads (raw reads) was compared between conditions using Kruskal-Wallis test (R package Base, significance of 5%). Differential gene expression

analysis was carried out using the R package DESeq2 (Love, Anders, & Huber, 2014) and a discrepancy of 2-fold and padj (FDR) <0.05 between groups was considered as statistical significance.

The up or down-regulated set of genes from each group were then annotated as gene ontology (GO) terms (using the gene2GO map for S. mutans UA159, downloaded from Uniprot https://www.uniprot.org/). The GO term enrichment analysis carried out using the R package topGO determines the GO terms that are over-represented or under-represented within the selected experimental groups. The visualization of these GO terms was done using REVIGO (Supek, Bošnjak, Škunca, & Šmuc, 2011), using GO terms within the biological process or molecular function category.

RESULTS

Results showed the TY_S_NaF group has the lowest number of reads. The median number of reads was 2,462,647 (range = 1,434,691-14,163,810) for the TY; 19,585,165 (range = 4,744,092-28,927,683) for the TY_S, and 150,823 (range = 108,035-190,255) for the TY_S_NaF (Kruskal-Wallis test; p=0.04). Higher variance in the TY_S_NaF group was observed when compared to the other two groups (Figure 1; supplementary figure 2). The proportion of genes expressed (considered as ">0 reads") was high in all groups (medium control = 99%; sucrose control = 98%; and test group = 95%).

The number of genes differentially expressed by comparison was TY vs. TY_S = 458 genes differentially expressed; TY vs. TY_S_NaF = 113 genes; and TY_S vs. TY_S_NaF = 117 genes. The up or down-regulated set of genes from each group was annotated as gene ontology (GO) terms. The GO term enrichment analysis is shown in figure 2, where the largest circles represent the merging of 6 GO terms within the biological process or molecular function category. The findings showed that most PEP:PTS systems and other metabolic transporters were up-regulated in the TY_S_NaF, as well as glutamate metabolism (Figure 2, A); and molecular functions such as transporters activity, glycosyltransferase and PTS activity were also up-regulated in the TY_S_NaF when compared to a medium without sucrose (Figure 2, B). When compared to the TY_S, TY_S_NaF up-regulated functions such as response to stress and defences response, as well as some peptidades and amino-acid molecular functions activities and transporters of bacteriocin, amide and proteins (Figure 1, C and D).

Down-regulation of regulatory functions and catabolic processes in the presence of fluoride could be observed (Suppl. figures 3 and 4).

We observed some relevant findings based on the classification of Argimon & Caufield, 2011(Argimón & Caufield, 2011) to categorize S. mutans genes as virulence related ones. Table 1 shows the results of the comparisons of TY vs TY_S; TY vs TY_S_NaF; and TY_S vs TY_S_NaF. Results of other genes affected by fluoride as described in literature were also explored in table 1.

Acidogenicity and sugar transport

Regarding those genes related to acidogenicity, scrA (Enzyme II scr) was up-regulated in the presence of NaF and sucrose when compared to sucrose only. The fruA (fructanase) was also up-regulated in the presence of NaF and sucrose (TY_S_NaF), but in the comparison with the media without sugar (TY). Many genes that code PTS components of maltose, mannose and fructose -specific transporters were up-regulated in the presence NaF and sucrose, when compared to media without sugar (TY). The ptsG (PTS system, maltose and glucose-specific) was up-regulated in the presence of sucrose, even in the presence of fluoride. The gene SMU_1961c involved in the PTS system, fructose- and mannose-inducible IIA component was up-regulated in the presence of NaF in both comparisons.

The glycolytic flux to D-lactate may be reduced in the presence of fluoride: pfl (pyruvate formate-lyase, log_2FC= 3.37 and log_2FC= 2.93) was down-regulated in the TY_S_NaF when compared to TY and TY_S.

Adhesion and biofilm formation

Genes related to S. mutans adhesion, such as gtfB (Glucosyltransferase GTF-I) and gtfC (Glucosyltransferase GTF-SI) were up-regulated in the presence of sucrose, even when fluoride was present, suggesting a utilization of sucrose following treatment with 100ppm/NaF (table 1). Considering the gtfBCD, only gtfD was expressed similarly in all groups, being one of the top 30 most expressed genes. The gbpC (Glucan-binding protein C) and wapA (Wall-associated protein A) as well as the same gtfB and gbpC were up-regulated in TY_S when compared to TY group. Competence histidine kinase (ciaH) and luxS (involved in synthesis of AI-2) were up-regulated in TY when compared to TY_S.

