

Divergent meta-transcriptional response of the upper and lower jaw dental plaque biofilm after sucrose exposure

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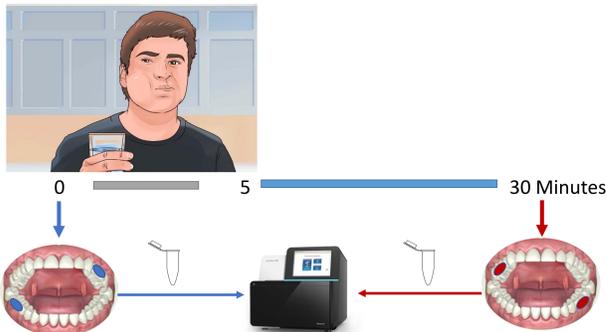
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INTRODUCTION

The intake of sucrose poses a risk for the development of dental caries. Intake of sucrose can be fermented by bacteria in the dental biofilm leading to the formation of lactic acid, and lowering of the pH. Localized accumulation of acids can result in dental demineralization, a key actor in dental decay. In this pilot study we aimed to gain understanding of this process by analysis of the transcriptional response of the dental biofilm upon exposure to sucrose.

METHODS

Study design: One human volunteer was requested to rinse the mouth for five minutes with a 10% sucrose solution. At the start of the pilot study and 30 minutes after the rinse, dental plaque biofilm samples were collected from lingual molar surfaces at contralateral quadrants of the upper and lower jaw. Samples were snap frozen and stored until further analysis.



Metatranscriptome analysis: RNA was extracted from samples and enriched for mRNA (RiboZero). After preparation of metagenomic libraries (TruSeq), samples were analysed by sequencing. Sequence data was quality filtered and merged. Ribosomal sequence reads were removed in SortMeRNA. Meta-transcriptomic reads were then mapped against the non-redundant protein database through BLASTX using DIAMOND (V.0.9.9). Mapping results were parsed in MEGAN (v. 6) and linked to KEGG functional modules (KEGG reference library december2017). Statistical analysis was performed in DeSeq2.

RESULTS

The sucrose rinse resulted in significant meta-transcriptional changes, revealing a modest shift in for the lower jaw plaque biofilm and larger changes for the upper jaw biofilm (Figure 1). The upper and lower jaw plaque biofilm showed different taxonomic representation in the meta transcriptomic reads (Figure 2). We identified 493, (20%) upregulated and 486 (20%) downregulated gene orthologues (Figure 3 & Table 1). Prominent were changes in expression of PTS metabolite transporters, glycoside hydrolases, a downregulation of oxidative phosphorylation and increased expression of gene groups related to dissimilatory sulfate reduction, concomitant with a decrease in the expression of gene groups related to nitrate reduction. When plotting transcriptional changes of upper and lower jaw plaque biofilm, we found that for some gene groups transcriptional changes were similar for both ecosystems. However, we also found cases where genes were upregulated in one but downregulated in the other (figure 4).

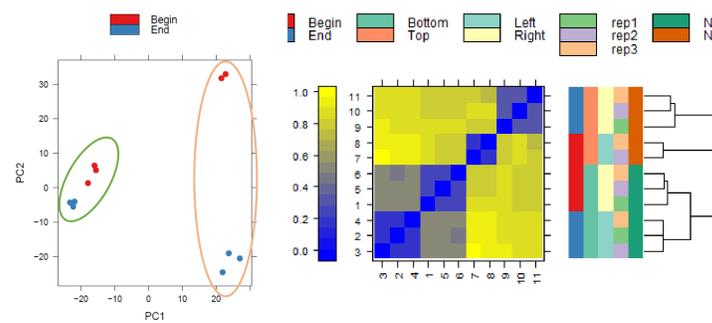


Figure 1. PCA plot and hierarchical heat map of transcriptional profile (KO orthologues) show profound changes in the upper and lower jaw buccal plaque biofilm transcriptome of following a sucrose rinse. While the upper and lower jaw plaque biofilms differ in meta transcriptional profile at the start of the experiment, they also respond differently towards the sucrose rinse.

