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Full Paper

The hydrophobicity of enterobacteria and their co-aggregation with *Enterococcus faecalis* isolated from Serbian cheese

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In this paper, we investigated the hydrophobicity, ability to adhere to solvents and the pig epithelium and co-aggregation of members of family Enterobacteriaceae and *Enterococcus faecalis* KGPMF 49. The bacteria used in this study were isolated from traditionally made autochthonous cheese from Southeastern Serbia (Sokobanja). The percentage of adhered bacteria was different in three solvents (chloroform, ethyl acetate and xylene). The highest percentage was detected in the presence of chloroform, and the lowest percentage was detected in the presence of xylene (chloroform < ethyl acetate < xylene). A different degree of co-aggregation of enterobacteria with *E. faecalis* KGPMF 49 was observed. *Klebsiella ornithinolytica* KGPMF 8 demonstrated the highest percentage of co-aggregation with *E. faecalis* KGPMF49 (32.29%). *Klebsiella pneumoniae* KGPMF 13, *K. ornithinolytica* KGPMF 9 and *Serratia marcescens* biogp 1 KGPMF 19 were found to have the ability to adhere to the pig epithelium, whereas *Escherichia coli* KGPMF 22 showed no such ability. The ability to co-aggregate with other species and the ability to adhere to the pig epithelium are very important characteristics of the isolated bacteria.

Key words: enterobacteria, autochthonous cheese, adhesion properties, *Enterococcus faecalis*

INTRODUCTION

In addition to lactic acid bacteria (LAB), the natural microflora of cheese includes other types of bacteria that can be detected in raw milk. Raw, fresh, uncooked milk is a source of various types of bacteria, regardless of the animal species from which the milk was obtained. Bacteria from the family Enterobacteriaceae are co-dominant in sheep and goat cheese. Since enterobacteria may affect the quality and taste of cheese, enterobacteria are characterized as one of the most common food spoilage agents [1]. The authors indicated that enterococci and enterobacteria could be isolated from cheese. *Citrobacter braakii, Enterobacter sakazakii*, *Escherichia coli*, *Kluyver* sp., *Salmonella enterica* sp. *arizonae* and *Serratia odorifera* were isolated from a local Italian cheese [2].

Potential interactions between LAB and enterobacteria were examined. The effect of *Lactobacillus curvatus* on *Enterobacter cloacae* was investigated, and it was observed that the growth of *L. curvatus* reduced the pH of the substrate preventing the development of *E. cloacae*. The authors of the study concluded that probiotic species could control or prevent food spoilage by bacteria from Enterobacteriaceae and Enterococcaceae [3]. Various studies have focused on the interaction of lactobacilli with *Klebsiella* sp. and *E. coli* [4]. However, there have only been a few studies on the adhesion of a pathogen to the intestine and the inhibition of pathogen adhesion to the intestine as well [5]. It is known that *Enterococcus faecium* inhibits adhesion of enterotoxigenic *E. coli* to porcine small intestine mucus [6]. One study indicated that the percentage of hydrophobicity directly reflected the adhesion ability of LAB to enterocytic cellular lines [7]. Therefore, this was considered to be the major criterion for selecting LAB for *in vitro* investigation of the adhesion ability to the pig epithelium. The ability to adhere and colonize intestinal epithelium cells of the host is an important characteristic that plays a role in the inhibition of the colonization of pathogenic strains [8]. The adhesion ability of *Salmonella typhimurium* was investigated on various surfaces. Despite the fact that disparate degrees of fimbriation and roughness of the cell surface were observed, as well as varied cell hydrophobicity, constant negative and positive charge values were obtained. High hydrophobicity values constantly coincided with enhanced adhesion to mineral particles. The negative charge of the bacterial surface as measured by electrostatic interaction chromatography appeared to play no role in the occurrence of adhesion. However, the positive charges on the cell surface contributed to the adhesion process [9].

