journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIII, Issue 3 – 2014, pag. 214 – 217

STUDY ON THE ADHESION OF LACTOBACILLUS PLANTARUM STRAINS WITH PROBIOTIC PROPERTIES TO MDCK

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Received August27th 2014, accepted September 15th 2014

Abstract: *One of the requirements for probiotic strains is to adhere to epithelial cells or cell lines. The presence of S-layer proteins in three Lactobacillus plantarum strains with probiotic properties was examined as well as their ability to adhere to the epithelial monolayer model non-cancerous cell line MDCK. The three strains lacked S-layer proteins, but Lactobacillus plantarum X2 and Lactobacillus plantarum LBRZ12 adhered to the cells of MDCK, while Lactobacillus plantarum F3 didn't. Along with their other probiotic properties these make them suitable for inclusion in the composition of probiotics and probiotic foods.*

Keywords: *adhesion, Lactobacillus, MDCK, probiotic, S-layer proteins*

1. Introduction

Probiotics are live microorganisms that have beneficial effects on the health of the host, when administered in adequate amounts [1, 2]. Due to their proven health beneficial effect the strains most frequently included in the composition of probiotics belong to the genera *Lactobacillus*, *Bifidobacterium* and *Propionibacterium* [3]. Probiotic microorganisms contribute to the restoration and maintenance of intestinal balance, play an important role in maintaining the overall health condition of the organism and improve significantly the quality of the foods they are incorporated into [4]. Not all strains can be included in probiotic formulas but only those that meet certain requirements [5]. One of these requirements for probiotic strains is to be able to adhere to the intestinal mucosa and cell lines, thereby competitively excluding pathogens [3; 6]. Adhesion may be either non-specific, related to specific physico-chemical factors, or based on specific adhesion molecules located on the surface of the microbial cells and receptor molecules on the surface of the epithelial cells.

Adhesion is species specific. Lactic acid bacteria possess moderate to good adhesion properties on human cell lines [7]. Adhesion of probiotic strains to the surface of the gut is of milestone importance as it is related to the subsequent colonization in the human intestinal tract which in turn determines the longer retention time of probiotic bacteria in the intestinal tract and the realization of their inherent immunomodulatory action. A number of in vitro studies demonstrating adherence of different strains of lactic acid bacteria to human epithelial cells have been conducted [8]. S-layer proteins are a strain-specific trait. Surface layer (S-layer) proteins with molecular masses between 40 and 60 kDa [9, 10], responsible for their

adhesion to epithelial cells or cell lines, are described in lactobacilli [11].

The purpose of the present study was to examine the presence of S-layer proteins and the ability of three strains of *Lactobacillus plantarum* with probiotic properties to adhere to the cells of the epithelial monolayer model non-cancer cell line MDCK.

2. Materials and methods

Microorganisms

The studies in this work were performed with three strains of *Lactobacillus plantarum* with probiotic properties designated as *Lactobacillus plantarum* X2 and *Lactobacillus plantarum* F3, isolated from spontaneously fermented sourdough, and *Lactobacillus plantarum* LBRZ12, isolated from spontaneously fermented vegetables.

Determination of the adhesion of the Lactobacillus plantarum strain to MDCK

The used non-cancer, kidney cell line MDCK (Madin-Darbey Canine Kidney) is cultured as a monolayer in a 24-well plate in DMEM without antibiotics.

The single-strain cultures of each *Lactobacillus plantarum* strain were incubated overnight at 37°C and centrifuged thrice, the biomass sludge was washed with PBS-buffer. 0,5 cm^3 of each cell suspension, suspended in PBS-buffer, with concentration of 10^9cfu/cm^3 was resuspended in 1 cm^3 DMEM and 0,5 cm^3 of the mixture were pipetted into the wells with the developed monolayer cell line MDCK. After 3 hours of incubation at 37°C the MDCK monolayer was washed with 250 µl PBS-buffer. 250 µl of fixating agent (96% ethanol : and acetic acid = 3: 1) were pipetted in each well and the plate was incubated for 20 minutes at room temperature. It was washed once with saline solution. A few drops of Gimza stain (diluted in a ratio of 1: 7) as to cover the bottom of the wells were pipetted in each well. After 5 minutes, the dye was removed and the cells were washed twice with 300 µl of saline solution. A light microscope (Dundee), at a magnification of 100x, was used for each well of the plates

Characterization of surface layer proteins by SDS-PAGE [9].

Untreated bacterial cells were resuspended in 1% SDS, incubated for 30 min at 37ºC for the isolation of surface layer proteins, centrifugedfor 5 min at 9000 x g and the supernatant was analyzed by SDS-polyacrylamide gel electrophoresis using 10% polyacrylamide gel.

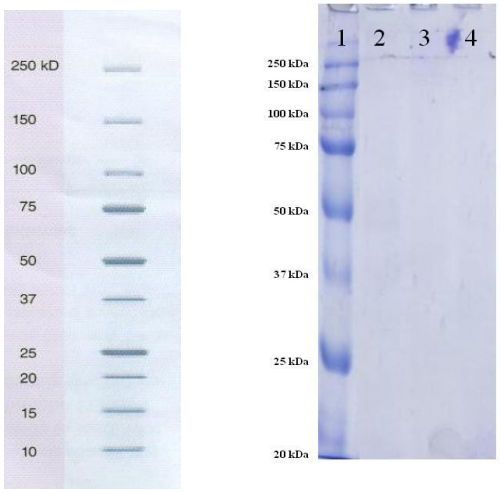
The used marker for the electrophoresis was Precision Plus Protein™ Standards (BioRad, Cat. # 161-0373). Protein bands were visualized by staining with Coomassie Blue R-250.