Fluoride resistance mechanisms

SMU_1290 and SMU_1289, both coding for EriC-type fluoride/proton exchange protein, presented no

differential expression, although the median of the number of reads was 10^2 in TY_S_NaF while it was 10^4 in TY_S and TY. F-ATPase system presented no differential expression as well.

Acid tolerance

No gene related to acid tolerance was significantly differentially expressed between groups. These included the atpA (F-ATPase proton pump), fabM (trans-2, cis-3-Decenoyl-ACP isomerase), sloR (Metalloregulatory protein), vicK (Histidine kinase), and htrA (Serine protease/chaperone).

Intracellular polysaccharides accumulation

Genes down-regulated in the absence of sucrose when compared to the media with sucrose included glgB (glycogen branching protein), glgC (glucose-1-phosphate adenylyltransferase) as well as glgP (glycogen phosphorylase). The glgP was also down-regulated in the absence of sucrose when compared to a media with NaF and sucrose. Conversely, the gene dltA, coding for D-alanine-activating enzymes, and related to regulation of non-PTS sugar internalization, was up-regulated ($\log_2$FC=1.47; p=0.003) in the TY when compared to TY_S_NaF.

Galactose metabolism

We showed down-regulation of lacACDG in the TY_S_NaF when compared to TY_S. However, it was also up-regulated in the TY_S when compared to the media containing no sucrose, suggesting that the result could be related to the presence of sucrose.

DISCUSSION

Sodium fluoride is effective in reducing caries and inhibits carbohydrate utilization of oral microorganisms by blocking enzymes involved in the bacterial glycolytic pathway (Bradshaw, Marsh, Hodgson, & Visser, 2002). However, the molecular mechanism of plaque biofilm's response to fluoride and the effect of fluoride in the presence of dietary carbohydrates are still little understood. This study has shown from the sequencing data that S. mutans growing in a medium containing 100 ppm/NaF in the presence of sucrose has a decreased overall gene expression level in S. mutans UA159. This might be explained by the decrease in cell number and metabolic activity caused by the deleterious effect of fluoride, which leads to lower number of total reads, and therefore lower gene expression overall. The aim of this manuscript was to carry out a descriptive analysis of S. mutans transcriptome. The presence

of 100 ppm/NaF indicates the up-regulation of genes related to response to stress, and sucrose/fructose/mannose-specific PEP:PTS transporters, and down-regulation of genes related to glycolytic flux to D-lactate and galactose metabolism. Genes related to S. mutans adhesion (gtfB and gtfC), acidogenicity and sugar transport were up-regulated in the presence of sucrose (TY_S) and sucrose/fluoride (TY_S_NaF) as well, showing that the fluoride did not influence in its expression.

In this study we aimed to further investigate the effect of fluoride on S. mutans cells in the presence of sucrose in various cariogenic traits, including the ones related to acidogenicity. It has been demonstrated previously that S. mutans up-regulates genes related to acidogenicity in the presence of sucrose such as comYA (late competence protein), SMU_1077 (phosphoglucomutase pgm), glgD, and pfl when compared to a medium with glucose (Zeng & Burne, 2015). However, we showed that comYA were also found to be overexpressed in the TY_S_NaF group (down-regulated in the presence of only sucrose). Interestingly, scrA was up-regulated in the presence of NaF and sucrose when compared to sucrose only. According to Zeng et al., the scrA, a sucrose-specific PTS, which internalizes and concomitantly phosphorylates sucrose and scrB, which cleaves S-6-P into glucose-6-PO4 (G-6-P) and fructose are the preferred internalization method for sucrose. Our results suggest that scrA (Enzyme II scr) expression is positively affected by the presence of NaF and sucrose (scrA was up-regulated in TY_S_NaF when compared to sucrose only). It could be a mechanism of interference in acid production. Zeng et al. showed that the expression of the scrA and scrB genes was higher in cells growing in the presence of sucrose, although the level of increase in scrA transcripts was larger than that for scrB transcripts (Zeng & Burne, 2015). This could explain our results where no difference was found for scrB. Furthermore, we showed fluoride/sucrose down-regulated pfl (pyruvate formate-lyase): the glycolytic flux to D-lactate may be reduced in the presence of fluoride, as it was found a $\log_2$FC of pfl 3.37 and 2.93 in the TY_S_NaF when compared to TY and TY_S, respectively.

