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Microbial DNA extraction methods for microbial screening – saliva vs biofilm comparison

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Introduction

Several factors may affect oral microbiome profiles obtained from different oral samples such as sample collection technique, processing, and extraction approaches. Regarding extraction methods. the variety of DNA extraction kits available often lead to different efficiencies and purity grades affecting microbiome composition results and consequently comparisons (Yu et al. 2023). Biofilms such as the oral pose specific challenges due to the high amount of polysaccharide from the matrix, and a high prevalence of Gram(+) bacteria with thick cell walls. These issues are solved by researchers with different approaches and currently it is not easy to find a standard procedure for microbial DNA extraction for oral microbiome characterization which makes study comparison difficult.

This work aims to stablish a guideline for microbial DNA extraction procedure for microbiome sequencing and for qPCR quantification of different bacteria species. Twenty-five unstimulated whole saliva (UWS) and biofilm samples from healthy volunteer donors were processed using an extraction-buffer [(QuickExtract DNA Extraction Solution® (QE-Buffer)] and 3 different column-based DNA extraction kits [NZYSoil gDNA Isolation Kit® (NZY_soil), ZymoBIOMICS DNA Miniprep Kit® (ZYMO_DNA) and NZYSoil gDNA Isolation Kit® (NZY_gDNA)]. The influence of the preservation of UWS in RNAlater solution® in the DNA extraction was also evaluated.

Results

UWS *vs* Biofilm Microbial DNA extraction comparison

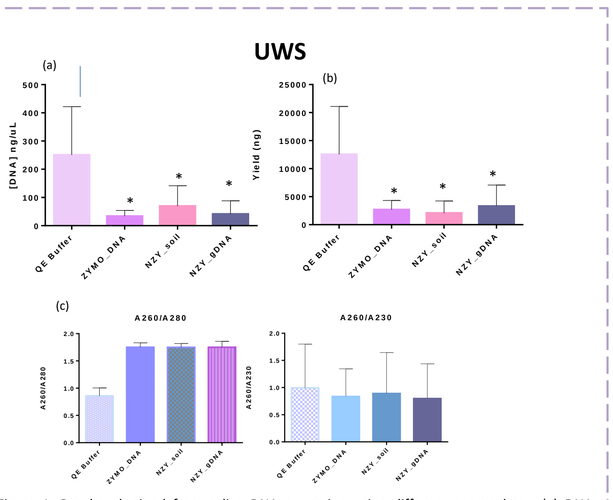

Figure 1. Results obtained from saliva DNA extraction using different approaches. (a) DNA concentration, (b) total yield and (c) purity. (n= 25) * results compared with QE buffer - p<0.05 (ANOVA). No significant differences was achieved between results attained with ZYMO_DNA, NZY_soil and NZY_gDNA.

vs

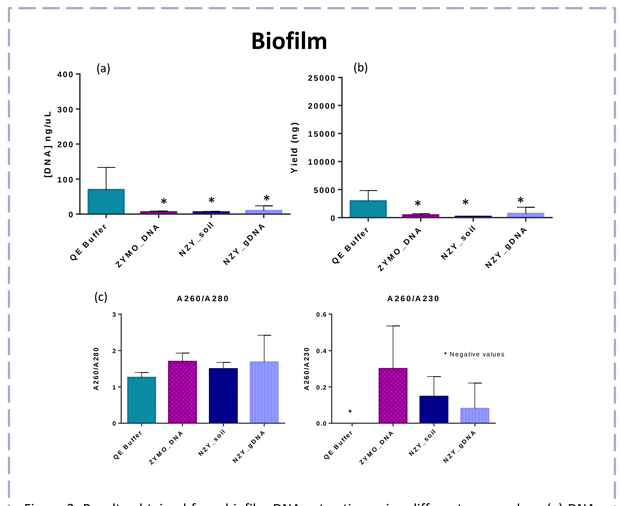

Figure 2. Results obtained from biofilm DNA extraction using different approaches. (a) DNA concentration, (b) total yield and (c) purity. (n= 25) * results compared with QE buffer - p<0.05 (ANOVA). No significant differences was achieved between results attained with ZYMO_DNA, NZY_soil and NZY_gDNA.

Table 1. Quality control of DNA microbial extraction with Microbial Community Standard*

	[DNA] ng/mL	Yield (ng)	A260/A280	A260/A230
MicrobialStand+PBS	18.8	940.0	1.79	0.5824
MicrobialStand+UWS+ QE	369.8	18490	1.395	Negative value
MicrobialStand+UWS+ ZYMO_DNA**	17.62	1409.6	1.802	0.8168
MicrobialStand+UWS+ NZY_soil	82.37	2471.1	1.761	1.282
MicrobialStand+UWS+ NZY_gDNA	35.51	2840.8	1.769	1.303

* Microbial Comunity Satandard from ZymoBIOMICS® containing *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *S. enterica*, *L. fermentum*, *E. faecalis*, *S. aureus*, *B. subtilis*, *S. cerevisiae* and *C. neoformans.* ** ZymoBIOMICS® DNA Miniprep Kit is used in the protocol of ZymoBIOMICS® Microbial Community Standard to assess quality control.

