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IN VITRO EVALUATION OF PROBIOTIC PROPERTIES OF A LACTIC ACID BACTERIA ISOLATED FROM PIGLET

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Abstract

The aim of this study was to evaluate the probiotic aspects of lactic acid bacteria (LAB) isolated from faeces of a healthy piglet (30-d-old, Topigs). Cultural, morphological and biochemical characteristics by standard API 50 CHL Biomerieux strips of isolated strain was assayed as well. The strain was conserved as *Lactobacillus fermentum* 1 IBNA 95 and can be found in the collection of INCDBNA. It is a Gram positive, non-spore forming, aerotolerant bacteria. It appears isolated and grew at 37°C in Oxoid MRS broth and MRS agar medium. The functional properties tested were to survive at pH 3.0 and to present resistance at addition of 0.3% bile salts (w/v). The strain was able to survive at a low pH value and to tolerate 0.3% bile salt solution during 3h exposure under according simulated gastrointestinal condition. *Lactobacillus fermentum* 1 IBNA 95 was presenting some probiotic features, thus can be a promising feed supplement for piglet's nutrition.

Key words: LAB, probiotic, weaning piglet, pH, bile salts

INTRODUCTION

The piglet's microbiota from gastrointestinal tract (GIT) is unstable and this characteristic can lead to frequent diseases such as diarrhea or even death [3]. Across the growth stages of pigs, health improvement and homeostasis installation were made with the help of feed additives which contains *Lactobacillus* spp. [7]. Previous studies have demonstrated the beneficial use of some *Lactobacillus* species as an alternative to antibiotics. Some antibiotics have been shown to cause harm in animal health, while using probiotic microorganisms is more feasible considering that they are environment friendly, natural and inexpensive [1]. To be characterized as a probiotic, a lactic acid bacteria must fulfill a series of requirements such as: they should be generally recognized as safe [1]; they should be tolerant at acid and bile salts [2]; they should be able to keep their viability during storage and processing [2, 3].

Therefore, this study objective is to evaluate some probiotic properties of *Lactobacillus fermentum* 1 IBNA 95 in order to obtain a feed additive for piglet's nutrition.

MATERIALS AND METHODS

Lactobacillus fermentum 1 IBNA 95 used in this study was isolated from faeces of a healthy piglet (30-d-old, Topigs) and incubated in stationary state at 37°C, for 24 h in MRS broth medium (Oxoid). The isolated strain was stored at -80°C in MRS medium with 20% glycerol.

At the optical microscope the strain appears short or medium in size Gram positive bacilli, straight, with straight ends, non-sporulated, arranged isolated, in diplo or in short chains.

On MRS agar the strain form big colonies, 2.0-3.0 mm in diameter, semi-transparent, S tip, round, with regular edges. On MRS broth the strain shows a moderate turbidity, homogenous, a very low and scattered deposit without surface formations.

The isolate was identified using apiweb API 50 CHL V5.1, resulting *Lactobacillus fermentum* 1, 95,9% ID.

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Acid tolerance

The method used was modified from that of Shokryazdan *et al.* [5]. Cells from a 24 h culture were inoculated into MRS broth medium (Oxoid) which was adjusted to pH 3 with HCl 1N 37% and incubated anaerobically for 3 h, at 37°C. The control was represented by a 24 h culture inoculated into MRS broth adjusted to pH 7. From the culture inoculated into MRS broth adjusted to pH 3 tenfold serial dilutions up to 10^{-6} were performed using PBS (phosphate-saline buffer) at time 0, 1:30h and 3 h for simulating the time that the bacterial cells cross the gastrointestinal tract of the piglet and to quantify the evolution of bacterial culture development. From 10^{-4} , 10^{-5} and 10^{-6} dilutions of each time interval, 100 μ l were spread-plated on MRS agar medium and incubated anaerobically at 37°C for 24 h. After incubation, colonies on the plates were counted and CFU/ml was calculated. Acid tolerance of *Lactobacillus fermentum* 1 was determined by comparing the values obtained after exposure to acidic and normal conditions.

Bile salts tolerance

For this assay Shokryazdan *et al.* [5] method with modifications was used. 1 ml of 24 h bacterial culture was inoculated into MRS broth medium with or without (control) 0.3% oxgall (bile salts) and incubated anaerobically at 37°C, for 3 h. Tenfold serial dilutions up to 10^{-6} were prepared using PBS for time 0, 1:30 h and 3 h. Then 100 μ l of 10^{-4} , 10^{-5} and 10^{-6} dilutions of each time interval

were spread-plated on MRS agar and incubated anaerobically at 37°C, for 24 h. Rate of survival of *Lactobacillus fermentum* 1 was quantified comparing CFU/ml values after exposure at bile salts and normal conditions.

