Study of the adhesion of *Lactobacillus acidophilus* strains with probiotic properties to MDCK

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Study of the adhesion of Lactobacillus acidophilus strains with probiotic properties to MDCK. *Probiotic strains are required to have the ability to adhere to epithelial cells or cell lines. The presence of S-layer proteins in three Lactobacillus acidophilus strains with probiotic properties was examined. Their ability to adhere to the epithelial monolayer model non-cancerous cell line MDCK was studied as well. The strains Lactobacillus acidophilus A2, Lactobacillus acidophilus Ac and Lactobacillus acidophilus Z10 possess S-layer proteins and adhere to the cells of MDCK. Along with their other probiotic properties these make them suitable for inclusion in the composition of probiotics and probiotic foods.*

Key words: *Lactobacillus*, S-layer proteins, adhesion, MDCK, probiotic

INTRODUCTION

Probiotics are live microorganisms that have beneficial effects on the health of the host, when administered in adequate amounts [1, 11]. Some strains of the genera *Lactobacillus*, *Bifidobacterium* and some representatives of *Propionibacterium* are currently included in the composition of foods and probiotics due to their proven health effects [2]. Probiotic microorganisms contribute to the restoring of intestinal balance, play an important role in maintaining health and improve the quality of the foods they are incorporated into [4]. Only strains that meet certain requirements can be included in the composition of probiotics and probiotic foods [8]. One requirement for probiotic strains is their ability to adhere to the intestinal mucosa and cell lines, thereby competitively excluding pathogens [2, 9].

The adhesion may be non-specific, related to specific physico-chemical factors, and based on adhesion molecules on the surface of the cells and receptor molecules on the epithelial cells. In general the strains applied in the manufacture of fermented milk foods, are not characterized by the best adhesion properties, while probiotic bacteria show strong adhesion properties. Adhesion is species specific. According to their ability to adhere lactic acid bacteria demonstrate moderate to good adhesion properties on human cell lines [6]. Adhesion of probiotic strains to the surface of the gut and the subsequent colonization in the human intestinal tract determine the longer retention time of probiotic bacteria in the intestinal tract and realization of their inherent metabolic processes with pronounced immunomodulatory action. Thus, reaction with the mucosa is achieved, promoting the contact with the gut-associated lymphoid tissue, which in turn provides the stabilization of the intestinal mucosa, which performs barrier function. In turn, intestinal-associated lymphoid tissue can come in contacted with the cells of the probiotic strains and their components, thus having positive influence on the host immune system [7]. A number of in vitro studies demonstrating adherence of different strains of lactic acid bacteria to human epithelial cells have been conducted [10]. According to several authors, surface-located cellular protein structures are a strain-specific trait. In lactobacilli surface layer (S-layer) proteins with molecular masses between 40 and 60 kDa are described [3, 12]. They are considered responsible for the adhesion of lactobacilli strains to epithelial cells or cell lines [5].

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

MATERIALS AND METHODS

Microorganisms

The studies in this work were performed with three strains of *Lactobacillus acidophilus* with probiotic properties designated as *Lactobacillus acidophilus* A2 and *Lactobacillus acidophilus* Ac of human origin; *Lactobacillus acidophilus* Z10, isolated from spontaneously fermented sourdough.

Media

LAPTg10 - broth. Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6 - 6.8, and Tween 80 is added - 1cm^3/dm^3. Sterilization - 20 minutes at 121ºC.

Determination of the adhesion of a Lactobacillus acidophilus 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 acidophilus* 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. Each well of the plates was microscoped under a light microscope at a magnification of 100x.

Characterization of surface layer proteins by SDS-PAGE [3].

Untreated bacterial cells were resuspended in 1% SDS, incubated for 30 min at 37ºC for the isolation of surface layer proteins, centrifuged for 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.

RESULTS AND DISCUSSION

Identification and characterization of surface layer proteins in Lactobacillus acidophilus A2, Lactobacillus acidophilus Ac and Lactobacillus acidophilus Z10

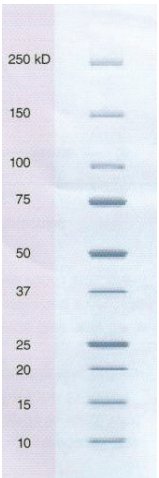
The presence of surface layer (S-layer) proteins with molecular masses between 40 and 60 kDa is described in lactobacilli. The strains *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus acidophilus* Z10 possessed S-layer proteins with a molecular weight between 37 kDa and 50 kDa (Fig. 1). Their presence is a prerequisite for further studies on their adhesion to epithelial cell lines, for example the model epithelial monolayer non-cancerous cell line MDCK.

