PROTOCOL
In-Vivo

Protection/Restoring the Oral Microbiome against alcohol

Background

The oral microbiome is a strong and diverse community of 700 different species. When the oral microbiota is in balance, the teeth and gums are protected. In that case, bacteria responsible for:

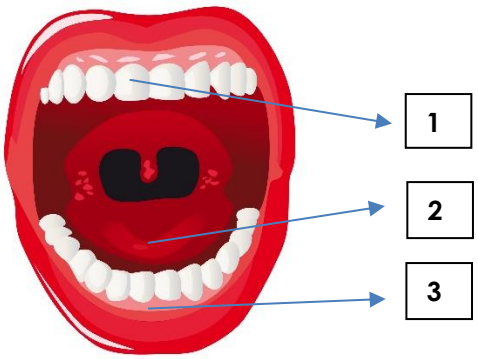
- Caries – Cariogenic bacteria
- Tooth decay – Periodontitis
- Sensitive gums – Gingivitis

have no chance to grow and perform their destructive work.
Balanced oral microbiota also prevents bad breath because the bacteria producing bad-smell molecules are suppressed.
The main protective bacterium is *Streptococcus salivarius*. This bacterium has anti-microbial peptides against almost all pathogenic microorganisms.
Very few things can disturb the oral microbiota including:

- Fermentable sugars such as glucose, Fructose, Sucrose
- Anti-microbial agents such as alcohol

Scope of the test

This test demonstrates the support of preBIULIN ORAL to the oral microbiome after it has been challenged with ethanol. Ethanol has an antimicrobial effect on all micro-organisms so it will weaken the protective oral microbiome. To investigate the effect of alcohol on the oral microbiota, the mouth was rinsed with a 10% solution of ethanol in water.

Test Laboratoria	S&C Laboratory (PL) BASECLEAR (NL)
Test period	2019
Test area	 1 – Teeth 2 – Tongue 3 - Gum
Application	20 g of the test product is used
Design of the study	3 Hours before the test and during the test the volunteers didn't eat or drink anything. Only water was allowed. All volunteers had healthy teeth, and gums and they had no bad breath problems. Before the treatment, the oral microbiota was collected with DNA swabs. The oral cavity was rinsed with the test product for 1 minute.

1 hour later the oral microbiota was collected with DNA swabs.
The oral microbiota before rinsing with alcohol and 1h after rinsing was compared.

T0	Before the treatment, the DNA of the Oralskin microbiota was collected with a DNA swab. The DANN was collected by swabbing 20 seconds on the tongue, 20 seconds on the teeth and 20 seconds on the gums.
T1	1 hour after the treatment the DNA of the Oral microbiota was collected with a DNA swab. The DNA was collected by swabbing for 20 seconds on the tongue, 20 seconds on the teeth and 20 seconds on the gums.

The oral microbiota was profiled through detailed analysis and interpretation of the sequencing results sequencing-based 16S or IST profiling experiment.
Short sequence reads were generated using the Illumina MiSeq platform. Assignment of sequence reads into Operational Taxonomic Units (OTUs) was performed using a proprietary pipeline (BASECLEAR). Classification of bacterial organisms is based on a combination of 16S gene databases. The result is a list of OTUs and corresponding frequencies.

Results interpretation

The test person had a healthy oral microbiota. It can be assumed that the oral microbiota before the treatment was in balance and was protecting the oral cavity.
After the treatment, each specie was compared to its initial population. This was performed by determining the abundance of each specie/genus.

Tested Formula

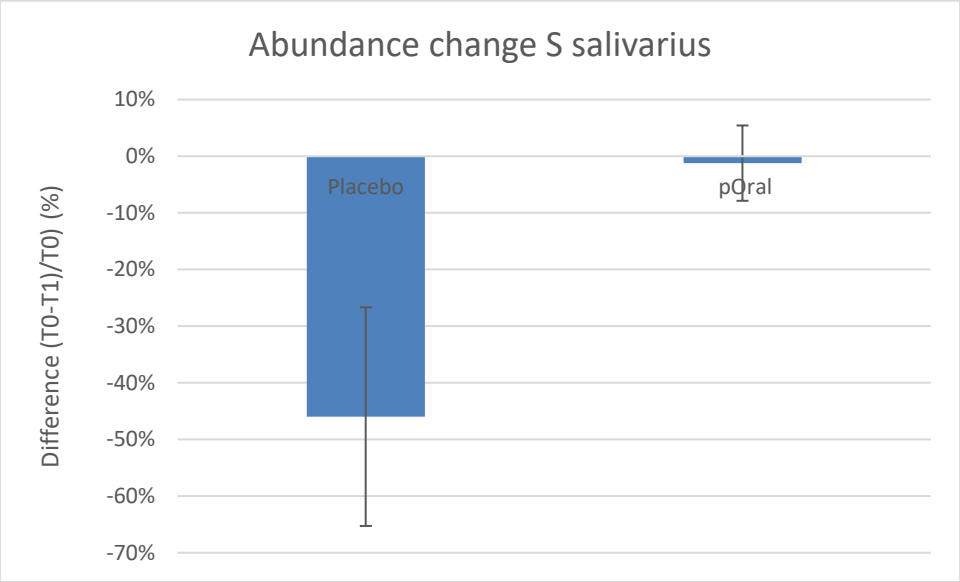
Ingredients	**Placebo**	**3.3%pORAL**
Aqua	90	86.7
Alcohol Denat	10	10
preBIULIN ORAL	-	3.3