3. Results and discussion

The presence of surface layer (S-layer) proteins with molecular masses between 40 and 60 kDa is described in lactobacilli. The examination of the presence of S-layer proteins showed that the strains *Lactobacillus plantarum* X2, *Lactobacillus plantarum* F3 and *Lactobacillus plantarum* LBRZ12 did not possess S-layer proteins (Fig. 1).

The ability of *Lactobacillus plantarum* X2, *Lactobacillus plantarum* F3 and *Lactobacillus plantarum* LBRZ12 to adhere to the cells of the monolayer model non-cancerous epithelial cell line MDCK was examined. In the control the cells were arranged in a dense monolayer, their shape and intercellular contacts being clear.

The strains *Lactobacillus plantarum* X2 and *Lactobacillus plantarum* LBRZ12 adhered to MDCK cells, while *Lactobacillus plantarum* F3 didn't (Fig. 2).



Marker

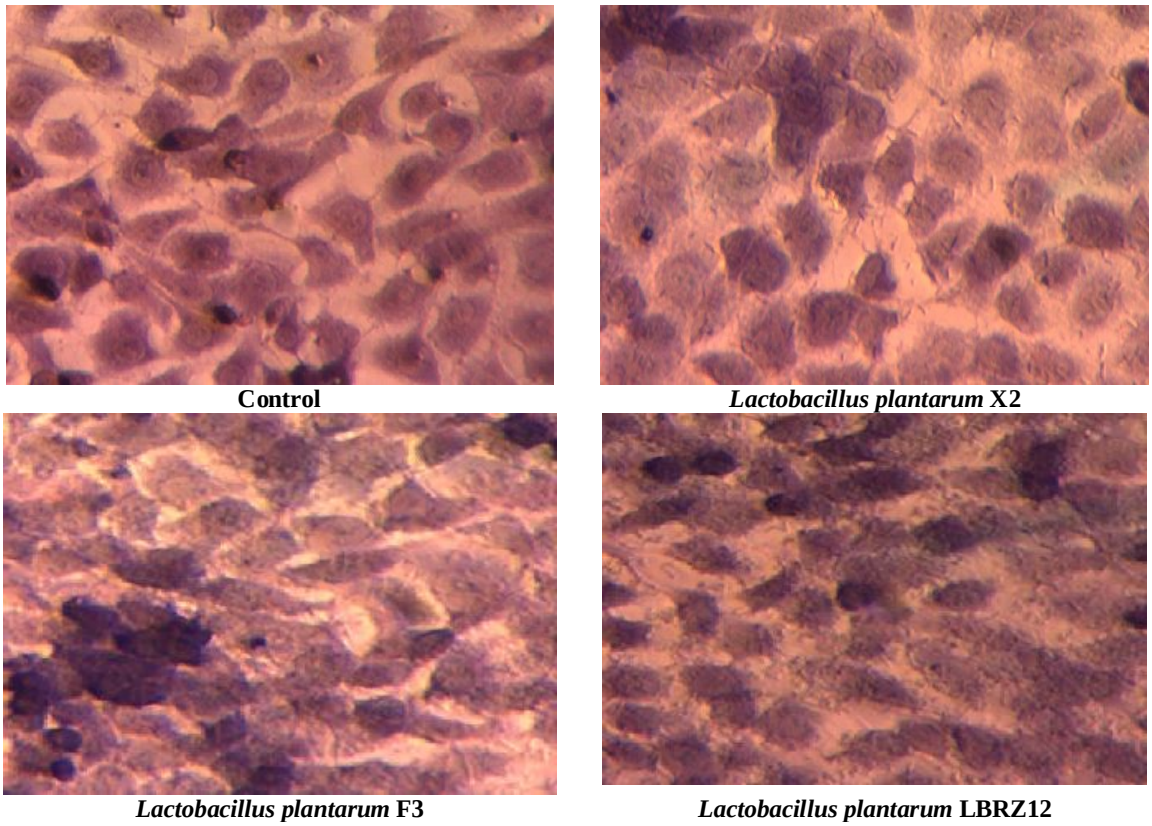
1. Marker
2. *L. plantarum* F3
3. *L. plantarum* LBRZ12
4. *L. plantarum* X2

Fig. 1. Presence of S-layer proteins in *Lactobacillus plantarum* X2, *Lactobacillus plantarum* F3 and *Lactobacillus plantarum* LBRZ12

When examining the adhesion of *Lactobacillus plantarum* LBRZ12 and *Lactobacillus plantarum* X2 to the cells of MDCK there was visible mounting of the cells of the *Lactobacillus plantarum* strains to the cells of the monolayer.
Furthermore disturbances in the structure, as well as the appearance of individual cell line cells were observed.

4. Conclusion

The ability of *Lactobacillus plantarum* strains of different origin with probiotic properties to adhere to the epithelial monolayer model non-cancerous cell line MDCK was studied.



Control | ***Lactobacillus plantarum* X2**

***Lactobacillus plantarum* F3** | ***Lactobacillus plantarum* LBRZ12**

Fig. 2. Adhesion of *L. plantarum* strains to MDCK cell line

The three studied strains did not possess S-layer proteins, but *Lactobacillus plantarum* X2 and *Lactobacillus plantarum* LBRZ12 adhered to the monolayer model non-cancerous cell line MDCK, which is a prerequisite for their inclusion in the composition of probiotics and probiotic foods.

5.References

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