Genotypic and phenotypic characterization of different probiotic strains of *Lactobacillus* spp. and *Bifidobacterium* spp.

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INTRODUCTION

The beneficial effects of probiotic strains on human health are well documented. One such benefit is the capacity of certain probiotic strains to decrease serum cholesterol levels in the presence of bile salts, via activity of the bile salt hydrolase (BSH) enzyme. Another benefit is associated with the capacity several such strains may have in producing CLA, a compound associated with anticarcinogenic and antiatherogenic effects in animal models, as well as immunomodulating and fat reduction activities, due to the presence of conjugated linoleic acid (CLA) isomerase.

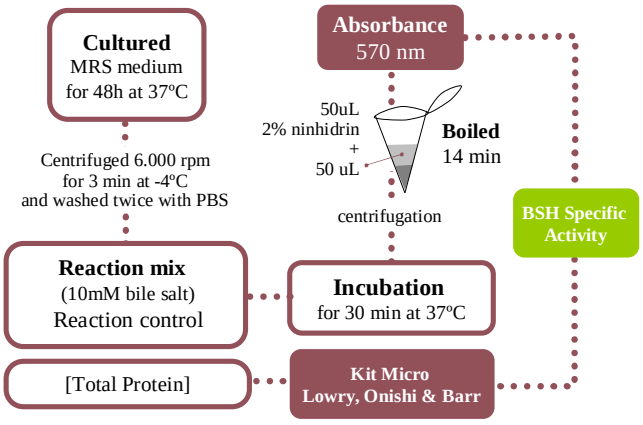
There are many tools to evaluate these functional properties, including conventional methods – associated to phenothypic characterization –, and other emergent technologies like molecular biology.

Hence, the objective of this study involved the genotypic and phenotypic characterization of 10 strains of microorganisms selected from *Lactobacillus* and *Bifidobacterium* spp., in what concerns, several of the abovementioned biochemical parameters, including BSH and CLA isomerase, and discuss the relationship and the potential of different methods to detect probiotic features.

Materials & Methods

Cultures were routinely subcultured in De Mann-Rogosa-Sharpe (MRS) medium and incubated at 37 ºC under anaerobic conditions.

•**Bile salt hydrolase assay** was carried out by the ninydhrin method according to Sridevi et al. (2009), with some modifications – explained briefly in the following scheme:



•**Protein sequences** were obtained by NCBI search, primers were designed by *Oligoperfect designer* (Invitrogen, USA) and synthetised by Eurofins MWG Operon (Ebersberg, Germany).

•**Extraction of DNA** was carried out using the guanidine isothiocyanate method described by Cunny and Witte (1996).

•**Production of CLA** in ewe's and goat's milks inoculated with single cultures of *B. animalis* Bo or mixed cultures of either *B. animalis* Bo+*L.acidophilus* Ki and *B. animalis* B94 + *L. acidophilus* Ki, was assessed by GC-MS (Perkin-elmer)

References

Sridevi N, Vishwe P, Prabhune A (2009) Hypocholesteremic effect of bile salt hydrolase from *Lactobacillus buchneri* ATCC 4005. Food Research International. 42:516-520.

Cuny C, Witte W (1996) Typing of Staphylococcus aureus by PCR for DNA Sequences Flanked by Transposon Tn916 Target Region and Ribosomal Binding Site. Journal of Clinical Microbiology, 34:1502-1505.

Acknowledgments

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Results & Discussion

The results for BSH activity – defined as the amount of enzyme needed to release 1 nmole of amino acid per minute, registered by each strain are shown in Figure 1.