Results

Influence on *S salivarius* (protective)

	Placebo			3.3%pORAL		
Volunteer	**Abundance T0 (%)**	**Abundance T1 (%)**	**Difference (%)**	**Abundance T0 (%)**	**Abundance T1 (%)**	**Difference (%)**
V1	12,47%	6,75%	-45,89%	3,90%	3,99%	2,19%
V2	3,67%	1,11%	-69,66%	1,33%	1,19%	-10,52%
V3	4,97%	3,86%	-22,37%	8,85%	9,26%	4,66%
Average			-45.97%			-1.22%

p-value	0,041549	p-value < 0,05: statistically significant difference



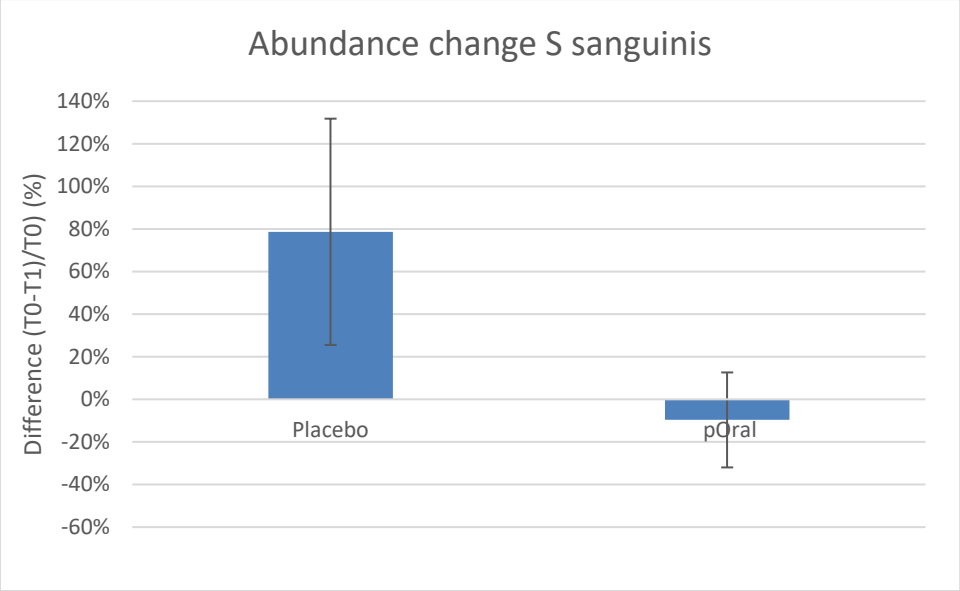
The protective microorganism S salivarius is significantly affected by only 10% alcohol. preBIULIN ORAL protects S salivarius by keeping its abundance stable. This results in

- A more efficient protection of teeth against caries
- A more efficient protection of gums
- Reduction of bad breath

Influence on *S sanguinis* (Cariogenic)

	Placebo			3.3%pORAL		
Volunteer	**Abundance T0 (%)**	**Abundance T1 (%)**	**Difference (%)**	**Abundance T0 (%)**	**Abundance T1 (%)**	**Difference (%)**
V1	1,02%	1,14%	12%	1,33%	1,26%	-5,%
V2	3,63%	6,62%	82%	4,53%	2,78%	-39%
V3	1,81%	4,39%	142%	0,96%	1,10%	15%
Average			+79%			-10%

