Cent. Eur. J. Biol. • 5(1) • 2010 • 103–110
DOI: 10.2478/s11535-009-0078-1

VERSITA

Central European Journal of **Biology**

Influence of carbohydrates on cell properties of *Lactobacillus rhamnosus*

Research Article

Jelena Begovic*, Djordje Fira, Amarela Terzic-Vidojevic, Ljubisa Topisirovic

Laboratory for Molecular Genetics of Industrial Microorganisms,
Institute of Molecular Genetics and Genetic Engineering, University of Belgrade,
11010 Belgrade, Serbia

Received 25 August 2009; Accepted 12 October 2009

Abstract: Lactobacilli represent normal commensals of the human body, particularly in the gut and vagina where they protect these environments from incoming pathogens *via* a variety of mechanisms. The influence of the carbohydrate source present in reconstituted MRS growth medium on the different cell properties of two *Lactobacillus rhamnosus* strains were examined. Two human vaginal isolates, BGHV719 and exopolysaccharide producer strain BGHV954 were analyzed. The results demonstrated that unlike in reconstituted MRS with glucose as a carbon source, the presence of fructose, mannose, or rhamnose, significantly reduced cell surface hydrophobicity of both strains. In addition, differences in cell wall protein composition of *L. rhamnosus* BGHV719 and alterations in colony mucoidity of *L. rhamnosus* BGHV954 were also demonstrated. Light and SEM microscopy revealed differences on the cellular level when BGHV719 was cultivated in the presence of different sugars. The results of this study point out the importance of complex relationships between growth medium composition and the different aspects of bacterial behavior, and call for more detailed analyses of versatile bacterial responses to the changes in the environment, including vaginal ecosystem. This is especially important since lactobacilli are amongst the most widely used of probiotics.

Keywords: *Vaginal lactobacilli • Lactobacillus rhamnosus • Cell surface properties • Cell wall proteins • Medium composition*

1. Introduction

Lactobacilli are lactic acid bacteria (LAB) ubiquitous in nature and widespread in humans as commensal bacteria, where they play a significant role in general health maintenance [1]. Lactobacilli are the dominant bacteria of a healthy human vagina where they prevent the growth of pathogen species through different mechanisms [2]. Many studies have shown a correlation between the disruption of the normal genital microflora, particularly of the *Lactobacillus* species, and an increase in genital and bladder infections [3]. Lactobacilli are being widely used by humans as biological food processors, dietary supplements and in the treatment of different disorders such as bacterial vaginosis and urinary infections [4]. They are also amongst the most widely used of probiotics. One of the clinically best-studied probiotic organisms, *Lactobacillus rhamnosus* GG (ATCC 53103) has been attributed with various health effects, such as the prevention and treatment of acute diarrhea in children, the prevention of antibiotic-associated diarrhea, the prevention and treatment of allergy, as well as occasionally beneficial effects for other disorders like those related to children's dental health [5,6]. The use of lactobacilli as probiotics offers a potential alternative approach to health restoration and maintenance in the vaginal tract. Several representatives of this genus, like *Lactobacillus rhamnosus* GR-1™ and *Lactobacillus fermentum* RC-14 have been shown efficient in treatment of bacterial vaginosis [7,8]. The ability of lactobacilli to adhere to epithelial cells is considered as an important feature in the process of colonization of the different human ecological niches such as the vagina. When selecting for potential probiotic strains, the question becomes which probiotic strains will persist in and colonize the vaginal epithel since this ecosystem is in a constant change. Colonization of the

* *E-mail: lab6@Eunet.rs*

vaginal environment is influenced by vaginal fluid and vaginal epithelium. HPLC analysis of free sugars in vaginal fluid revealed the presence of free glucose as the major sugar and also mannose [9].

Adhesion is also believed to be an important factor in the process of realization of some probiotic effects such as pathogen exclusion [4]. However, the mechanisms of bacterial attachment to epithelium are not completely clear.