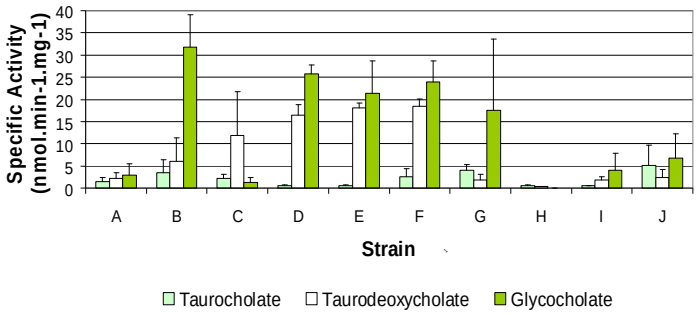

Figure 1. BSH specific activity for the strains under study. (A) *L. acidophilus* Ki, (B) *L. acidophilus* L10, (C) *L. acidophilus* ATCC 4356, (D) L. *plantarum* LMG 519557, (E) *L. pentosus* LMG 10755, (F) *L. brevis* LMG 6906, (G) *L. paracasei* LAFTI L26, (H) *B. animalis* Bb12, (I) *B. animalis* Bo, (J) *B. animalis* B94.

Glycocholate induced the highest specific activities. *Bifidobacterium animalis* Bb12 showed the lowest activity, whereas *Lactobacillus acidophilus* L10, *L. pentosus* LMG 10755 and *L. brevis* LMG 6906 showed the highest activity.

Bioinformatic and PCR analysis using orthologous primers were further conducted (see Figures 2 and 3) in order to evaluate the effect of genetic similarities and enzymatic activities.

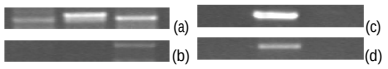

Figure 2. Electrophoresis gel for PCR primers (a) LaHsb - L10, Ki e ATCC; (b) LbreHsb – Bre, Pent, Plant; (c) BaHsb – Bb12, B94 e Bo; (d) LplantHsb – Plant, Pent, control.

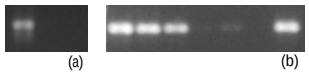

Figure 3. Electrophoresis gel for PCR primers (a) LaLin: - Ki, L10, ATCC; (b) LpLin: Plant, Bre, LMG, LP, Bo, Bb12, B94.

Both genotypic and phenotypic studies were conducted to evaluate CLA parameters, as shown in Table 1 and Figure 4.

Table 1. Concentration of CLA isomers (µg/g) in fermented milks

		Ewe milk		Goat milk	
		c9, t11 18:2	t10, c12 18:2	c9, t11 18:2	t10, c12 18:2
Bo	0d	ND	ND	ND	ND
	14d	0,020 ± 0,001	0,032 ± 0,001	0,018 ± 0,001	0,030 ± 0,001
	21d	0,415 ± 0,001	0,073 ± 0,001	0,352 ± 0,001	0,062 ± 0,001
Bo+Ki	0d	ND	ND	ND	ND
	14d	0,021 ± 0,001	0,033 ± 0,001	0,019 ± 0,000	0,030 ± 0,000
	21d	0,416 ± 0,001	0,074 ± 0,001	0,353 ± 0,001	0,064 ± 0,000
B94+Ki	0d	ND	ND	ND	ND
	14d	0,026 ± 0,001	0,037 ± 0,000	0,019 ± 0,000	0,028 ± 0,000
	21d	0,420 ± 0,001	0,078 ± 0,001	0,351 ± 0,001	0,063 ± 0,001
Control	0d	ND	ND	ND	ND
	21d	ND	ND	ND	ND

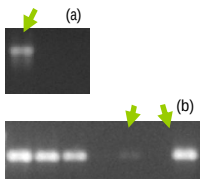

Figure 4. Electrophoresis gel for PCR primers (a) LaLin: Ki, L10, ATCC; (b)LpLin: Plant, Bre, LMG, LP, Bo, Bb12, B94.

The results of GC-MS showed that both ewe's and goat's fermented milks induced rising CLA values in the presence of probiotics, in particular by *B. animalis* strains. No significant differences between matrices were detected.

Conclusions

BSH enzyme activity was strongly strain dependent and CLA production was not apparently different between strains. Data suggest a strong evolutionary relationship between sequences, probably due to genetic conservation amongst different probiotic species and strains.

Similarities between genotypic and phenotypic analysis were found, but for the most part detection of the gene and/or evolutionary closeness did not correlate with enzymatic activities registered.

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