The internalization of sugar by S. mutans depend on the sugar available and it is mostly accomplished via the phosphoenolpyruvate-phosphotransferase transport system (PEP:PTS system), and less frequently by multiple-sugar metabolism as a part of the pathway for non-PTS sugars uptake (Slee & Tanzer, 1979). In the present study, NaF and sucrose significantly up-regulate the genes involved in mannose/fructose/sugar-specific components of the PTS compared to fluoride-free control medium,

with and without sucrose (TY and TY_S). Other interesting finding related to sugar transport, is that the results of the present study support the fact that there is some impact related to trehalose metabolism, due to the PTS system, trehalose-specific pttB down-regulation in the absence of sucrose. This trehalose-PTS enzyme II of S. mutans have also been shown to be capable of internalizing sucrose from the environment, albeit much less efficiently (Zeng & Burne, 2015).

Enolase and F-ATPase system were believed to be competitively inhibited by fluoride (Liao et al., 2017). However, none of them presented significant differential expression here when compared to fluoride-free mediums. This result means the expression of the protein is not affected by the presence of fluoride using the thresholds applied in this study and under the set of conditions used: the enolase gene may be translated into a protein but the enzyme may not be active due to the Mg+ capture by fluoride, which leads to enolase being left inactive. Enolase not only plays a role in the glycolytic process, but also catalyses the production of phosphoenolpyruvate (PEP) for glucose uptake through the PEP-dependent phosphotransferase system (PTS). Thus, the inhibition of the enolase activity by F– could also has a negative effect on glucose uptake (Liao et al., 2017). In our study, it is very interesting that no effect of gene expression of enolase was observed, but many genes that code PTS components of maltose, mannose and fructose -specific transporters were up-regulated in the presence NaF and sucrose, when compared to media without sugar (TY). Some attention should be presented to the gene SMU_1961c involved in the PTS system, fructose- and mannose-inducible IIA component. This gene was up-regulated in the presence of NaF in both comparisons (TY vs. TY_S_NaF and TY_S vs. TY_S_NaF), suggesting an addition effect of fluoride effect over sucrose.

The results of this study suggest that the expression of genes involved in an important cariogenic trait – adhesion – is not altered by the presence of fluoride when sucrose is present. Inhibition of S. mutans adhesion to the tooth surface is one of the main strategies for the control of caries development. Some microorganisms in the oral cavity require polysaccharides for their colonization and S. mutans can synthesize these polysaccharides using glucosyltransferases (GTFs), using sucrose as substrate. Inhibition of GTF activity in the presence of fluoride has been reported (Liao et al., 2017). However, in our study, the up-regulation of exo-beta-D-fructosidase and gtfBCD (which only gtfD was expressed similarly in all groups, although it was one of the 30 most expressed genes) suggest that the production

