

Studies on Probiotics Properties of Two Lactobacillus Strains

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ABSTRACT

"In vitro" studies were carried out for the selection of Lactobacillus strains with probiotics characteristics. Two strains of Lactobacillus were evaluated for the growth specific rate, generation time, acids, bile and antimicrobial resistances. "In vitro" tests indicated that the strains presented a potential for being used as probiotics.

Key words: Probiotics, Lactobacillus, selection

INTRODUCTION

The development of products with probiotic characteristics surges as a necessity of substituting the employment of antibiotics in animal feeding which are used to maintain a good balance of the gastrointestinal tract (GIT) microflora and to eliminate pathogenic microorganisms, facilitating the reduction of gastrointestinal upsets very frequent in animals. However antibiotics not only contribute to the destruction of beneficial gastrointestinal microflora but also produce residual effects in tissues and products of animal origin such as meat, eggs and milk. (Smoragiewicz *et al.*, 1993).

Probiotic has been defined as a live or dead organism from *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus* and *Saccharomyces* genera as well as microbial fermentation products, nucleotides and its metabolizable products, metabolites and organic acids (Ouweland *et al.* 1999).

Probiotics have been administered to animals in order to prevent infectious illness, reinforcing the barrier function of the intestinal flora or increasing the immune system. (Pascual *et al.*, 1996).

Most probiotics contain lactic acid bacteria, because they are components of the natural microflora of almost all organisms, are rarely pathogenic and present antagonistic properties against pathogenic microorganisms. (Fuller *et al.*, 1992)

Newly born piglets possess a relatively rich and diverse components of the natural microflora of almost all organisms, are rarely pathogenic and present antagonistic properties against pathogenic microflora in their GIT occurring bacterial concentrations of 10^7 - 10^9 microorganisms per gram of digesta in the small intestine. Lactic acid bacteria, mainly *Lactobacillus* and *Streptococcus* genera dominate the microflora. However modern methods of postnatal care limit the contact with the mother, keeping the animals under artificial conditions

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causing disturbs in the flora responsible for the resistance to illness. The use of probiotics in newly born piglets pretends not only to correct this deficiency without creating any other problems but also to re-establishing the full protective capacity of the GIT microflora.

Probiotics should be resistant to specific conditions occurring on the GIT, thus should be resistant for more than 4 hours to proteolytic enzymes, low pH values (1.8-3.2) prevailing in the stomach and to bile concentration, pancreatic juices and mucus which are part of the small intestine. Furthermore, bacterial strains to be used in probiotics obtention are supposed to be resistant to antibiotics eventually administered in animal diets and, are also to be a producers of antimicrobial substances such as lactic acid, hydrogen peroxide, bacteriocins, etc. (Gorbach & Goldin, 1991; Hoyos, 1997)

The aim of this study was to evaluate the “in vitro” capacity of two *Lactobacillus* strains (LB-12 and B/103-1-5) for their application as probiotics in animal feeding.

MATERIAL AND METHODS

Microorganisms and culture medium: The study was carried out using *Lactobacillus* sp. strain (LB-12) and *Lactobacillus acidophilus* strain (B/103-1-5) from ICIDCA Culture Collection. For counting as well as for cultivation, either the solid or liquid MRS medium (Man *et al.*, 1960) was used. MRS formulated with tomato juice, acetic acid and Oxgall Bile (MRS[®]) was employed for tolerance to bile assay (Gorbach *et al.*, 1991)

Specific growth rate and generation time determination: Culture were grown in MRS medium (700 mL in 1 L Erlenmeyer flasks) by inoculating with the cells at 10% (v/v) and incubating at 37°C for 24 hours under partial CO₂ (25%) atmosphere. Samples for estimating the growth and viable cells count were taken every two hour. Growth was estimated by dry weight measurement, and data were processed using lineal regression analysis.

Lactobacilli acid tolerance assay: Acid tolerance was tested using 30 mL MRS medium contained

in 250-mL erlenmeyers. The initial pH value was fixed at 3.0 with HCl 1N. Flasks were inoculated as above. Samples were taken at 0, 12 and 24 hour to determine viable cells. Simultaneously a control at optimal growth pH was developed.

Bile tolerance assay: Bile tolerance was tested as above for acid tolerance.

