AB-BIOTICS

AB:Dentalac®

ORAL COLONIZATION STUDY

March 19th, 2019

Jordi Espadaler, PhD

ABSTRACT

AIM: Primarily was to determine dental colonization by strains *L. brevis* CECT7480 (DSM32822) and *L. plantarum* CECT7481 (DSM32821); and secondarily, the safety and tolerability of the product.

METHODS: A randomized, parallel double-blinded, placebo-controlled intervention clinical trial was designed. A total of 40 volunteers (22 females, 18 males; age range 18-55 years) with healthy gingiva (mean GI < 1.3) were randomly allocated to receive probiotic (n=20) or placebo (n=20) chewing gum, *b.i.d* for 6 weeks. At baseline and 6 weeks of treatment, dental plaque and gum samples were collected, and probiotic colonization was analyzed by real-time PCR. Also, 10 predefined teeth were assessed by a professional dentist for dental plaque in each subject, at baseline and end of intervention (6 weeks). At the end of the intervention, volunteers filled a product satisfaction questionnaire.

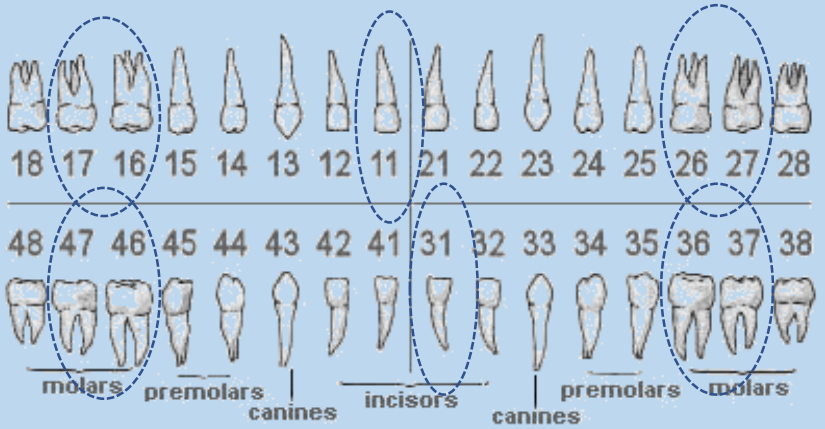
RESULTS: Treatment compliance was high (99%). After 6 weeks, volunteers receiving probiotic had a significant increase of both *L. brevis* ($p = 0.0077$) and *L. plantarum* ($p = 0.0014$) versus placebo. The effect remained significant after adjusting for age and gender. Intensity of aroma/taste was evaluated as lower in the probiotic group compared to the placebo group ($p = 0.0131$) and speed of disappearance of aroma/taste was fast in both groups. No adverse drug reactions were reported following the consumption of probiotic or placebo chewing gum. Of note, there was a 41 % significant net reduction of subjects with dental plaque within the probiotic group (7 of 17, $p = 0.0156$) without any differences by gender, and this reduction was significantly correlated to higher levels of *L. brevis* at study endpoint ($\rho = 0.55$, $p = 0.0215$) but not of *L. plantarum*. Conversely, no reduction in dental plaque was observed in the placebo group (3 of 18, $p > 0.10$).

CONCLUSION: *L. plantarum* CECT7481 and *Lactobacillus brevis* CECT7480 have been shown to are able to colonize the buccal microbiota. Moreover, higher colonization by *L. brevis* correlated to reduction in dental plaque.