The surface properties of bacteria are vital for their survival and interactions with the environment, including that with other microorganisms and host organisms [10]. Frequently, the behavior of LAB is dependent on the cell surface physicochemical properties and their chemical composition [11]. Albeit the influence of cell wall constituents on the bacterial surface properties has not been elucidated, they are influenced both by the surface proteins and exopolysaccharides. The surface hydrophobicity and composition of LAB cell wall have been studied both by microbial adhesion to hydrocarbons and biochemical analyses [12-14]. Nevertheless, the techniques routinely employed for characterizing the physicochemical nature of cells also include liquid drop contact angle measurement, X-ray photoelectron spectroscopy (XPS) and electrophoretic mobility [15]. Still, detailed analyses of the relationship between LAB cell wall constituents and physicochemical properties are slowly emerging. It has been demonstrated that components of growth medium (such as yeast extract and peptones) present an important factor in determining the surface properties of lactobacilli, including structure and physicochemical characteristics of bacterial cell wall [10]. A better knowledge of these aspects could help us understand the roles that different cell surface constituents have in bacterial behavior. This is important since lactobacilli, particularly *L. rhamnosus* are of considerable technological and commercial importance [2].

The medium in this study contained all principal components of a full-fermentation medium used for lactobacilli growth and the only difference between media was a carbohydrate source. In this way we examined the influence of the carbohydrates present in medium on different aspects of two human vaginal *Lactobacillus rhamnosus* strains including: (i) their cell surface characteristics of (ii) their cell wall proteins synthesis (iii) their colony mucoidity and (iv) their colony and cell morphology.

2. Experimental Procedures

2.1 Bacterial growth and preparation of bacterial cultures

Two human vaginal isolates, *L. rhamnosus* BGHV719 and exopolysaccharide producer *L. rhamnosus* BGHV954 were studied. Both strains were isolated from the vaginal swabs of two women during routine gynaecological examination. Strain identification was based on 16S rDNA sequencing and repetitive extragenic palindromic PCR analysis (data not shown). All media were based on reconstituted MRS medium as previously described [16] and components were prepared separately and combined together. The only difference between media was the sugar source where MRSglu indicates that MRS medium was supplemented with glucose (10 g/l), Mfru with fructose (10 g/l), Mman with mannose (10 g/l) Mrham with rhamnose (10 g/l). Overnight bacterial cultures (precultures) were cultivated under aerobic conditions at 37°C for 20 h in appropriate medium. Fermentation cultures used for further experiments were inoculated with precultures (5%, vol/vol) that had been grown in the same media. The time of cultivation depended on the time needed for the strain to reach middle log phase in a particular medium (Figure 1). The initial pHs of reconstituted MRS were 6.8. The final pH of cultures was determined by measuring pH at the beginning of the stationary phase depending on the growth medium (Hi9321 Microprocessor pHmeter, Hanna Instruments, USA).

2.2 Microbial adhesion to hexadecane (MATH)

The influence of carbohydrates in the medium on the cell surface hydrophobicity was measured using MATH test described by Reid *et al.*, [17]. In a brief, a bacterial suspension from the mid logarithmic phase of growth was added to 10 ml of 10 mM KH_2PO_4 buffer (pH=7.0) to the final optical density (OD_{600nm}) 0.5±0.05. After brief vortexing, 3 ml of bacterial suspension was transferred to 15 ml glass test tubes. Subsequently, 150 µl of hexadecane (Merck, Hohenbrunn, Germany) was added and the mixture was vortexed three times for 30 s with a 1 min pause between vortexing. Tubes were left for 30 min to allow the separation of hexadecane and aqueous phase. The lower aqueous layer was carefully removed by using Pasteur pipettes, and transferred to clean tubes. The optical density of the initial (OD_0) and extracted solution (OD_1) was measured at OD_{600nm} (Ultrospec 3300 Pro, Amersham Biosciences). Phosphate buffer without bacteria was used as a blank value.

The fraction of bacteria adhering to hexadecane/water interface was calculated as:

$\theta = OD_0\text{-}OD_1/OD_0$

For the evaluation of strain hydrophobicity previously defined values [14] were used as reference values: 0-35% marked low hydrophobicity, 36-70% medium hydrophobicity and 71-100% high hydrophobicity. The values of strain hydrophobicity reduction in Table 1 represent the reduction of MATH value in particular medium when compared with the MATH value obtained in MRSglu medium (%).