p-value	0,04799	p-value < 0,05: statistically significant difference

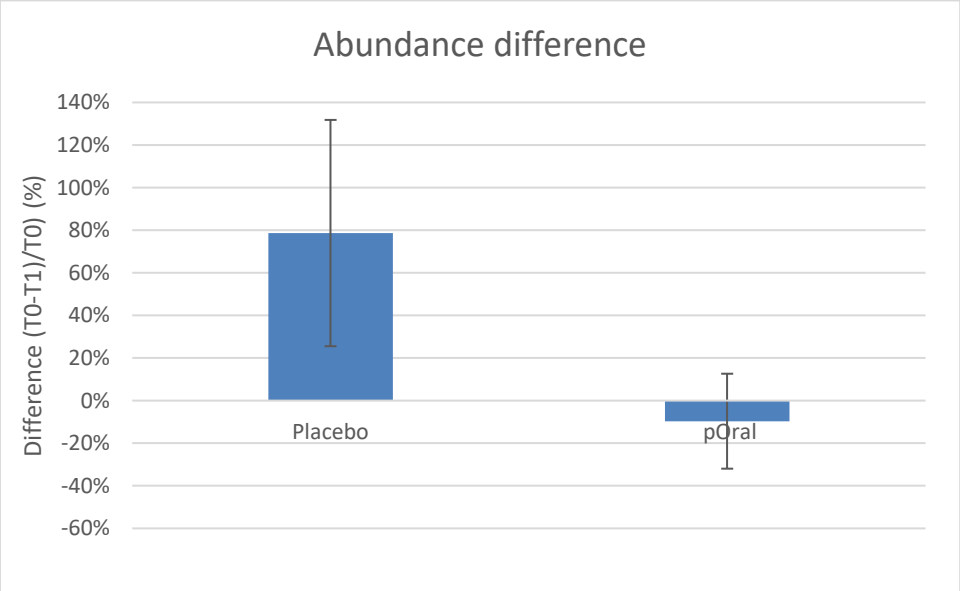


The main cariogenic microorganism *S sanguinis* is significantly stimulated by only 10% alcohol. A plausible explanation is that the alcohol is significantly reducing the *S salivarius* which keeps the *S sanguinis* under control. This results in more efficient protection of teeth against caries

Influence on cariogenic bacteria

	Placebo	3.3% pORAL
Volunteer	**Difference (%)**	**Difference (%)**
V1	64.5%	-15.2%
V2	35.5%	1.37%
V3	91.0%	-31.0%
Average	+64%	-15%

p-value	0,045143	p-value < 0,05: statistically significant difference



The cariogenic microorganisms are significantly promoted by a mouthwash containing 10% alcohol. The addition of preBIULIN ORAL keeps the cariogenic bacteria under control and hence helps to protect the health of the teeth significantly.

The following cariogenic bacteria have been monitored:

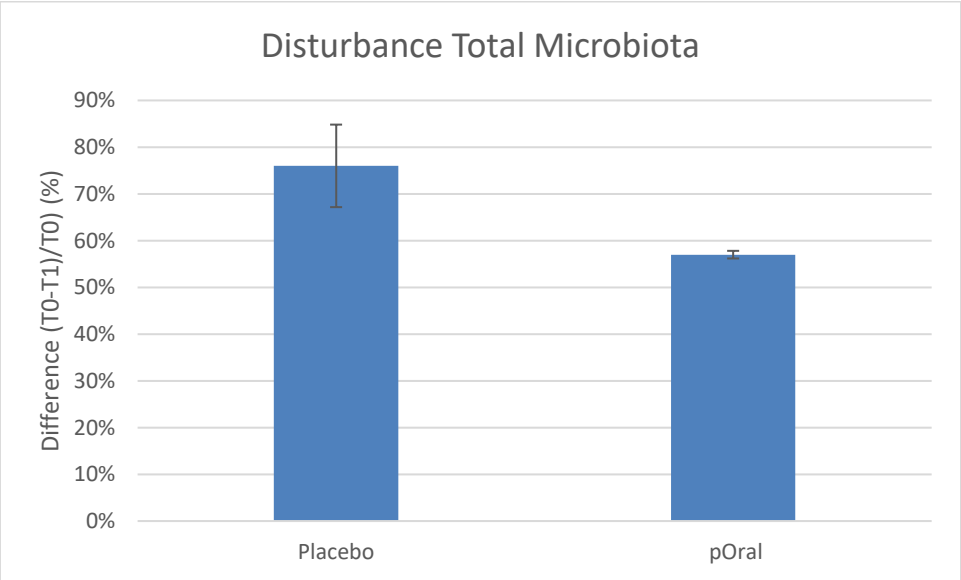
Streptococcus	**Bifidobacterium**	**Lactobacillus**	**Propionibacterium**	**Other**
S mutans *S sanguinis* *S sobrinus* *S oralis*	*B animalis* *Bi adolescentis* *B saguini* *B longum* *B bifidum* *B breve* *B biavatii* *B ruminantium*	*L acidophilus* *L reuteri* *L crispatus* *L plantarum* *L sucicola* *L vaginalis* *L casei* *L fermentum* *L mucosae* *L parafarraginis* *L johnsonii* *L gasseri* *L iners* *L helveticus* *L lactis*	*P namnetense* *P acidifaciens* *P australiense*	*Scardovia wiggsiae*

Disturbance in the total oral microbiota

A genus of microorganisms is disturbed when its abundance changed significantly. In this study, a change of 30% is considered significant. The number of genera that were disturbed by more than 30% was counted:

	Placebo			3.3%pORAL		
Genus	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Total number	135	164	156	124	166	132
Number disturbed	98	110	136	71	93	84
% Disturbed	73%	67%	88%	57%	56%	58%
Average disturbed	76%			57%		

p-value	0,039543	p-value < 0,05: statistically significant difference



Most of the oral microbiome is disturbed with 10% alcohol. This disturbance is significantly reduced with the use of 3.3% preBIULIN ORAL. Which results in an oral microbiome that can better protect the teeth and gums.

Conclusion

preBIULIN ORAL supports through a mouthwash the protective oral microbiome and simultaneously reduces the growth of cariogenic bacteria. The oral cavity remains healthier.

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