STUDY METHODOLOGY

- **Target population:** Volunteers with healthy gingiva (mean GI < 1.3)
- **Exclusion criteria:** Antibiotics within 8 wk, mouthwashes and other probiotics within 4 wk of study initiation. Current orthodontic or periodontal treatment, or more than 2 untreated caries. Current severe chronic disease.
- **Design:** Randomized. placebo-controlled. parallel double blinded clinical trial
- **Intervention:** Probiotic or placebo chewing-gum, *b.i.d*, for at least 15-20 minutes, ≥1h after a meal, for 6 weeks
- **Primary endpoint:** Colonization by AB-Dentalac® bacteria (assessed by species-specific, real-time PCR)
- **Secondary endpoints:** Change in clinical parameters and product satisfaction

Dental pieces evaluated in study subjects



AB-BIOTICS

BASELINE DATA FOR STUDY POPULATION

	PROBIOTIC (n=20)		PLACEBO (n=20)		P-Value
Age (median, range)	30.0	18-55	29.5	18-55	>0.20[A]
Gender (women/men)	14	6	8	12	0.111[B]
Tooth brushing/day (mean, SE)	2.25	0.14	2.15	0.13	>0.20[B]
Plaque Index (mean, SE)	0.18	0.03	0.19	0.06	>0.20[A]
Subjects with Plaque (y/n)	16	4	11	9	0.176[B]
Gingival Index (mean, SE)	0.18	0.03	0.29	0.08	>0.20[A]
LOG *L.brevis*	2.68	0.19	2.63	0.19	>0.20[A]
LOG *L.plantarum*	3.28	0.13	3.13	0.13	>0.20[A]
A) Student T-test; B) Fisher exact test					

- ✓ No significant differences at $p < 0.05$ (nor at $p < 0.1$!) at baseline for demographic, clinical or microbiological data ➔ groups are comparable at baseline
- ✓ Concentration of *L. plantarum* higher than of *L. brevis* at baseline

FOLLOW-UP AND PRODUCT SATISFACTION DATA

	PROBIOTIC			PLACEBO			P-Value
Drop-outs during study period (n)	3			1			>0.20[B]
Treatment compliance (mean, SE)	98.8%	0.9%		98.8%	0.8%		>0.20[A]
Global valuation of product [1-9 scale] (mean, SE)	6.0	0.4		6.5	0.2		>0.20[A]
Product aroma and taste [1-9 scale] (mean, SE)	6.1	0.4		7.0	0.2		**0.0794**[A]
Intensity of aroma/taste (too weak/a bit weak/just right)	6	4	7	0	10	9	**0.0131**[B]
Product texture [1-9 scale] (mean, SE)	5.1	0.5		5.7	0.3		>0.20[A]
Duration of aroma/taste (too short/a bit short/just right)	6	6	5	3	12	4	>0.20[B]
Final sensation [1-9 scale] (mean, SE)	5.9	0.4		6.3	0.3		>0.20[A]

A) Mann-Whitney test; B) Fisher exact test

- ✓ Trend for lower aroma/taste with probiotic vs. placebo
- ✓ Main cause for this difference appears to be the intensity of aroma (too weak), not the texture or duration

FOCUS ON PRODUCT SATISFACTION

Intensity	Too weak	A bit too weak	Just right	A bit too strong	Too strong	P-value
Probiotic	6 (35.3%)	4 (23.4%)	7 (41.2%)	0 (0%)	0 (0%)	P = 0.0131
Placebo	0 (0%)	10 (52.6%)	9 (47.4%)	0 (0%)	0 (0%)	
Speed	**Too slow**	**A bit too slow**	**Just right**	**A bit too fast**	**Too fast**	**P-value**
Probiotic	0 (0%)	0 (0%)	6 (35.3%)	6 (35.3%)	5 (29.4%)	P > 0.20
Placebo	0 (0%)	0 (0%)	3 (15.8%)	12 (63.2%)	4 (21.1%)	