of extracellular polysaccharides (EPS) by the use of sucrose in a low concentration of fluoride could be unaltered as well as when a media with sucrose and no fluoride (TY_S) is compared to a media without sucrose (TY). GtfB produce water-insoluble alpha-1- 3 glucosidic linkages, whereas GtfD produces water-soluble alpha-1-6 glucosidic linkages, and GtfC produces both. S. mutans (particularly GtfB and GtfC) bind to the tooth surface and to surfaces of bacteria being important to initiation of caries lesions (Jeon et al., 2009). It may explain the lack of differences of gene expression of gtfD. The up-regulation of genes related to EPS production corroborates with the findings of Dong et al. (2012): a dense biofilm with an extensive extracellular matrix was observed following treatment with sub-MICs of sodium Fluoride (NaF); this previous study of Dong et al. showed an upregulation of gtfB, gtfC, luxS, comD and comE in S. mutans following treatment with sub-minimal concentration of fluoride (625 mg/L) (Dong et al., 2012). This has been explained by persister cells trigger gene expression of stress response. Another study showed that the biomass of biofilm increased after treatment with 0.05% NaF (Ccahuana-Vásquez & Cury, 2010). On the other hand, a study showed that water-insoluble EPS of S. mutans biofilms were reduced by the treatments with NaF at 10, 50 and 125 ppm F (Pandit, Kim, Jung, Chang, & Jeon, 2011). Genes related to intracellular polysaccharides accumulation were down-regulated in the absence of sucrose when compared to the media with sucrose included glgB (glycogen branching protein), glgC (glucose-1-phosphate adenylyltransferase) as well as glgP (glycogen phosphorylase). These genes are involved in putative glycogen synthase that are linked to S. mutans IPS accumulation – intracellular polysaccharides (Spatafora et al., 1999). Conversely, the dltA gene, that encodes D-alanine-activating enzymes, and related to regulation of non-PTS sugar internalization (Spatafora et al., 1999) and also has function in the IPS accumulation was up-regulated in the absence of sucrose when compared to NaF and sucrose.

It has been shown that fluoride significantly inhibited several genes encoding enzymes of the galactose pathway (Shi, Li, White, & Biesbrock, 2018). Our study corroborates with this finding. We showed down-regulation of lacACDG in the TY_S_NaF when compared to TY_S, despite being up-regulated in the presence of sucrose. The previous study of Shi et al (2018) showed inhibition upon a 10-min exposure to NaF versus a negative control of the genes lacA and lacB (A and B subunits of the galactose-6-P isomerase), lacC (tagatose-6-P kinase), lacD (tagatose-1,6-bP adolase), galK

(galactokinase), galT (galactose-1-phosphate uridylyltransferase), and galE (UDP-glucose 4-epimerase). Authors suggested that gene expression inhibition of lacA, lacB, lacC, and lacD indicates that beside the fluoride-enzyme interaction, fluoride also inhibits gene expression in the tagatose pathway (Shi et al., 2018).

The virulence factor classification of Argimon & Caufield (2011) was used as a reference for our analysis of the putative virulence genes. A note of caution is due here since their study showed no correlation between the presence of these virulence factors and dental caries. It is important to point out that it was attempted to have a NaF alone group, however the bacterial growth is too limited to perform nucleotide sequencing. Future investigations will include qPCR tests to validate some of the results presented here and microscopy to observe cell organization, chain length and the presence of glucans in the samples. It is also important to note that growth rate may differ between culture samples, and impact gene expression (ref), despite similar trends observed (Supplementary figure 1). Future investigations will also include growth rate measurement of each sample used in RNA-Seq analysis.

In conclusion, NaF repressed the overall gene expression of S. mutans. However, its major effect seems to be the inducing of expression of genes involved in some PEP:PTS systems and other metabolic transporters which imply specific cellular internalisation of sugars, as well as response to stress. Also, it could have a role in decreasing galactose and trehalose metabolism as well as in the D-lactate flux due to the down-regulation of an enzyme involved in these systems. Knowledge of the mechanisms of the effect of fluoride on S. mutans transcriptome may provide tools to study the prevalence of fluoride response in the oral microbial community.

Acknowledgements

This is an original research, not under publication consideration elsewhere, and was partly funded by the Leeds Hospital Charitable Foundation (R&D/PP/12011).

REFERENCES

Ajdić, D., McShan, W. M., McLaughlin, R. E., Savić, G., Chang, J., Carson, M. B., . . . Ferretti, J. J. (2002). Genome sequence of Streptococcus mutans UA159, a cariogenic dental pathogen. Proc Natl Acad Sci U S A, 99(22), 14434-14439. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12397186. doi:10.1073/pnas.172501299

Ajdić, D., & Pham, V. T. (2007). Global transcriptional analysis of Streptococcus mutans sugar transporters using microarrays. J Bacteriol, 189(14), 5049-5059. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17496079. doi:10.1128/JB.00338-07

Argimón, S., & Caufield, P. W. (2011). Distribution of putative virulence genes in Streptococcus mutans strains does not correlate with caries experience. J Clin Microbiol, 49(3), 984-992. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21209168. doi:10.1128/JCM.01993-10