RESULTS AND DISCUSSION

Acid tolerance

For determining the acid tolerance of *Lactobacillus fermentum* 1, the control was represented by bacterial inoculum grew up into MRS broth adjusted to pH 7. After 48 h in anaerobically conditions at 37°C, 48 h, the *Lactobacillus fermentum* 1 IBNA 95 registered 7.43×10^8 CFU/ml. Our strain grows very well at normal pH. The ability of survival under low pH value of our isolate strain was examined, as well.

This examination included the resistance to pH 3, results which are shown in Figure 1. Analyzing the obtained data it can be observed that *Lactobacillus fermentum* 1 grows very well from a quantitative point of view and resist at acidic conditions. Melo *et al.* [4] analyzed a bacterial strain of *Lactobacillus fermentum* TCUESC01 with a similar method and the results have highlighted a development of cells in acidic conditions. The bacterial strains develop different degrees of sensibility by passed the GIT [8]. According to Dumitru *et al.* [9] to have good results, a bacterial strain to be used as a probiotic must to survive to the acid from the stomach.

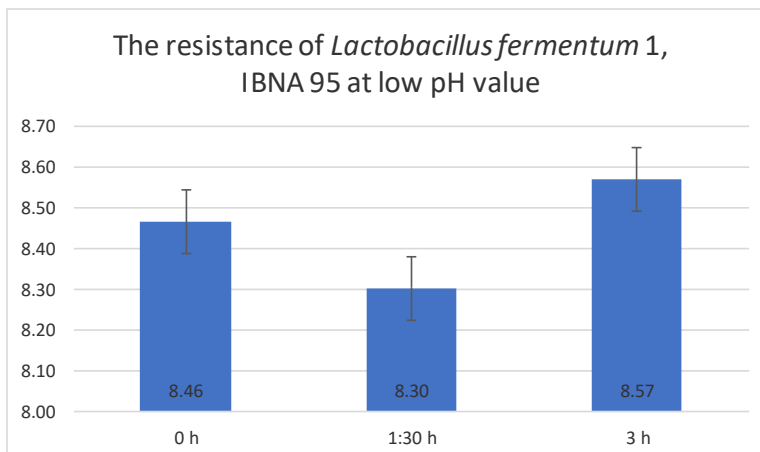


Fig. 1 The evolution of *Lactobacillus fermentum* 1, IBNA 95 at low pH, during 3 h of incubation

Bile salts tolerance

The results obtained by this *in vitro* test are presented in Figure 2. This assay revealed that

Lactobacillus fermentum 1 is resistant and grows up in MRS broth with 0,3% oxgall. The viability of the bacterial cells decreases when they are subject to bile salts, however they are able to grow in this environmental conditions thus *Lactobacillus*

fermentum 1 can survive through piglet's gastrointestinal tract. Tulumoğlu *et al.*[6] have proven that this strain can tolerate high bile salts concentrations from 0,25% to 1% and it can be viable in the gastrointestinal tract. According to Jain *et al.* [10], survival in bile salts is an important properties of lactic acid bacteria, for know if the addition of a bacterial probiotic can perform in the GIT.

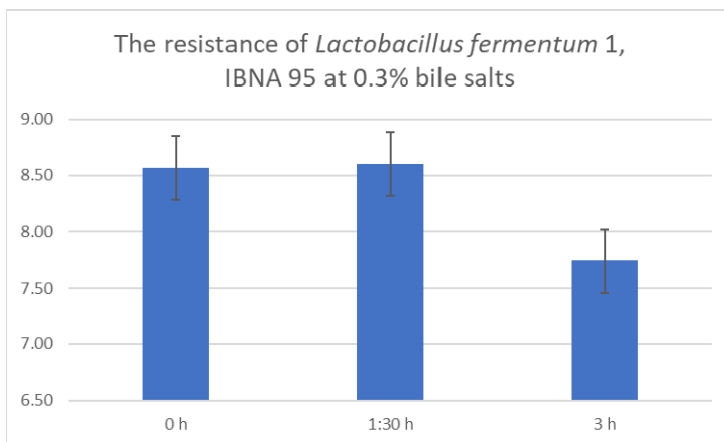


Fig. 2 The evolution of *Lactobacillus fermentum* 1, IBNA 95 at 0.3% bile salts, during 3 h of incubation

CONCLUSIONS

It can be concluded that *Lactobacillus fermentum* 1 IBNA 95 is able to survive in the environmental conditions of the gastrointestinal tract and it can bring a beneficial contribution to the homeostasis status of piglets. This strain should be studied further more for other probiotic properties that could improve the health status of piglets.

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