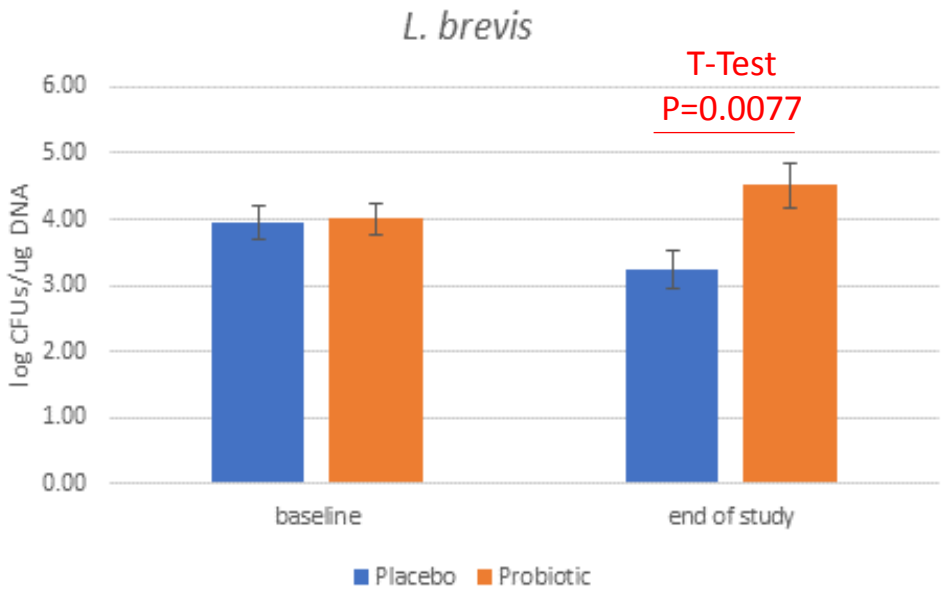
- ✓ Intensity of aroma/taste is on the low side, and the effect is stronger in the probiotic than in the placebo
- ✓ Speed of disappearance of aroma/taste is on the fast side (no differences between probiotic and placebo)

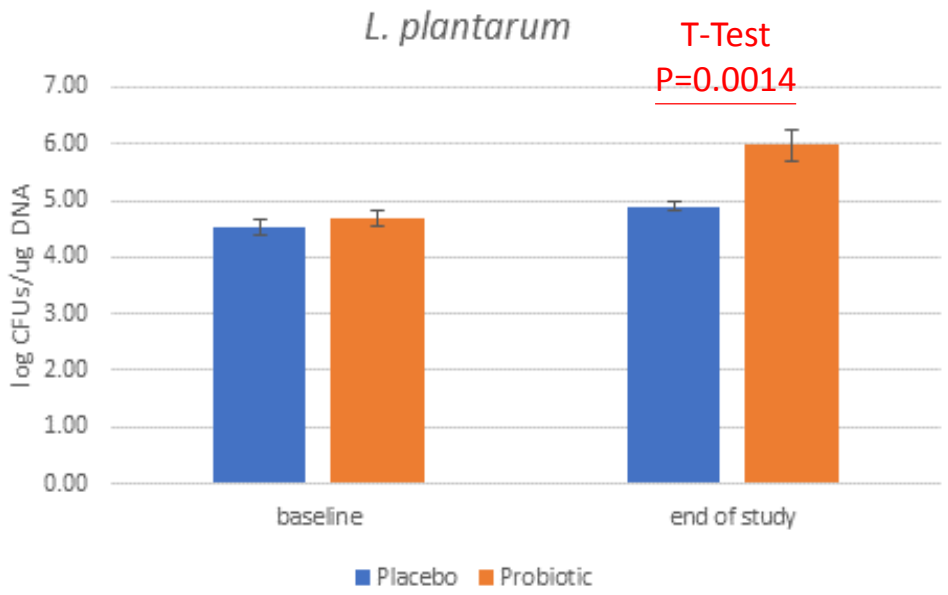
CLINICAL & MICROBIOLOGICAL DATA

	PROBIOTIC		PLACEBO		P-Value
Plaque Index (mean, SE)	0.12	0.04	0.19	0.07	>0.20[A]
Subjects with Plaque (y/n)	7	10	8	10	>0.20[B]
Gingival Index (mean, SE)	0.15	0.04	0.20	0.06	>0.20[A]
LOG *L.brevis*	3.12	0.34	1.99	0.19	0.0077[A]
LOG *L.plantarum*	4.58	0.28	3.50	0.09	0.0014[A]
A) Student T-test; B) Fisher exact test					