Due to the fact that no preservatives of any kind are added to Sokobanja cheese, except for a low concentration of salt, and that it is produced in a traditional way, the presence of enterobacteria

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in it is expected. The cheese is made without adding any bacterial starter culture, so most likely, natural LAB have a role in the safety and preservation of the cheese. According to the literature, *Enterococcus faecalis* KGPMF 49 showed an acidification ability as well as an antagonistic effect on the growth of selected isolates of enterobacteria, which were isolated from the same cheese [10]. Therefore, we assumed that bacteria from the genus *Enterococcus* as well as from the other genera that belong to LAB could interact with enterobacteria. It was expected that members of the *Lactobacillus* and *Lactococcus* genera have a co-aggregation ability, and there are many papers that describe this, but we wanted to examine and evaluate the interaction with enterobacteria of *E. faecalis* KGPMF 49 and its role in the product's safety. The aim of this research study was to investigate the co-aggregation ability of enterobacteria isolated from autochthonous cheese produced in Southeastern Serbia (Sokobanja region) with *E. faecalis* KGPMF 49, which was obtained from the same cheese. Furthermore, we evaluated the hydrophobicity of enterobacteria to different solvents and the adhesion ability of certain enterobacteria to the pig epithelium.

MATERIALS AND METHODS

Strains and growth conditions

The bacteria used in this study were *Klebsiella oxytoca* KGPMF 1, *K. oxytoca* KGPMF 2, *Klebsiella ornithinolytica* KGPMF 8, *K. ornithinolytica* KGPMF 9, *Klebsiella pneumoniae* KGPMF 10, *K. pneumoniae* KGPMF13, *E. coli* KGPMF 14, *E. coli* KGPMF 17, *E. coli* KGPMF 22, *E. coli* KGPMF 24, *S. odorifera* KGPMF 18 and *Serratia marcescens* biogp 1 KGPMF 19. The bacteria were previously isolated from Serbian cheese (Sokobanja region) and identified at the Laboratory for Microbiology, Faculty of Science, University of Kragujevac (KGPMF) [11, 12]. Moreover, we used *E. faecalis* KGPMF49 isolated from the same cheese [10]. The collected and identified bacterial species were kept in a 20% glycerol/medium mixture at −80°C. *E. coli* (clinical isolate), *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 70063 were used as positive controls. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 70063 were provided by the Microbiology Laboratory, Faculty of Science, University of Kragujevac, Serbia, while *E. coli* (clinical isolate) was a generous gift from the Institute of Public Health, Kragujevac.

Co-aggregation ability

The co-aggregation of tested enterobacteria with *E. faecalis* KGPMF 49 was examined because these bacteria were isolated from the same cheese [10–12]. Furthermore, it has been found that these bacteria can be detected together in the human gastrointestinal tract [13]. Co-aggregation was monitored using a modified method described by Ocaña and Nader-Macías [14]. Cells cultured overnight were settled by centrifugation at 5,000 rpm for 15 min, after which they were washed twice in PBS (Alfa Aesar GmbH & Co Karlsruhe, Germany) and then resuspended in 4 mL of the PBS so that the number of cells was approximately 10^8 CFU/mL. Than 2 mL of each suspension for which coaggregation was observed, were mixed by vortexing. After mixing, 200 µL from the surface of the suspension were transferred to a microtube containing 1800 µL of PBS, and the absorbance value was monitored at 600 nm (A_0). The same procedure was repeated after 2 hr (A_t). Percentage of co-aggregation was calculated using the following formula:

$$\text{Co-aggregation } \% = (A_0 - A_t)/A_0 \times 100,$$

where A_t represents the absorbance of supernatant after 2 hr.

In order to confirm the presence of both bacteria in the mixture, Gram staining was used. Figure 1 shows Gram-negative rod-shaped bacteria in co-aggregation with Gram-positive rod-shaped bacteria.

Hydrophobicity of bacteria

Hydrophobicity of bacteria was measured in accordance with the method of Rosenberg *et al.* [15], with certain modifications [16, 17]. After 24 hr of incubation in tryptic soy broth (TSB), the bacteria underwent centrifugation at 5,000 rpm for 15 min, were washed twice, and were resuspended in 0.1 M KNO_3 (pH 6.2) to approximately 10^8 CFU/mL. The absorbance of the cell suspension was measured at 600 nm (A_0). Subsequently, 1 mL of solvent was added to 3 mL of the cell suspension. After 10 min of incubation at room temperature, the two-phase system was mixed for 2 min using a vortex mixer. The aqueous phase was removed after 20 min of incubation at room temperature, and its absorbance at 600 nm (A_1) was measured. The percentage of hydrophobicity was calculated as $(1 - A_1/A_0) \times 100$.