2.3 Isolation of cell wall-associated proteins

Cell wall proteins were extracted from mid log phase of *L. rhamnosus* BGHV719 cultivated in four different media with 5 M LiCl, according to the procedure of Schär-Zammaretti *et al.*, [10] with minor adjustments. Overnight cultures were grown in the same medium as cultures used in this experiment. The fermentation was carried out in 250 ml bottles containing 100 ml of each fermentation medium. The bacteria were harvested by centrifugation (3,500 x g, 20 min, 4°C) and washed three times with 15 ml of 10 mM KH_2PO_4. Afterwards, the bacterial pellet was resuspended in 15 ml of KH_2PO_4 buffer. In order to obtain approximately the same quantity of bacteria from the logarithmic phase of growth, bacterial suspensions were adjusted OD_{600nm}=2.3 and checked by CFU count (data not shown). An equal volume (15 ml) of 5 M LiCl was added and the suspension was incubated for 1 h at a room temperature. Afterwards, bacteria and high-molecular-mass debris were sedimented by centrifugation (5,000 x g, 20 min, 4°C) and dialyzed overnight against 5 liters of 50 mM Tris-HCl (pH 7.4, room temperature). Three hours after the start of the dialysis the dialysis buffer was refreshed. The remaining high molecular mass debris was removed by centrifugation (5,000 x g, 20 min, 4°C). The final concentration of samples was done with MICROCON YM-10 vials with 10 000 Da protein cut-off (Millipore, USA). Final supernatants were used undiluted and analyzed on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).

2.4 SDS-PAGE analysis

The analysis of the supernatants was carried out using the reference molecular marker encompassing proteins of molecular masses between 10 kDa and 200 kDa (Page Ruler™ prestained Protein Ladder, Fermentas UAB, Lithuania). Supernatants were mixed with the loading buffer (125 mM Tris-HCl, pH 6.8), 10 mM disodium EDTA, and 4% sodium dodecyl sulphate (SDS), 25% glycerol, 5% 2-mercaptoethanol, 0.07% bromphoenol blue) at a 1:1 volume ratio. The samples were heated at 80°C for 10 min and run on a 10% polyacrilamide gel (SE 600 Vertical Slab Gel Unit, Hoefer Scientific Instruments, San Francisco USA) for 16 h at the constant current of 10 mA. Proteins were visualized after the staining of the gel with Coomassie brilliant blue R 250 (SERVA, Heidelberg, Germany).

2.5 Exopolysaccharide production

Mucoidity of *L. rhamnosus* BGHV954 colonies, which indicates exopolysaccharide production, was determined by visual appearance [18]. An indication of EPS production was taken for the strain showing weak pellet formation after centrifugation and the presence of diffuse capsules surrounding bacterial cells. For slime production, strain was streaked on the MRS medium and incubated at 37°C for 24 h. Ropiness of colonies on agar surfaces was tested with a loop to observe the formation of slime. Colonies were scored for the intensity of ropiness by picking with an inoculation loop. To demonstrate the presence of a gene involved in biosynthesis of the EPS repeating units, we performed PCR with degenerate primers G-*-Bact-a-F-36 (5'-TCATTTTATTCGTAAAA CCTCAATTGAYGARYTNCC-3') and G-*-Bact-a-R-27 (5'-AATATTATTACGACCTSWNAYYTGCCA-3') encompassing the glycosyltransferase (GT) gene according to Provencher *et al.*, [19]. Each hybrid primer consisted of a short 3' degenerate core region based on four highly conserved amino acids and a longer 5' consensus clamp region. The PCR mix contained 50 pmol of each primer, 10XPCR buffer, 4 mM $MgCl_2$, 200 µM deoxynucleoside triphosphate (Fermentas Life Sciences, Lithuania). The PCR program consisted of 45 cycles, after an initial incubation at 94°C for 5 min to allow complete denaturation of the DNA template. The first 5 cycles consisted of a denaturation step at 94°C for 30 s, an annealing step at 62°C for 30 s, and an elongation step at 72°C for 30 s. The last 40 cycles consisted of a denaturation step at 94°C for 30 s, an annealing step at 52°C for 30 s, an elongation step at 72°C for 30 s, and a final elongation step at 72°C for 10 min. PCR products were analyzed by electrophoresis on a 1% agarose gel containing ethidium bromide and visualized by CCD camera Biometra BDR2/5/6 (Biometra Biomedizinische Analytik GmbH, Goettingen, Germany).