Bender, G. R., Thibodeau, E. A., & Marquis, R. E. (1985). Reduction of acidurance of streptococcal growth and glycolysis by fluoride and gramicidin. J Dent Res, 64(2), 90-95. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2579114. doi:10.1177/00220345850640021701

Biswas, I., Drake, L., & Biswas, S. (2007). Regulation of gbpC expression in Streptococcus mutans. J Bacteriol, 189(18), 6521-6531. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17616585. doi:10.1128/JB.00825-07

Bradshaw, D. J., Marsh, P. D., Hodgson, R. J., & Visser, J. M. (2002). Effects of glucose and fluoride on competition and metabolism within in vitro dental bacterial communities and biofilms. Caries Res, 36(2), 81-86. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12037363. doi:10.1159/000057864

Ccahuana-Vásquez, R. A., & Cury, J. A. (2010). S. mutans biofilm model to evaluate antimicrobial substances and enamel demineralization. Braz Oral Res, 24(2), 135-141. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20658029.

Cury, J. A., do Amaral, R. C., Tenuta, L. M., Del Bel Cury, A. A., & Tabchoury, C. P. (2010). Low-fluoride toothpaste and deciduous enamel demineralization under biofilm accumulation and sucrose exposure. Eur J Oral Sci, 118(4), 370-375. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20662910. doi:10.1111/j.1600-0722.2010.00745.x

Deng, D. M., ten Cate, J. M., & Crielaard, W. (2007). The adaptive response of Streptococcus mutans towards oral care products: involvement of the ClpP serine protease. Eur J Oral Sci, 115(5), 363-370. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17850424. doi:10.1111/j.1600-0722.2007.00477.x

Do, T., Sheehy, E. C., Mulli, T., Hughes, F., & Beighton, D. (2015). Transcriptomic analysis of three Veillonella spp. present in carious dentine and in the saliva of caries-free individuals. Front. Cell. Infect. Microbiol. doi:10.3389/fcimb.2015.00025

Dong, L., Tong, Z., Linghu, D., Lin, Y., Tao, R., Liu, J., . . . Ni, L. (2012). Effects of sub-minimum inhibitory concentrations of antimicrobial agents on Streptococcus mutans biofilm formation. Int J Antimicrob Agents, 39(5), 390-395. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22421330. doi:10.1016/j.ijantimicag.2012.01.009

Guha-Chowdhury, N., Clark, A. G., & Sissons, C. H. (1997). Inhibition of purified enolases from oral bacteria by fluoride. Oral Microbiol Immunol, 12(2), 91-97. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9227132.

Jeon, J. G., Klein, M. I., Xiao, J., Gregoire, S., Rosalen, P. L., & Koo, H. (2009). Influences of naturally occurring agents in combination with fluoride on gene expression and structural organization of Streptococcus mutans in biofilms. BMC Microbiol, 9, 228. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19863808. doi:10.1186/1471-2180-9-228

Klein, M. I., DeBaz, L., Agidi, S., Lee, H., Xie, G., Lin, A. H., . . . Koo, H. (2010). Dynamics of Streptococcus mutans transcriptome in response to starch and sucrose during biofilm development. PLoS One, 5(10), e13478. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20976057. doi:10.1371/journal.pone.0013478

Li, D., Shibata, Y., Takeshita, T., & Yamashita, Y. (2014). A novel gene involved in the survival of Streptococcus mutans under stress conditions. Appl Environ Microbiol, 80(1), 97-103. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24123744. doi:10.1128/AEM.02549-13

Liao, Y., Brandt, B. W., Li, J., Crielaard, W., Van Loveren, C., & Deng, D. M. (2017). Fluoride resistance in. J Oral Microbiol, 9(1), 1344509. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28748043. doi:10.1080/20002297.2017.1344509

Love, M., Anders, S., & Huber, W. (2014). Differential analysis of count data – the DESeq2 package. In: bioRxiv.

