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Functional and biotechnological characterization of selected *Lactobacillus* and *Bifidobacterium* strains for their application in dairy products conceived for specific categories of consumers

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INTRODUCTION AND AIM

During the last decades, the selection of tailored cultures has been developed in order to design functional foods, conceived as food strategy to increase the *well-being of specific types of consumers*.

In this framework, the first two years of research have been focused on the biotechnological and functional characterization of selected ***Lactobacillus*** and ***Bifidobacterium*** strains, isolated from **breast milk** and **vagina** of healthy women to include them into foods designed for infants and women in order to deliver specific functionalities **(A).**
Another aim of my work was to evaluate the potential of ***spray-drying*** as a technological approach to vehiculate selected strains into specific **functional foods** **(B)**.

MATERIALS AND METHODS

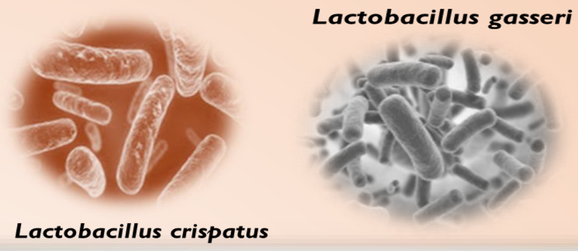
A)

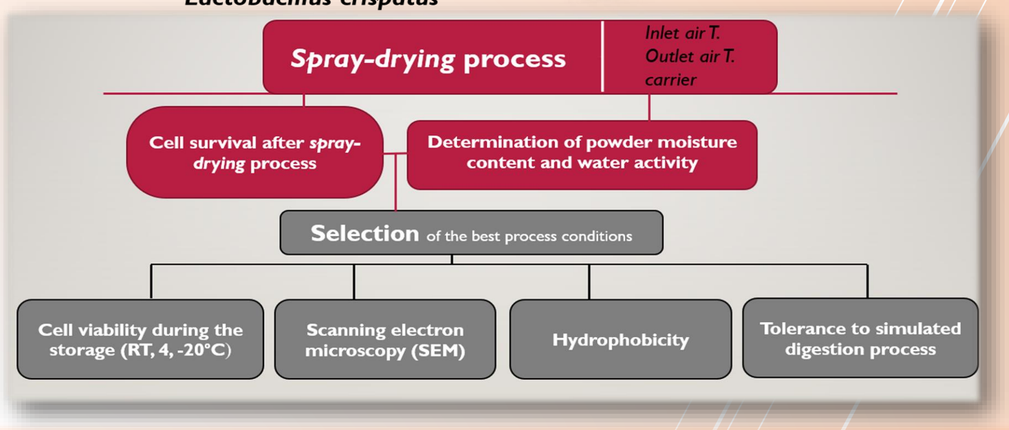
Strains	*Species*	*Isolation source*
BC1, BC3, BC4, BC5, BC6, BC7, BC8	*L. crispatus*	*Female genital tract*
BC9, BC10, BC11, BC12, BC13, BC14,	*L. gasseri*	*Female genital tract*
BC16, BC17	*L. vaginalis*	*Female genital tract*
3.6D, 11.3 C, M6C, 29.0L, 31.0 C	*L. plantarum*	*Breast milk*
32.0 C, 33.1 G, 34.0 B, 35.0 B.bis, 30 b6A	*L. plantarum*	*Breast milk*
32.0 A, 34.0 C, g.1, CFl11	*L. gasseri*	*Breast milk*
32.0 B.bis	*B. longum*	*Breast milk*
BL6	*B. longum*	*Breast milk*

METABOLIC POTENTIAL: Biolog phenotype microarray analysis **[1]**
SAFETY ASPECTS: antagonistic activity**, antibiotic** susceptibility **[2]**
FUNCTIONALITY: hydrophobicity, **auto-aggregation**, **Caco-2 cells,** simulated **digestion process [1]**

B)

Microencapsulation by spray-drying on mixed cultures





RESULTS

A)

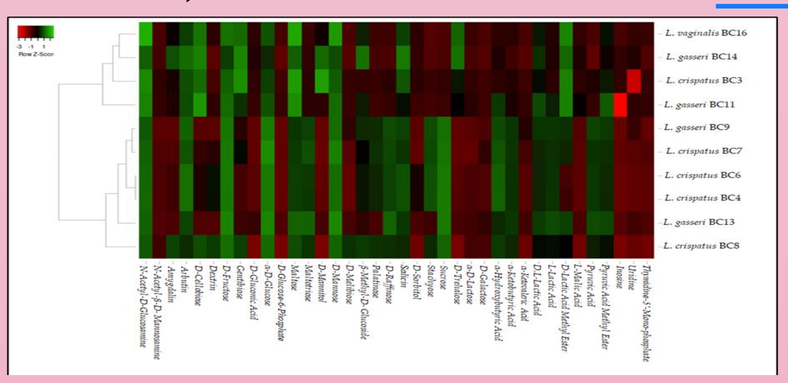


Fig. 1 A. Heat map showing the **metabolic differences** among selected strains with green referring to high catabolic activity and red to low activity.