2.6 Microscopic observation of colonies

The slide preparations of mid log cultures of *L. rhamnosus* strains cultivated in different media were prepared as follows: 50 µl of the bacterial suspension (each from the log phase of growth, depending on the medium) was fixed to the slide by heat and stained

Strain	Medium	Mucoidity	CFU/ml ± SD	pH ± SD	MATH ± SD	Hydrophobicity reduction
BGHV719	Mglu	-	$2.7 \times 10^9 \pm 0.6$	4.68 ± 0.04	28 (L) ± 1	0%
	Mfru	-	$2.3 \times 10^9 \pm 1.1$	4.75 ± 0.07	22 (L) ± 2	22%
	Mman	-	$1.0 \times 10^9 \pm 0.6$	4.30 ± 0.06	19 (L) ± 2	32%
	Mram	-	$5.7 \times 10^8 \pm 0.6$	5.40 ± 0.07	16 (L) ± 2	43%
BGHV954	Mglu	++	$2.6 \times 10^9 \pm 1.5$	4.58 ± 0.04	40 (M) ± 1	0%
	Mfru	+	$2.3 \times 10^9 \pm 1.5$	4.80 ± 0.01	28 (L) ± 1	30%
	Mman	+	$1.1 \times 10^9 \pm 0.6$	4.22 ± 0.08	25 (L) ± 2	38%
	Mram	-	$5.9 \times 10^8 \pm 0.6$	5.56 ± 0.06	22 (L) ± 1	45%

Table 1. Cell properties of human vaginal *L. rhamnosus* BGHV719 and BGHV954.

SD - standard deviation of means of three independent measurements; NP - mucoidity not detected, ++ marks intensive mucoidity, + marks reduced mucoidity and – marks a total visible reduction of mycoididty: L – low hydrophobicity; M – medium hydrophobicity.

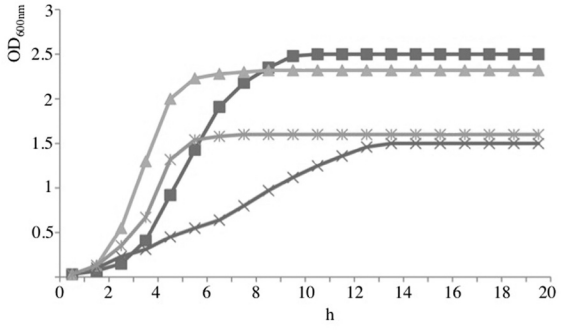

Figure 1. Growth curve of human vaginal *L. rhamnosus* BGHV719 in four different reconstituted media: MRSglu (square), MRSfru (triangle), MRSram (star) and MRSman (cross).

according to Gramm's method. Cell morphology was examined under the microscope (Olympus U-RFL-T, GmbH, Hamburg, Germany) and photographed.

2.7 Scanning electron microscopy (SEM)

Since small changes in cell morphologies of bacteria are difficult to observe under the light microscope, scanning electron microscopy (SEM) was used to examine the minor changes in cell morphology of the bacterial populations that have been cultured in different media.

Bacterial colonies (16 hour old) were picked up from Petri dishes with the appropriate medium and coated with gold particles in a Bal-Tec SCD 005 sputter coater (BAL-TEC AG, Liechtenstein). The cell morphology of the tested populations was examined JEOL JSM-35CF SEM instrument (JEOL Ltd., Japan).

2.8 Statistical analysis

A Student's t test was used to compare the results.

3. Results

3.1 Bacterial growth

Depending on the growth media used for the cultivation of the strain, the final cell count values varied. Nevertheless, values obtained for the particular growth medium were similar for both strains (Table 1). During the cultivation in the different media, cultures differed in the duration of log phase reaching a stationary phase between approximately 6 h and 13 h (Figure 1). Additionally, initial pH of all media used in the study was adjusted at 6.8. After onset of stationary phase, depending on the growth medium, pH of all liquid cultures became more acidic (Table 1). Nevertheless, in Mram PH values were higher than in other cultures reaching 5.7 (BGHV719) and 5.9 (BGHV954).

