

Real-time PCR based assessment of colon colonisation by 4 lactobacilli in an orally consumed synbiotic formula

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INTRODUCTION

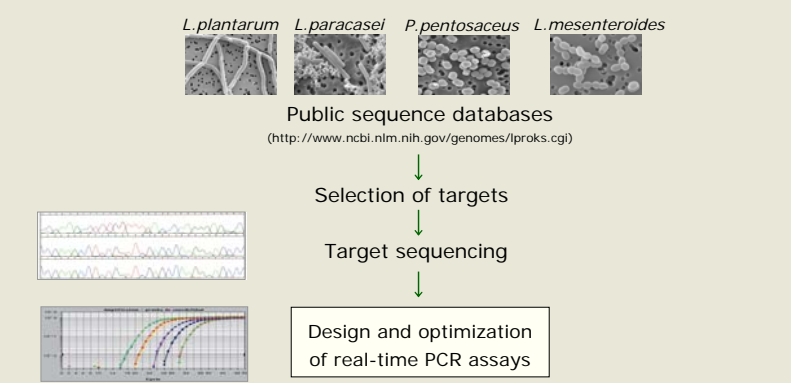
Probiotics and prebiotics have been reported to have beneficial effects on human health in several clinical situations. The synbiotic formula *Synbiotic 2000* (Medipharm) combines 4 different lactic acid bacteria (LAB) strains belonging to *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* species (probiotics) with various vegetable fibres (prebiotics). *In vitro* preliminary studies and pilot tests indicated that this synbiotic composition lead to significant clinical improvement to certain types of ulcerative colitis patients. Prospective assays are currently carried out with patients suffering from active Crohn's disease. These studies include traceability of the 4 LAB strains using quantitative real-time PCR.

OBJECTIVE

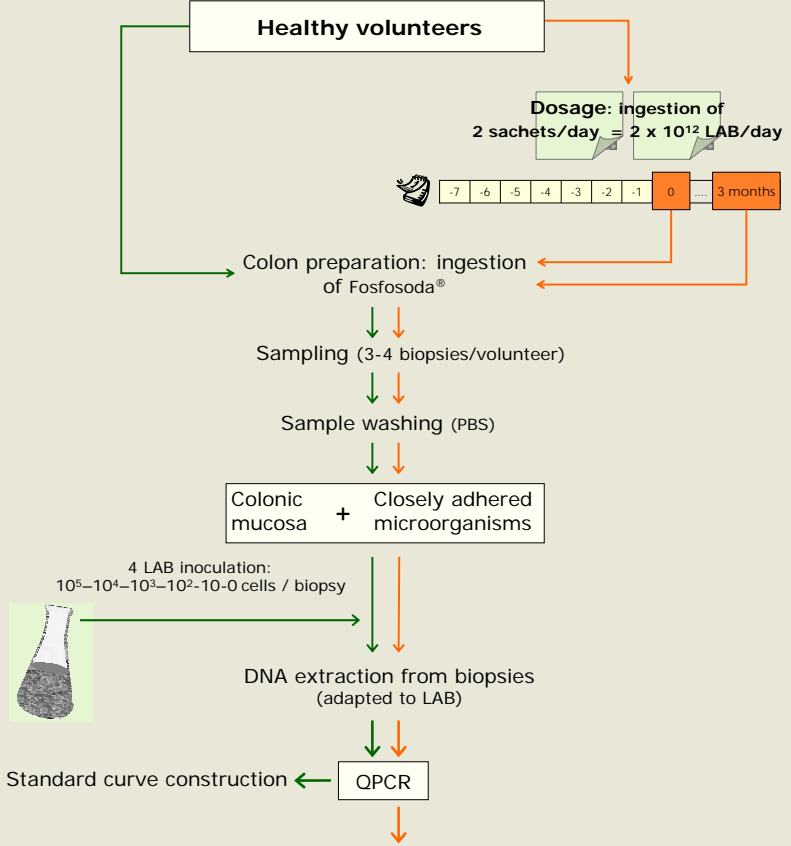
Development and evaluation of real-time PCR based methods for quantitative detection of 4 LAB strains in colon biopsies. Assessment of colon colonization.