Most of these strains were able to catabolise D-cellobiose → potential PREBIOTIC activity

B)

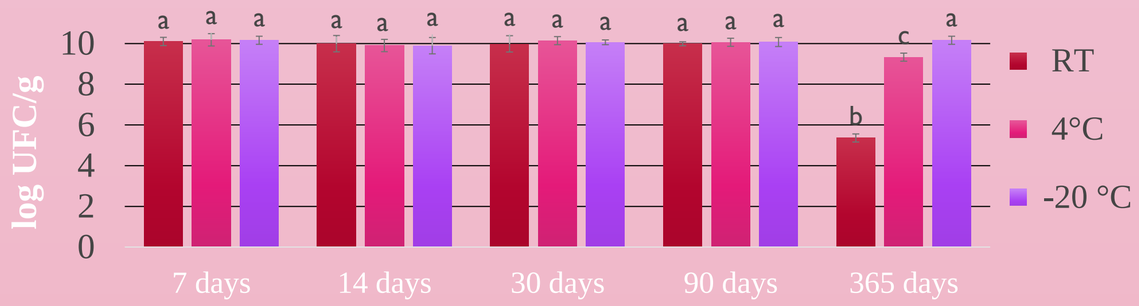


Fig. 1 B. Cell loads (log UFC/g) of *L. crispatus* BC1 + *L. gasseri* BC9 microencapsulated after 7, 14, 30, 90, 365 days of storage (RT, + 4 ° C and -20 ° C).

Fig. 2 A. Antibiotic susceptibility

Amoxicillin, Ampicillin, Erythromycin → High bactericidal effect → **Vaginal strains**

Tetracycline, Erythromycin, Clindamycin → High bactericidal effect → **Strains from breast milk**

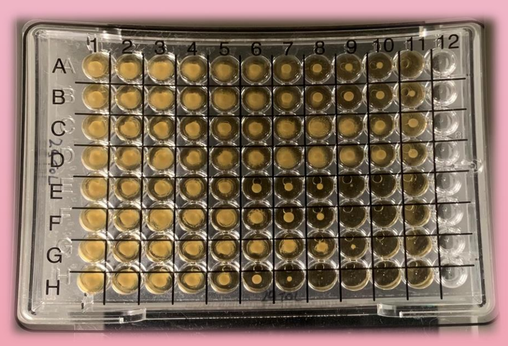
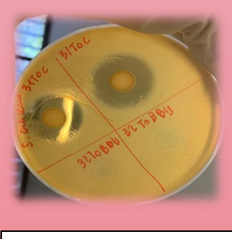


Fig. 3 A. Antagonistic activity of selected strains vs. pathogens



Most of these strains showed the **highest antagonistic activity** (inhibition 6-10 mm) vs.: ***L. monocytogenes* Scott A, *E. coli* 555, *S. aureus* DSM 20231, *E. coli* ECET H10407, *S. enterica* and *Y. enterocolitica.*** Only ***L.gasseri* BC11-BC12**, ***L. vaginalis* BC11** and ***B. longum* 32.0 B.bis** showed the lowest inhibitory activity (≤ 6-1 mm) vs. ***S. aureus* DSM 20231**

As regards to **FUNCTIONAL PARAMETERS,** most of these strains have demonstrated high values of **hydrophobicity** and **auto-aggregation** (over 70 %) and good **resistance to digestion process,** considering *L. rhamnosus* GG as reference (*data not showed*)

On the other hand, strains from breast milk have revealed higher values of adhesiveness than vaginal strains

Strains	*Isolation source*	*Adhesion (lb. cells/Caco-2 cell)*
BC1, BC3, BC4, BC5, BC6, BC7, BC8	Vaginal tract	**0.63; 0.45; 0.24; 0.43; 0.74; 5.14**
BC9, BC10, BC11, BC12, BC13, BC14,	Vaginal tract	**0.26; 0.27; 0.75; 0.15**
BC16, BC17	Vaginal tract	**0.34; 2.32**
3.6D, 11.3 C, M6C, 29.0L; 31.0C	Breast milk	**22.88; 14.29; 25.71; 34.18;24.71**
32.0 C, 33.1 G, 34.0 B, 35.0 B.bis, 30 b6A	Breast milk	**21.17; 20.18; 28.70; 17.11;24.84**
32.0 A, 34.0 C, g.1, CFl11	Breast milk	**14.68; 27.73; 13.94; 4.39**
32.0 B.bis	Breast milk	**22.52**
BL6	Breast milk	**13.7**

Future goal of my third-year PhD :
Production of functional foods, including dairy, for infants and women, characterized also for their interaction on specific microbiome using human model systems.