Resistance to antimicrobial substances assays: Solutions with antimicrobial substances in the following concentrations were prepared: furasolidona (10 mg/10mL), baijonot (10mg/10 mL), nitrovin (20mg/100 mL), cooper sulphate (20mg/10 mL) and dimetridazol (100 mg/10 mL). Test tubes of 8 mL of capacity containing MRS medium were inoculated with 16 h old culture and after adding 1 mL antimicrobial solutions were incubated for 24 hour employing the same conditions as above. Microbial growth (by optical density analysis) and viable cells counting were done at the end of the cultivation. Cells were also cultivated in a 5L Marubishi fermentator with 3L of MRS medium under the similar conditions of pH, temperature and inoculation.

Assays: Dry weight was carried out by centrifugation, and drying at 60°C until constant weight. Viable cells counting was performed by the serial dilution method and depth inoculation on MRS-agar media. Plates were incubated anaerobically at 37°C for 48 hours. Optical density measurements were made in a digital spectrophotometer (LKB) at 650 nm was used and total reducing sugar was done by 3,5 dinitro salicylic acid method. (Miller, 1959).

RESULTS AND DISCUSSION

Specific growth rate and generation time:

Table 1 - Specific growth rate and generation time of LB-12 and B/103-1-5 strains at laboratory level.

Strain	(μ), h ⁻¹	Correlation Coefficient	T _G (h)
LB-12	0.5480	0.9715	1.2
B/103-1-5	0.4930	0.9871	1.4

μ : Specific growth rate T_G: Generation time

Specific growth rate and generation time of both strains are shown in Table 1. Strain LB-12 showed higher specific growth rate and thus minor generation time under the established experimental conditions. These values are comparable to those reported by Gorbach & Goldin, (1991) who reported generation time of one hour for lactobacilli.

Acid and bile tolerance: It is important that the probiotic microorganisms are able to reach the GIT and remain viable there for 4 h or more (Marshall, 1997). Figure 1 shows the results corresponding to acid tolerance assay at pH 3 for the strains. Strain LB-12 was able to achieve small cell concentration increments during 24 h of fermentation. Strain B/103-1-5 showed a lower viable cell counting during the same fermentation time. However it maintained an acceptable final cell concentration level. These results are comparable to those reported by Conway (1987).

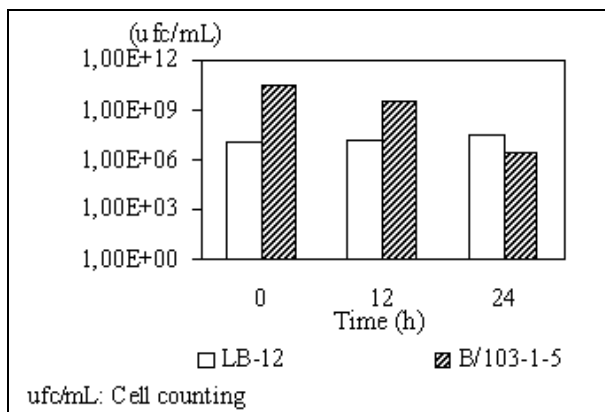


Figure 1 - Stability of LB-12 and B/103-1-5 strains in acid presence at laboratory level.

Bile addition effects on the evaluated strains are represented in Figure 2. Strain LB-12 was able to grow and stay viable in the presence of bile although a diminishing of cell concentration was observed when this behaviour was compared with the control. Nevertheless, the manifested behaviour allowed to predict the potentiality of this strain as a probiotic, since it was able to grow in a bile concentration similar to those existing in the small intestine.

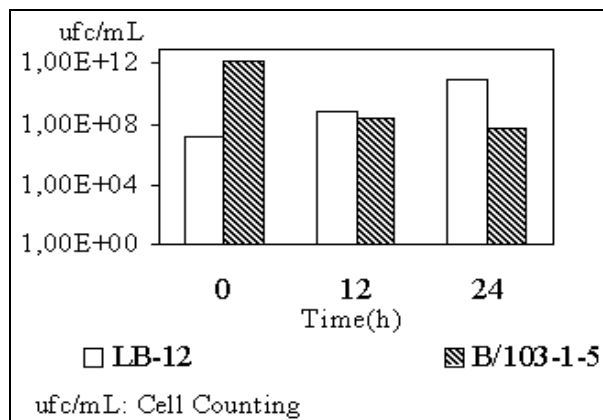


Figure 2 - Stability of LB-12 and B/103-1-5 strains in bile presence at laboratory.