METHODOLOGY

Design and optimization of the real-time PCR assays



Quantitative detection of the 4 LAB strains in colon biopsies



EVALUATION OF THE PRESENCE OF THE 4 LAB IN COLON BIOPSIES

CONCLUSIONS

Real-time PCR based analyses of colon biopsies taken from volunteers after ingestion of *Synbiotic 2000* suggested transient colon colonization by the 4 LAB strains. They also indicated lack of permanent establishment upon interruption of their ingestion. Additionally, the method described in this study brings a new tool for on-going clinical studies of the dynamics of probiotics colonization.

RESULTS

Performance of the 4 real-time PCR assays
Efficiency (E); linearity (R²); and limits of detection (LOD) and quantification (LOQ) values obtained with genomic DNA of the 4 synbiotic LAB components.

LAB	Target	E (%)	R ²	LOD	LOQ
<i>L. plantarum</i>	<i>luxS</i>	81.0	0.99	1 (40%)	30
<i>L. paracasei</i>	<i>pcrA</i>	97.6	0.99	1 (70%)	10
<i>P. pentosaceus</i>	<i>gene 1158</i>	98.6	0.99	1 (50%)	30
<i>L. mesenteroides</i>	<i>acetate kinase</i>	98.0	0.99	1 (70%)	10

LOD is expressed as the number of target copies giving positive results. In each case, the percentage of replicate reactions that give a positive result is indicated into brackets. For all QPCR assays, at least 95 % replicate reactions with 3 genomic DNA target copies were positive.

LOQ is expressed as the number of target copies giving quantitative results with precision values below 25% thus complying with the accepted thresholds.

Quantitative detection of the 4 LAB in colon biopsies

LAB	E (%)	R ²	LOD (LAB/biopsy)	LOQ
<i>L. plantarum</i>	85.2	0.99	10 (50%)	100
<i>L. paracasei</i>	99.9	0.99	10 (75%)	100
<i>P. pentosaceus</i>	96.2	0.99	10 (25%)	100
<i>L. mesenteroides</i>	95.8	0.98	10 (75%)	100

The QPCR assays developed for quantitative detection of the 4 LAB exhibit specificity and performance parameters within the accepted values.

The assays are capable to accurately quantify down to 100 target LAB cells/biopsy with detection limit of 10 cells (30% probability).

Assessment of colon

Volunteer 1				
LAB	Biopsy 1	Biopsy 2	Biopsy 3	mean
<i>L. plantarum</i>	1.5x10 ⁵	3.8x10 ⁴	7.1x10 ⁴	8.5x10 ⁴
<i>L. paracasei</i>	9.8x10 ⁴	2.3x10 ⁴	4.9x10 ⁴	5.7x10 ⁴
<i>P. pentosaceus</i>	3.4x10 ⁴	8.2x10 ³	2.5x10 ⁴	2.2x10 ⁴
<i>L. mesenteroides</i>	4.8x10 ⁴	1.5x10 ⁴	1.9x10 ⁴	2.8x10 ⁴
Volunteer 2				
LAB	Biopsy 1	Biopsy 2	Biopsy 3	mean
<i>L. plantarum</i>	1.4x10 ⁵	5.1x10 ⁵	1.9x10 ⁶	8.5x10 ⁵
<i>L. paracasei</i>	6.2x10 ⁴	3.0x10 ⁵	1.0x10 ⁶	4.7x10 ⁵
<i>P. pentosaceus</i>	3.1x10 ⁴	8.9x10 ⁴	3.6x10 ⁵	1.6x10 ⁵
<i>L. mesenteroides</i>	1.9x10 ⁴	2.7x10 ⁴	1.1x10 ⁵	5.0x10 ⁴

The 4 LAB were detected in all biopsy replicates from each volunteer that orally consumed the synbiotic.

Whereas, after 3 months of consuming the last synbiotic dosage none of the 4 LAB was detected.

Analyses of colon biopsies from volunteers that did not consume the synbiotic gave negative results.