- ✓ No significant differences between groups for clinical parameters
- ✓ After 6 weeks, subjects receiving probiotic have higher levels of both *L. brevis* and *L. plantarum*
- ✓ For both Lactobacilli, the difference between groups is of ~1log

COLONIZATION BY AB-DENTALAC STRAINS





- ✓ After 6 weeks, subjects receiving probiotic have higher levels of both *L. brevis* and *L. plantarum* ($p < 0.01$)
- ✓ Repeated measures analysis adjusted for gender is significant for both *L. brevis* and *L. plantarum* ($p < 0.01$)

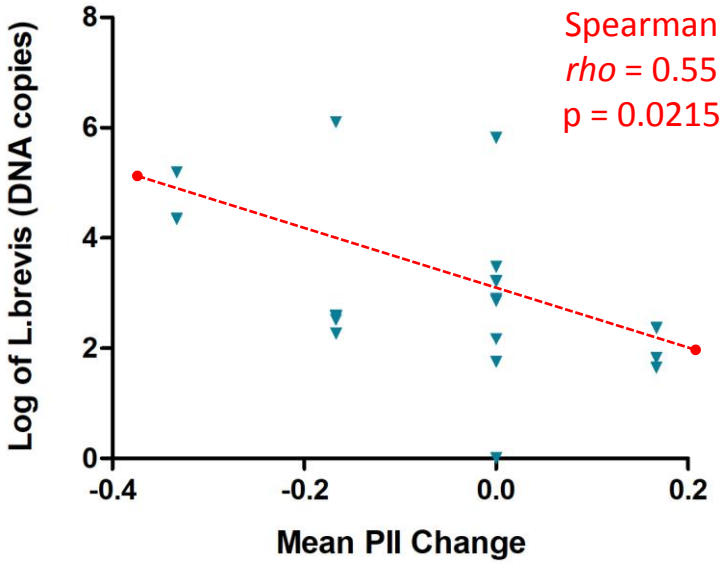
EVOLUTION OF DENTAL PLAQUE WITHIN STUDY GROUPS

Study Group	Plaque Disappears	Plaque de novo	No changes	Net reduction	P-Value*
Probiotic (n=17)	7 (41.2%)	0 (0.0%)	10 (58.8%)	7 of 17	0.0156
Placebo (n=18)	5 (27.8%)	2 (11.1%)	11 (61.1%)	3 of 18	>0.10

*) McNemars exact test

- ✓ Significant net reduction of subjects with dental plaque within the probiotic group
- ✓ Subjects whose plaque disappeared were 2 men (of 6) and 5 women (of 14), thus no difference for gender
- ✓ No significant net reduction of subjects with dental plaque within the placebo group

CORRELATION BETWEEN DENTAL PLAQUE CHANGE AND L.BREVIS



- ✓ In further exploratory analyses, change in dental plaque score (PII) significantly correlated to final levels of *L. brevis* after 6 weeks **in probiotic group only**
- ✓ No other significant correlations observed for baseline levels of *L. brevis*, baseline or final levels of *L. plantarum*, nor in placebo group

SUMMARY

- ✓ No differences between groups at baseline, nor in drop-out or compliance during study
- ✓ Treatment compliance was high in both probiotic and placebo (average 99%)
- ✓ After 6 weeks, subjects receiving probiotic have higher levels of both *L. brevis* ($p = 0.0077$) and *L. plantarum* ($p = 0.0014$). Difference between treatments remains significant after adjusting for gender.
- ✓ Significant reduction of subjects with dental plaque within the probiotic group (7 of 17, $p = 0.0156$) but not in placebo group (3 of 18)
- ✓ Change in mean Plaque Index (PlI) correlates to final levels of *L. brevis* ($p = 0.0215$) in probiotic group
- ✓ Intensity of aroma/taste is on the “low” side, and is lower in probiotic than in placebo ($p = 0.0131$)
- ✓ Speed of disappearance of aroma/taste is on the “fast” side (no significant differences between probiotic and placebo)

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