3.2 MATH

In MRSglu medium, relatively low percentage (28%) of *L. rhamnosus* BGHV719 bacteria adhered to this non-polar solvent indicating a low hydrophobicity of the strain (Table 1). On the other hand, significantly higher number of *L. rhamnosus* BGHV954 adhered to hexadecane (40%), classifying it as a medium hydrophobic strain. In addition, all MATH values obtained for this strain were slightly higher than those observed for BGHV719 when cultivated in the respective medium ($P<0.05$). According to the results from this study, the MATH values of both

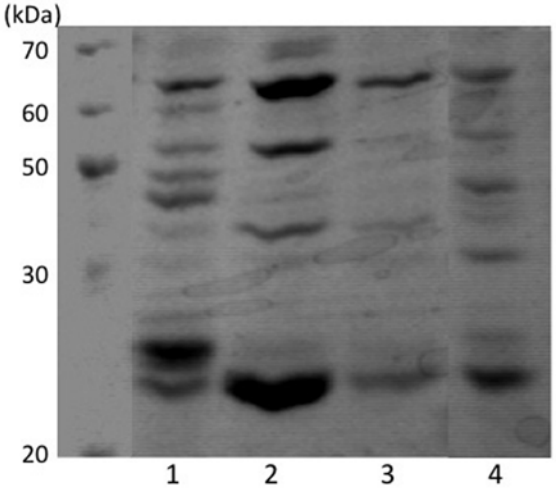

Figure 2. SDS-PAGE analysis of cell wall extracts of human vaginal *L. rhamnosus* BGHV719 cultured in four different media. L - protein marker. Lanes: 1-Mram, 2-Mfru, 3-Mman, 4-Mglu.

strains, when compared to MATH values obtained in MRSglu medium, were reduced in media with different sugar sources. Interestingly, although the level of hydrophobicity in MRSglu differed between two tested strains, the highest hydrophobicity reduction for both strains was detected when cultivated in Mram.

3.3 SDS-PAGE analysis of cell wall proteins

From the SDS-PAGE analysis of cell wall protein extracts of *L. rhamnosus* BGHV719 the molecular mass of the extracted cell wall proteins varied between 25 kDa and 100 kDa. The protein profiles of cultures from Mfru and Mglu were similar, with bands being more intense for Mfru (Figure 2). When culture was cultivated in Mman a unique band pattern was obtained (Figure 2). It was also evident that in the presence of rhamnose, the greatest number of cell wall proteins was observed. Due to the problems of cell wall protein isolation of exopolysaccharide producer *L. rhamnosus* BGHV954, SDS-PAGE analysis of this strain was not conducted.

3.4 Microscopic observation of *L. rhamnosus* BGHV719

Different sugar sources had a visible impact on the cell shape and cell organization appearance of *L. rhamnosus* BGHV719 strain (data not shown). In Mglu and Mfru, lactobacilli tended to make aggregate-like structures. Nevertheless, the largest aggregates, visible even by naked eye in test tubes, formed when rhamnose was added to the medium. On the other hand, in Mman medium, bacteria tended to form long chains and have more coccoid shape. For *L. rhamnosus* BGHV954 these differences were not so evident and under all conditions aggregate-like structures were observed (data not shown).

3.5 SEM

The cell morphology of BGHV719 strain from late logarithmic phase of growth on appropriate medium was examined by scanning electronic microscopy (SEM). The cell morphology of the Mglu and Mfru cultures were