Moye, Z. D., Zeng, L., & Burne, R. A. (2014). Modification of gene expression and virulence traits in Streptococcus mutans in response to carbohydrate availability. Appl Environ Microbiol, 80(3), 972-985. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24271168. doi:10.1128/AEM.03579-13

Pandit, S., Kim, J. E., Jung, K. H., Chang, K. W., & Jeon, J. G. (2011). Effect of sodium fluoride on the virulence factors and composition of Streptococcus mutans biofilms. Arch Oral Biol, 56(7), 643-649. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21241981. doi:10.1016/j.archoralbio.2010.12.012

Parahitiyawa, N. B., Scully, C., Leung, W. K., Yam, W. C., Jin, L. J., & Samaranayake, L. P. (2010). Exploring the oral bacterial flora: current status and future directions. Oral Dis, 16(2), 136-145. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19627515. doi:10.1111/j.1601-0825.2009.01607.x

Shi, Y., Li, R., White, D. J., & Biesbrock, A. R. (2018). Stannous Fluoride Effects on Gene Expression of Streptococcus mutans and Actinomyces viscosus. Adv Dent Res, 29(1), 124-130. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29355427. doi:10.1177/0022034517737027

Slee, A. M., & Tanzer, J. M. (1979). Phosphoenolpyruvate-dependent sucrose phosphotransferase activity in Streptococcus mutans NCTC 10449. Infect Immun, 24(3), 821-828. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/468377.

Spatafora, G. A., Sheets, M., June, R., Luyimbazi, D., Howard, K., Hulbert, R., . . . Hudson, M. C. (1999). Regulated expression of the Streptococcus mutans dlt genes correlates with intracellular polysaccharide accumulation. J Bacteriol, 181(8), 2363-2372. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10197997.

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One, 6(7), e21800. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21789182. doi:10.1371/journal.pone.0021800

Zeng, L., & Burne, R. A. (2015). Sucrose- and Fructose-Specific Effects on the Transcriptome of Streptococcus mutans, as Determined by RNA Sequencing. Appl Environ Microbiol, 82(1), 146-156. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26475108. doi:10.1128/AEM.02681-15

Zeng, L., Choi, S. C., Danko, C. G., Siepel, A., Stanhope, M. J., & Burne, R. A. (2013). Gene regulation by CcpA and catabolite repression explored by RNA-Seq in Streptococcus mutans. PLoS One, 8(3), e60465. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23555977. doi:10.1371/journal.pone.0060465

TABLES

Table 1. List of putative virulence genes (adapted from Argimón & Caulfied, 2010) and other genes affected by fluoride in S. mutans and the corresponding results of the R package DESeq2 results. log2FC = log2 fold-change; NS = Non-significant (FDS>0.05 and >2-fold). TY = medium control group; TY_S = sucrose control group; TY_S_NaF = sodium fluoride and sucrose test group.

Locus tag	S. mutans putative virulence genes		log2FC	
			TY_S vs. TY	TY_S vs. TY_S_NaF
	Adhesion			
SMU_1004	gtfB	Glucosyltransferases GTF-I	3.59	NS
SMU_1005	gtfC	Glucosyltransferases GTF-SI	2.46	NS
SMU_2112	gbpA	Glucan-binding protein A	NS	NS
SMU_22	gbpB	Glucan-binding protein B	NS	NS
SMU_1396	gbpC	Glucan-binding protein C	1.10	NS
SMU_772	gbpD	Glucan-binding protein D	NS	NS
SMU_987	wapA	Wall-associated protein A	1.69	NS
SMU_610	spaP	Antigen I/II	NS	NS
SMU_1247	eno	Enolase	NS	NS
SMU_2042	dexA	Dextranase	NS	NS
	Acidogenicity			
SMU_1536	glgA	Glycogen synthase	NS	NS
SMU_1689	dltC	D-Alanyl carrier protein	NS	NS
SMU_1841	scrA	Enzyme II(scr)	NS	-1.54
SMU_1843	scrB	Sucrose-6-phosphate hydrolase	NS	NS
SMU_879	msmF	Multiple sugar ABC transporter, permease protein MsmF	NS	NS
SMU_78	fruA	Fructanase	NS	NS
	Acid tolerance			
SMU_1527	atpA	F-ATPase proton pump	NS	NS
SMU_186	sloR	Metalloregulatory protein	NS	NS
SMU_486	vicK	Histidine kinase	NS	NS
SMU_2164	htrA	Serine protease/chaperone	NS	NS
	Biofilm formation			
SMU_1916	comD	Competence histidine kinase	NS	NS
SMU_1917	comE	Competence response regulator	NS	NS
SMU_1997	comX	Competence alternate sigma factor	NS	NS
SMU_1128	ciaH	Competence histidine kinase	-1.19	NS
SMU_474	luxS	Enzyme involved in synthesis of AI-2	-1.22	NS
	Other putative virulence genes			
SMU_361	pgk	Putative phosphoglycerate kinase	NS	NS