DNA Integrity Number (DIN)

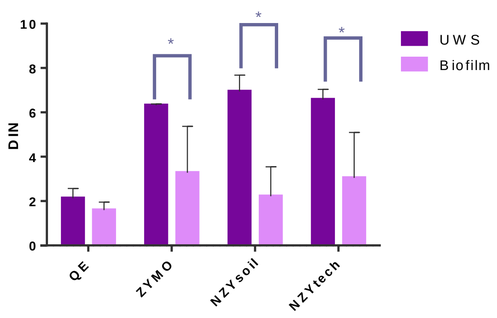

Figure 3. DIN of saliva and biofilm samples after DNA extraction using different approaches. (a) DNA concentration, (b) total yield and (c) purity. (n=8) *, p<0,05 (ANOVA).

Effect of UWS preservation in RNA later in Microbial DNA extraction

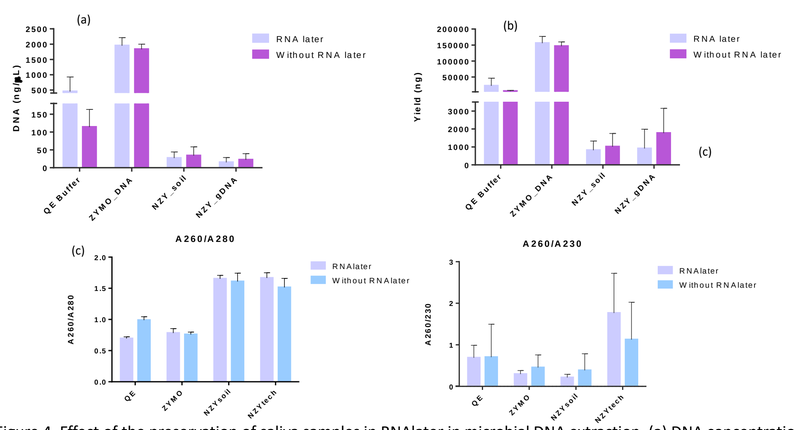

Figure 4. Effect of the preservation of saliva samples in RNAlater in microbial DNA extraction. (a) DNA concentration, (b) total yield and (c) purity. (n= 4) No significant differences was observed between conditions with RNAlater *vs* without RNAlater

qPCR quantification of total bacterial load in saliva samples – an example of downstream applications

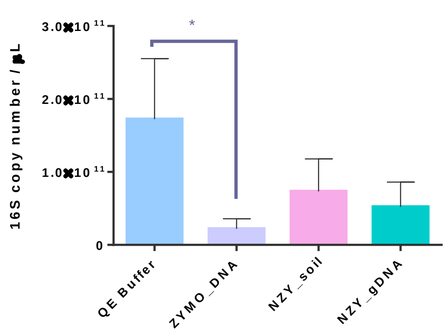

Figure 5. Total bacterial load quantification (16S copy number) in saliva samples using different approaches. (n= 4) *p<0,05 (ANOVA). 16S Primers used: 926F AAACTCAAAKGAATTGACGG and 1062R CTCACRRCACGAGCTGAC

Discussion and Conclusion

The results show that DNA extraction from saliva was more efficient when compared with biofilm, resulting in higher DNA concentrations, yields, purities and integrity. Although QE buffer yielded higher amounts of DNA, the purity ratios and DNA integrity number were lower. No significant differences was achieved between the 3 commercial kits, however DIN of UWS samples extracted with NZYSoil gDNA Isolation Kit® were higher, which can suggest that it may be better for microbiome sequencing downstream applications.

The results also showed no improvement in DNA extraction of UWS samples preserved in RNAlater solution®, lowering the cost of sampling and storage of samples.

Interestingly, besides the low purity and DIN of UWS DNA samples extracted with QE buffer, when total bacterial load was quantified using qPCR, this condition allowed an efficient 16S copy number/mL quantification, without significant differences when compared to the results achieved with NZY_soil and NZY-gDNA kits. This buffer could be a cost-effective solution for downstream applications, such as qPCR quantification of different bacterial species.

References

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Ethical declaration

This work has been approved by the Comissão de Ética para a Saúde of Universidade Católica Portuguesa (project CES133 – Microbioma Oral Humano).

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