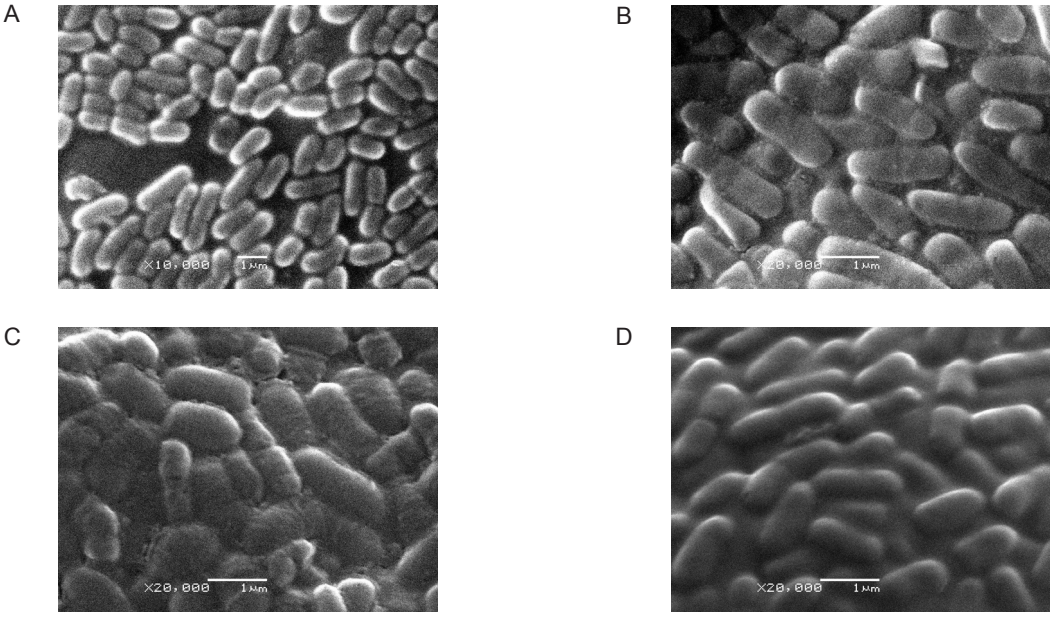

Figure 3. SEM micrographs of human vaginal *L. rhamnosus* BGHV719 cultivated in four different media. 3a-Mglu, 3b-Mfru, 3c-Mman, 3d-Mram.

apparently bacilli like cell forms with no visible matrix around cells (Figure 3a). Nevertheless, some granules in the bacterial surroundings were visible around lactobacilli grown in Mfru medium (Figure 3b). In Mman, bacterial cells tended to be more deformed with a visibly rough surface (Figure 3c). When cultivated in Mram, cells were immersed in the matrix and appeared as one layer of cells (Figure 3d).

3.6 EPS production

PCR results confirmed the presence of the glycosyltransferase gene required for the biosynthesis of the EPS as a DNA fragment of the expected size (276 bp, data not shown) was obtained when DNA from BGHV954 served as a template in the PCR reaction. According to DNA sequencing and BLAST analysis (www.blast.ncbi.nlm.nih.gov), the obtained nucleotide sequence shared 100% homology with *L. rhamnosus* ATCC 21052 glycosyltransferase gene. The mucoidity of *L. rhamnosus* BGHV954 colonies changed when different sugars were used as a carbon source in the growth medium. The complete loss of colony mucoidity, as seen by the naked eye, was observed when the strain was grown in Mram media. In Mfru and Mman media, a visible reduction of *L. rhamnosus* BGHV954 mucoidity occurred. The highest degree of mucoidity was obtained when the strain was cultivated in Mglu.

4. Discussion

Several studies have shown that lactobacilli, normally present in the human vagina, can protect the host against incoming pathogens that are constantly invading this ecosystem [2]. The vagina represents a unique and dynamic ecosystem that is continually fluctuating and is influenced, among other factors, by the menstrual cycle, infections and pregnancy [20]. Although much has been published on the histology of the human vagina and its changes, little is still known about its biochemistry and its metabolism. It is known that the vaginal fluid contains carbohydrates such as glucose and mannose and the vaginal environment also includes glycogen [9]. The cell wall represents a significant structural component of bacteria and differences in their outer layer reflect the adaptations of the organism to specific environmental conditions [21]. In this study, changes in different aspects of bacterial properties due to the variation in carbohydrate source in the growth medium were demonstrated.

Although both strains were able to grow in all tested media, variations in the carbohydrate source had a significant influence on the cell density measured in the stationary phase and on the growth kinetics of the strains. These differences, most probably, reflect physiological changes undergoing in strains as a response to different capabilities in metabolizing diverse carbon sources. Although all cultures became significantly more acidic after 20 hours of cultivation in the presence of all tested sugars, when rhamnose was added to the reconstituted MRS medium, pH remained relatively high in comparison to other cultures. This may be due to the lower cell count and the inability of efficient rhamnose fermentation.

In spite of several studies on cell hydrophobicity of lactobacilli [10,22], this physicochemical aspect remains poorly understood. The ability of bacteria to adhere to tissues is considered an important factor in the colonization of different human environments. It has been proposed that bacterial adherence is the result of two different mechanisms: specific and nonspecific binding [23]. It has been reported that bacterial recognition of specific sites by receptors on microorganisms, cell-surface charge and hydrophobicity influence the strength of adhesion [14,24]. Schär-Zammareti *et al.*, 2005, demonstrated that composition of fermentation medium significantly influences and determines surface properties of *L. acidophilus* NCC2628 and even small changes in MRS composition changes the hydrophobicity of this strain. We have determined that *L. rhamnosus* BGHV719 exhibited low hydrophobicity, which is in accordance with previous results and the changes induced by the presence of different carbohydrates could be related to differences in concentration of nitrogen or carbon in carbohydrate form [11,17,22]. Nevertheless, strain *L. rhamnosus* BGHV954 exhibited higher hydrophobicity, suggesting that this feature could be strain dependent. Although hydrophyllic character of the strain might be related to the presence of polysacharides on lactobacilli surface, lower hydrophyllic character of this EPS producer could not be explained in this way [22]. Rather the presence of other cell wall constituents - like hydrophobic polypeptides may be responsible for this difference. On the other hand, Ocaña *et al*., (1999) analyzed 134 vaginal lactobacilli and showed that most of the strains including *L. rhamnosus* were highly hydrophobic [14]. This may indicate the influence of either the origin of strains or the source of components used for the preparation of medium on the results of the measurements.