On the other hand, strain B-103-1-5 showed a different behaviour, diminishing its cell concentration during the microbial growth period; however, the final cell concentration value was adequate for the utilisation of this strain as probiotic.

Resistance to antibiotics: Figure 3 shows the optical density determinations. The behaviour of the strain B/103-1-5 appeared very similar to those obtained by viable cells counting, showing a maximal O D. after 12 h of cultivation followed by a decrease in the absorbance during the remaining incubation time. These results coincided to those reported by Mattila-Sandholm (1999) who described that many *Lactobacillus* strains were able to survive in the presence of antibiotic.

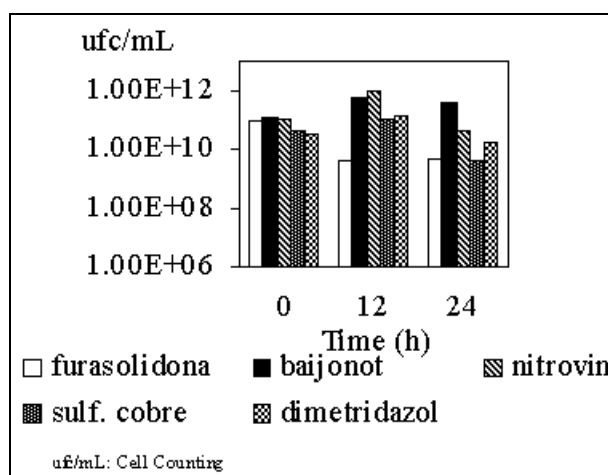


Figure 3 - Stability of B/103-1-5 strain in antimicrobial substances presence

The presence of Baijonot produced a viable cells increment during the first 12 hours of fermentation, then the culture stayed practically stable until the end of the fermentation period. The rest of the evaluated antimicrobial substances influenced B/103-1-5 culture in a similar way to that of Baijonot.

Table 2 shows the results corresponding to the parameters estimated from the growth kinetic of both evaluated strains in fermentators. It was observed that the values obtained were in coincidence with the results at laboratory level.

Table 2 - Specific growth rate and generation time of LB-12 y B/103-1-5 strains at fermentators scale.

Strain	(μ), h ⁻¹	Correlation Coefficient	T _G (h)
LB-12	0.5435	0.9940	1.2
B/103-1-5	0.5120	0.9850	1.3

μ : Specific growth rate T_G: Generation time

Figure 4 shows the growth curves for the two strains in the presence of acid. It was observed that between 8 to 12 h, both strains possessed an adequate behaviour referred to the proposed objective. Strain LB-12 exhibited a superior behaviour in relation to growth rate, productivity and cell concentration when it was compared with strain B/103-1-5.

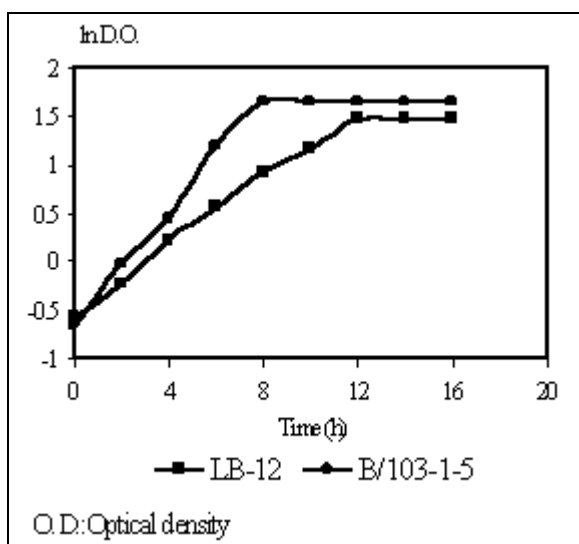


Figure 4 - Growth of LB-12 and B/103-1-5 strains in acid media at fermentators

The results achieved in relation to the growth rate,

generation time, tolerance to acid and bile and antimicrobial substances indicated that the LB-12 and B/103-1-5 strains presented a good potential for being used as probiotics.

RESUMO

Foram realizados estudos “in vitro” para selecionar cepas de *Lactobacillus* com características probióticas. Duas cepas de *Lactobacillus* foram avaliadas quanto as características específicas que definem sua potencialidade como probióticos (velocidade específica de crescimento, tempo de geração, e resistência a ácido, bile e substâncias antimicrobianas). Os testes “in vitro” indicaram que ambas as cepas apresentaram um grande potencial para ser utilizadas na obtenção de probióticos.

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