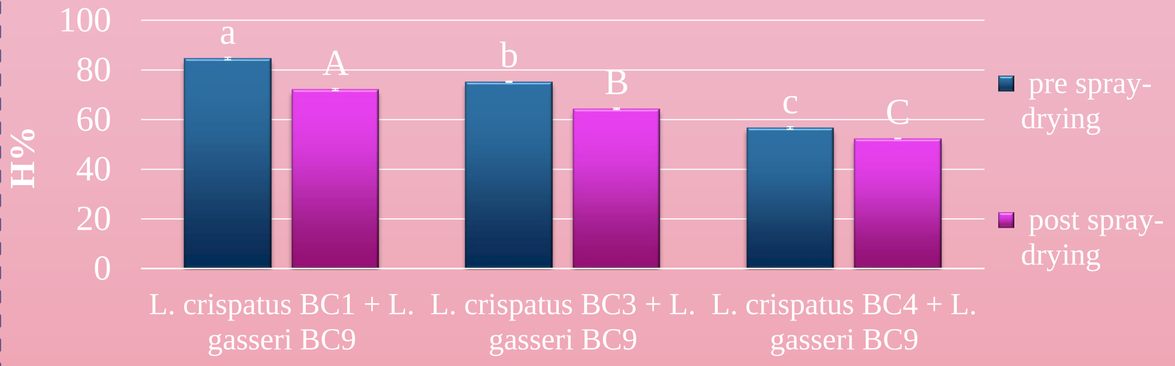


Fig. 2 B. Cell hydrophobicity of pre and post spray-drying samples.

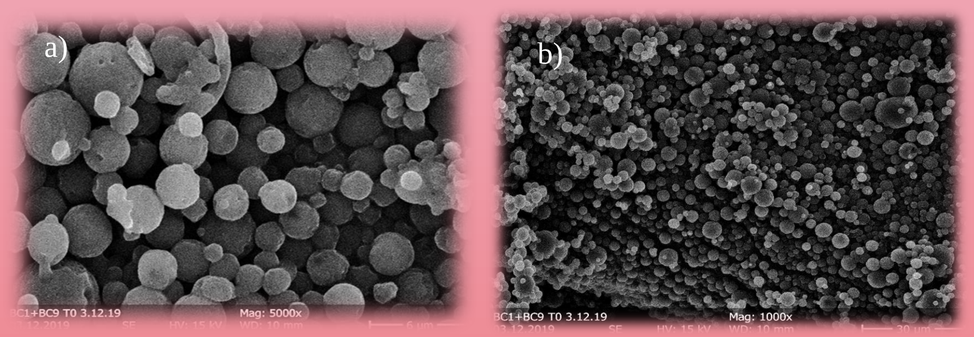


Fig. 3 B. SEM images of microparticles of *L. crispatus* BC1 + *L. gasseri* BC9 : a) at x 5000 magnification, b) at x 1000 magnification

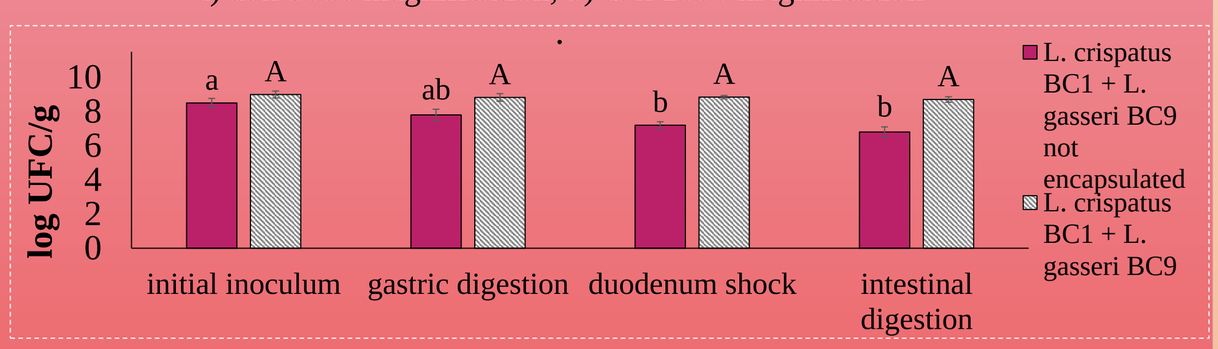


Fig. 4 B. Cell loads (log UFC/g) of *L. crispatus* BC1+ *L. gasseri* BC9 unencapsulated and microencapsulated **after stomach-duodenum passage.**

References:[1] D'Alessandro, M., Parolin, C., Bukvicki, D., Siroli, L., Vitali, B., De Angelis, M.,& Patrignani, F. (2021). Probiotic and Metabolic Characterization of Vaginal Lactobacilli for a Potential Use in Functional Foods. *Microorganisms*, *9*(4), 833. **[2]** Siroli, L., Patrignani, F., Serrazanetti, D. I., Parolin, C., Ñahui Palomino, R. A., Vitali, B., & Lanciotti, R. (2017). Determination of antibacterial and technological properties of vaginal lactobacilli for their potential application in dairy products. *Frontiers in Microbiology*, *8*, 166.