The reduction of hydrophobicity of both strains in reconstituted MRS media most probably reflects large changes in complete cell physiology of both strains. The presence of different sugars in the medium influences composition of the isolated cell wall protein of *L. rhamnosus* BGHV719. We can only speculate that either composition of cell wall proteins is changed or

that the export of some proteins is also influenced by the changes in the cell physiology. Maybe due to non-optimal growth conditions bacteria express some other proteins like specific adhesion factors as suggested by Piette *et al.*, 1992 [23]. Further identification of cell wall proteins influenced by the presence of different carbohydrates is needed in order to answer these questions. Additionally, potential surface layer proteins that had a molecular mass between 30 and 60 kDa were isolated and should be determined for this strain.

The total yield of EPS produced by LAB depends on the medium composition and conditions in which the organisms grow [12]. Although still preliminary, our results showed that exopolysaccharide production expressed as colony mucoidity of *L. rhamnosus* BHGV954 depends on the carbon source present in the medium. The results obtained when the strain was cultivated in Mglu and Mfru were in accordance with previous work, where glucose was the most efficient carbon source for EPS production [12]. The absence of mucoidity of this strain when cultivated in Mram may be due to its slower growth.

Due to the carbon deficiency in the growth medium, the cell surface of some bacteria changed its appearance [25]. Results obtained from our study showed that the medium composition also influences cell and colony appearance of *L. rhamnosus* BGHV719 reflecting changes in bacterial physiology and adaptations to the different environmental conditions. These results were confirmed by SEM microscopy where differences on a cellular level were demonstrated

In conclusion, the presence of different sugars in the fermentation medium has a significant impact on different properties of the human vaginal isolates *L. rhamnosus* BGHV719 and *L. rhamnosus* BGHV954. Different sugars influence both cell wall protein composition and mucoidity of bacterial cells indicating the alterations in cell wall content. These changes are followed by changes in cell shape and appearance. Further research should be focused on the influence of these changes on the adhesion properties of these strains. Since lactobacilli are widely used in the food, chemical and pharmaceutical industry, the results presented in our study call for more detailed analyses of complex relationship between bacteria and their environment.

Acknowledgments

This work has been supported by Ministry of Science and Tecnological Development of Republic of Serbia, Grant No 143036. The authors would like to thank dr Baltasar Mayo from Instituto de Productos Lácteos de Asturias (CSIC), Asturias, Spain, for the comments on the manuscript.

References

[1] Felten A., Barreau C., Bizet C., Lagrange P.H., Philippon, A., *Lactobacillus* species identification, H2O2 production, and antibiotic resistance and correlation with human clinical status, J. Clin. Microbiol., 1999, 37, 729-733

[2] Zárate G., Santos V., Nader-Macias M.E., Protective effect of vaginal Lactobacillus paracasei CRL 1289 against urogenital infection produced by Staphylococcus aureus in mouse animal model, Infect. Dis. Obstetrics Gynecol., 2007, DOI:10.1155/2007/48358

[3] Redondo-Lopez V., Cook R.L., Sobel J.D., Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora, Rev. Infect. Dis., 1990, 12, 856-872

[4] Reid G., Bruce A.W., Urogenital infections in women: can probiotics help?, Postgrad. Med. J., 2003, 79, 428-432

[5] Nase L., Hataka K., Savilahti E., Saxelin M., Ponka A., Pousa T., et al., Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children, Caries Res., 2001, 35, 412-420

[6] Doron S., Snydman D.R., Gorbach S.L., *Lactobacillus* GG: bacteriology and clinical applications, Gastroenterol. Clin. N. Am., 2005, 34, 483-498