In vitro determination of the adhesive properties of Lactobacillus acidophilus A2, Lactobacillus acidophilus Ac and Lactobacillus acidophilus Z10

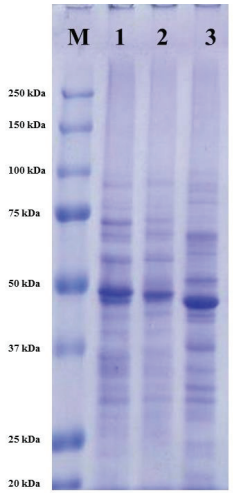
The ability of *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus acidophilus* Z10 to adhere to the cells of the monolayer model non-cancerous epithelial cell line MDCK was examined.

In the control wells the cells were arranged in a dense monolayer and their shape and intercellular contacts were clear.

When examining the adhesion of the *Lactobacillus acidophilus* strains to the cells of MDCK there was not only visible mounting of the cells of the *Lactobacillus acidophilus* strains to the cells of the monolayer, but also disturbances in the structure, as well as the appearance of individual cell line cells were observed.



Marker



M. Marker
1. *Lactobacillus acidophilus* A2
2. *Lactobacillus acidophilus* Ac
3. *Lactobacillus acidophilus* Z10

Fig.1 Presence of S-layer proteins in *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus acidophilus* Z10

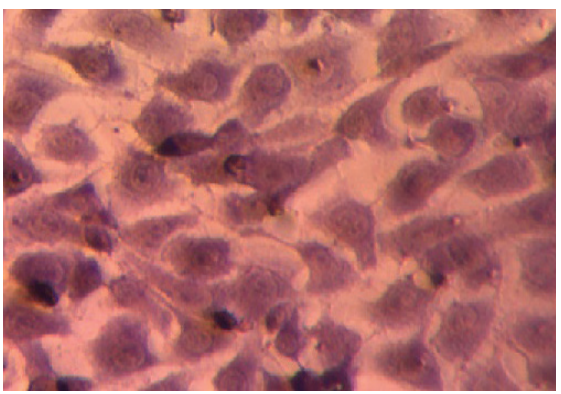
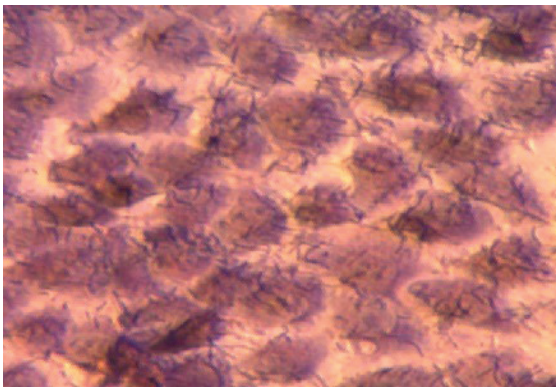
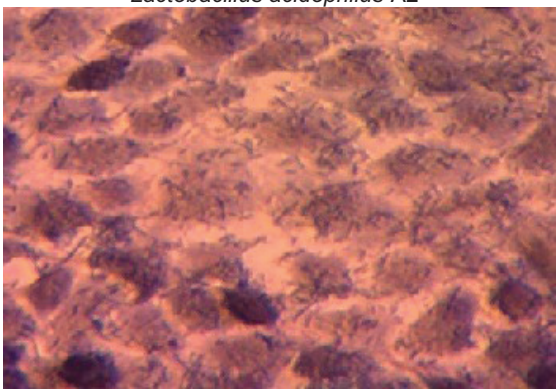


Fig. 2 Control MDCK cell line

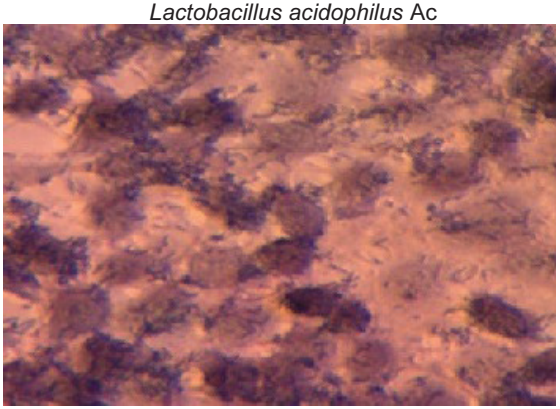
The strains *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus acidophilus* Z10 adhered to MDCK cells (Fig. 3).



Lactobacillus acidophilus A2



Lactobacillus acidophilus Ac



Lactobacillus acidophilus Z10

Fig. 3 Adhesion of *Lactobacillus acidophilus* strains to MDCK cell line

CONCLUSION

The ability of *Lactobacillus acidophilus* strains of different origin with probiotic properties to adhere to the epithelial monolayer model non-cancerous cell line MDCK was studied. *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus acidophilus* Z10 were characterized by the presence of S-layer proteins with a molecular mass between 37 kDa and 50 kDa. The three strains 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.

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This paper has been reviewed