KEGG	Functional description	Log2 Fold Change
K00823	4-aminobutyrate aminotransferase	-3.7
K00691	maltose phosphorylase	-3.1
K01838	beta-phosphoglucomutase	-3.0
K01464	dihydropyrimidinase	-2.9
K10254	oleate hydratase	-2.7
K01486	adenine deaminase	-2.7
K00261	glutamate dehydrogenase (NAD(P)+)	-2.5
K05825	2-aminoadipate transaminase	-2.5
K02261	cytochrome c oxidase subunit 2	-2.4
K02256	cytochrome c oxidase subunit 1	-2.4
K12567	titin	-2.3
K02262	cytochrome c oxidase subunit 3	-2.3
K07248	lactaldehyde dehydrogenase / glycolaldehyde dehydrogenase	-2.3
K01745	histidine ammonia-lyase	-2.2
K01953	asparagine synthase (glutamine-hydrolysing)	-2.2
K00371	"nitrate reductase / nitrite oxidoreductase, beta subunit"	-2.2
K12527	putative selenate reductase	-2.1
K01478	arginine deiminase	-2.1

KEGG	Functional description	Log2 Fold Change
K02744	"PTS system, N-acetylglucosamine-specific IIA component"	5.1
K02771	"PTS system, fructose-specific IID component"	4.6
K03332	fructan beta-fructosidase	2.6
K02810	"PTS system, sucrose-specific IIC component"	2.1
K19405	protein arginine kinase	1.9
K16960	L-cystine transport system ATP-binding protein sulfite reductase (NADPH) hemoprotein beta-component	1.8
K00381	putative acetyltransferase	1.7
K19286	FMN reductase [NAD(P)H]	1.7
K05352	ribitol-5-phosphate 2-dehydrogenase	1.6
K02046	sulfate transport system permease protein	1.5
K01361	lactocarpin	1.5
K13497	anthranilate synthase/phosphoribosyltransferase	1.5
K01193	beta-fructofuranosidase	1.4
K03462	nicotinamide phosphoribosyltransferase	1.4
K01533	Cu ²⁺ -exporting ATPase	1.4
K03705	heat-inducible transcriptional repressor	1.4
K00209	enoyl-[acyl-carrier protein] reductase	1.4

Table 1. Top 15 up- and downregulated gene orthologues.

CONCLUSIONS

Sucrose rinse induced a shift in the expression of carbon uptake and hydrolase genes, including upregulation of sucrose and fructose-specific PTS transporters. We found down regulation of genes for components of oxidative phosphorylation perhaps reflecting overflow metabolism. Importantly, upper and lower jaw biofilms displayed striking differences in transcriptional response. While for many gene-orthologues, transcription of upper and lower jaw plaque biofilms was in concordance, for others this was not the case showing divergent transcriptional responses, e.g. upregulated in upper jaw biofilm and down regulation for the lower jaw biofilm. The transcriptional profile of the upper and lower jaw plaque microbiota showed significant differences in taxonomic representation.

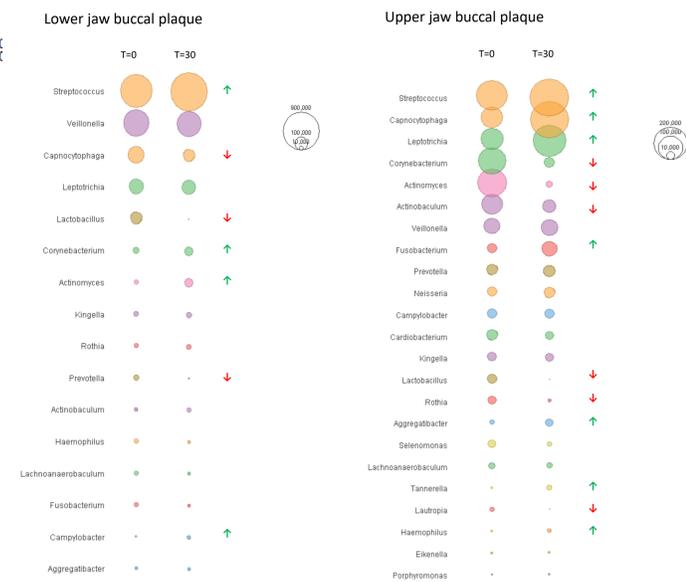


Figure 2. Taxonomic representation of the plaque biofilm meta-transcriptomic reads (genus level). The transcriptional profile of the lower jaw biofilm is less diverse compared to the upper jaw biofilm, and dominated by *Streptococcus* and *Veillonella* species. The upper jaw biofilm is more diverse and transcriptional changes following sucrose rinse are represented by a larger group of genera.

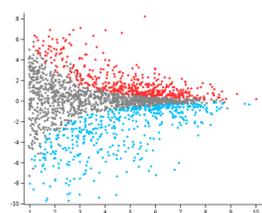


Figure 3. Mean-Difference Plot of Expression Data.

Figure 3. KEGG orthologue changes in expression of before and after sucrose rinse of the upper jaw plaque microbiota plotted against that of the lower jaw plaque microbiota. The boxplot show examples of the orthologous groups of the four quadrants.