[7] Gardiner G.E., Bruce A.W., Beuerman D., Reid G., Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA, Clin. Diagn. Lab. Immunol., 2002, 9, 92-96

[8] Falagas M.E., Betsi G.I., Athanasiou S., Probiotics for the treatment of women with bacterial vaginosis, Clin. Microbiol. Infect., 2007, 13, 657-654

[9] Rajan N., Cao, Q., Anderson B.E., Pruden, D.L., Sensibar, J., Duncan J.L., et al., Roles of Glycoproteins and Oligosaccharides Found in Human Vaginal Fluid in Bacterial Adherence, Infect. Immun., 1999, 67, 5027-2032

[10] Schär-Zammaretti P., Dillmann M.L., Damico N., Affolter M., Ubbnik J., Influence of fermentation

medium composition on physicochemical surface properties of *Lactobacillus acidophilus*, Appl. Environ. Microbiol., 2005, 71, 8165-8173

[11] Boonaert C., Rouxhet P.G., Surface of Lactic acid bacteria: relationship between chemical composition and physicochemical properties, Appl. Environ. Microbiol., 2000, 66, 2458-2554

[12] Cerning J., Renard C.M.G.C., Thibault J.F., Bouillanne,C., Landon, M., Desmazeaud M., Carbon source requirements for exopolysaccahride production by Lactobacillus casei CG11 and partial structure analysis of the polimer, Appl. Environ. Microbiol., 1994, 60, 3914-3919

[13] Buck B.L., Altermann E., Svingerud T., Klaenhammer T.R., Functional Analysis of Putative Adhesion Factors in *Lactobacillus acidophilus* NCFM, Appl. Environ. Microbiol., 2005, 71, 8344-8351

[14] Ocaña V.S., Bru E., de Ruiz Holgado A.A.P., Nader-Macías M.E., Surface characteristics of lactobacilli isolated from human vagina, J. Gen. Appl. Microbiol., 1999, 45, 203-212

[15] Pembrey R.S., Marshall K.C., Schneider R.P., Cell surface Analysis Techniques: What do cell preparation protocols do to cell surface properties?, Appl. Environ. Microbiol., 1999, 65, 2877-2894

[16] de Man J.C., Rogosa M., Sharpe M.E., A medium for the cultivation of lactobacilli, J. Appl. Bacteriol., 1960, 23, 130-135

[17] Reid G., Cuperus P.L., Bruce A.W., van der Mei H.C., Tomeczek L., Khouru A.H., et al., Comparison of contact angels and adhesion to hexadecane of urogenital dairy, and poultry lactobacilli: effect of serial culture passages, Appl. Environ. Microbiol., 1992, 58, 1549-1553

[18] Raus-Madeido P., de los Reyes-Gavilán C.G., Invited review: Methods for screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria, J. Diary Sci., 2005, 88, 843-856

[19] Provencher C., La Pointe G., Siros S., Van Calsteren M.R., Roy D., Consensus-degnerate hybrid oligonucleotide primers for amplification of priming glycosyltransferase genes of the exopolysaccharide locus in strains of *Lactobacillus casei* group, Appl. Environ. Microbiol., 2003, 69, 3299-3307

[20] Zhou X., Bents S.J., Schneider M.G., Davis C.C., Islam M.R., Forney L.J., Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods, Microbiology, 2004, 150, 2565-2573

[21] Beveridge T.J., Graham L.L., Surface layers of bacteria, Microbiol. Rev., 1991, 55, 684-705

[22] Pelletier C., Bouley C., Cayela C., Bouttier S., Bourlioux P., Bellon-Fontaine M.N., Cell surface characteristics of *Lactobacillus casei* subsp. *casei*, *Lactobacillus paracasei* subsp *paracasei* and *Lactobacillus rhamnosus* strains, Appl. Environ. Microbiol., 1997, 63, 1725-1731

[23] Piette J.P.G., Idziak E.S., A model study of factors involved in adhesion of *Pseudomonas fluorescens*, Appl. Environ. Microbiol., 1992, 58, 2783-2791

[24] Savage D.S., Fletcher M., Bacterial adhesion: mechanisms and physiological significance, 1985, Plenum Press, New York

[25] Kjellberg S., Hermasson M., Starvation induced effects on bacterial surface characteristics, Appl. Environ. Microbiol., 1984, 48, 497-503