SMU_1302	adcA	Putative surface adhesin/Zn-binding lipoprotein	NS	NS
SMU_360	gapC	Putative glyceraldehyde-3-phosphate dehydrogenase	2.84	2.09
SMU_1541	pulA	Putative pullulanase	NS	NS
SMU_399	-	Putative C3-degrading proteinase	NS	NS
SMU_583	-	Putative hemolysin	NS	NS
SMU_761	-	Putative collagenase	NS	NS
SMU_759	-	Putative collagenase	NS	NS
	F-ATPase			
SMU_1534	atpH	ATP synthase F0 sector subunit c (EC 3.6.3.14)	-2.40	NS
	Competence			
SMU_1987	comYA	Late competence protein ComGA, access of DNA to ComEA	-2.34	-2.84
	Sugar transport			
SMU_872	-	PTS system, fructose-specific	1.94	NS
SMU_1491	lacE	PTS system, lactose-specific IIB and IIC components	3.14	2.53
SMU_1492	lacF	PTS system, lactose-specific IIA component	2.99	NS
SMU_1185	mtlA1	PTS system, mannitol-specific	3.66	2.66
SMU_1183	mtlA2	PTS system, mannitol-specific IIA component	2.14	NS
SMU_1877	ptnA	PTS system, mannose-specific IIA and IIB components	1.55	NS
SMU_1878	ptnC	PTS system, mannose-specific IIC component	NS	NS
SMU_2047	ptsG	PTS system, maltose and glucose-specific	3.86	2.03
SMU_2038	pttB	PTS system, trehalose-specific	4.99	3.68
SMU_114	-	PTS system, tagatose-specific IIB and IIC components	2.79	NS
SMU_115	-	PTS system, tagatose-specific IIA component	3.69	NS
SMU_1879	-	PTS system, mannose-specific IID component	1.44	NS
SMU_1956c	-	PTS system, fructose- and mannose-inducible putative EII component	NS	NS
SMU_1957	-	PTS system, fructose- and mannose-inducible IID component	NS	NS
SMU_1960c	-	PTS system, fructose- and mannose-inducible IIB component	NS	NS
SMU_1961c	-	PTS system, fructose- and mannose-inducible IIA component	NS	-3.90
	Galactose metabolism			
SMU_1496	lacA	Galactose-6-phosphate isomerase, LacA	3.21	2.53

		subunit (EC 5.3.1.26)		
SMU_1490	lacG	6-phospho-beta-galactosidase (EC 3.2.1.85)	3.53	2.77
SMU_1494	lacC	Tagatose-6-phosphate kinase (EC 2.7.1.144)	3.14	2.34
SMU_1493	lacD	Tagatose 1,6-bisphosphate aldolase (EC 4.1.2.40)	3.58	2.66
SMU_886	galK	(galactokinase),	NS	NS
SMU_887	galT	galactose-1-phosphate uridylyltransferase	NS	NS
SMU_888	galE	UDP-glucose 4-epimerase	NS	NS

FIGURE LEGENDS

Figure 1. Heatmap of the normalized gene expression profiles of the samples. The mean expression values (as displayed by the coloured gradient scale) were calculated from the variance stabilizing transformation of the count data. TY=medium control, TY_S=sucrose control, TY_S_NaF=sodium fluoride and sucrose test.

Figure 2. Gene ontology (GO) terms for biological processes (top figures) and molecular functions (bottom figures) **up-regulated** in S. mutans UA159 in the **TY_S_NaF** test group. Gene expression levels were compared between **TY** and **TY_S_NaF** (A and B); and between **TY_S** and **TY_S_NaF** (C and D). Significant differentially expressed genes with adjusted p-values (FDR)<0.05 were selected for GO term enrichment analysis using topGO, and visualised in REVIGO. The circle size correlates to the number of merged GO terms.