Three different solvents were tested in this study: xylene (Sineks, Belgrade, Serbia), which is a polar solvent; chloroform (Alkaloid, Skopje, Macedonia), a monopolar and acidic solvent; and ethyl acetate (Zorka Sabac, Sabac, Serbia), a monopolar and basic solvent. Only bacterial adhesion to xylene demonstrated cell surface hydrophobicity or hydrophilicity. Bacterial adhesion in the presence of chloroform and ethyl acetate was regarded as an indicator of electron donor ability (basic) and electron acceptor ability (acidic), respectively [16]. According to Ocaña and Nader-Macías [14], the percentage of hydrophobicity was expressed as follows: 0–35%, low hydrophobicity; 36–70%, medium hydrophobicity; and 71–100%, high hydrophobicity.

In vitro test for adhesion to pig intestinal epithelium

The adhesion ability of *K. pneumoniae* KGPMF13, *K. ornithinolytica* KGPMF9, *S. marcescens* biogp 1 KGPMF19 and *E. coli* KGPMF 22 to the pig intestinal epithelium was tested in accordance with the method described by Kos *et al.* [17], with some modifications. These bacteria were selected based on their ability to adhere to solvents. Ileal samples were collected from 9-month-old pigs. Immediately after sacrificing an animal, the intestinal epithelium was stored at 4°C in a refrigerator. Before the experiment, the intestinal epithelium was cut to an appropriate length of 1 cm^2 and held for 30 min in phosphate-buffered saline (PBS) at 4°C in a refrigerator in order to loosen surface mucus. Furthermore, the epithelium was washed three times in PBS, with mixing on a rotary shaker (PSU-20I, England), in order to remove excess fat. Prepared samples were aseptically transferred to Erlenmeyer flasks, which contained 20 mL of TBS (Torlak, Belgrade, Serbia), previously inoculated with 200 µL of overnight bacterial culture. Bacterial cultures in Erlenmeyer flasks were incubated for 24 hr at 37°C. After incubation, the ileal samples were washed using sterile saline to remove free-floating bacteria and then fixed with methanol. After drying, the samples were subjected to fluorescent staining with acridine orange for 2 min [18]. Excess color was removed by washing samples with distilled water. The samples were then examined and

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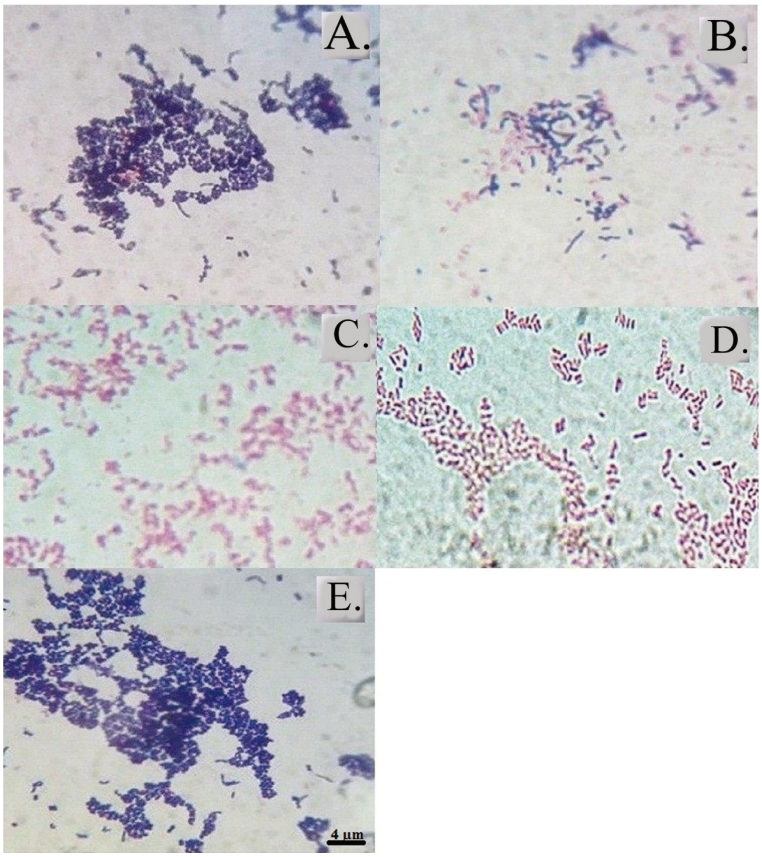

Fig. 1. Gram staining of tested bacteria: A. *K. ornithinolytica* KGPMF 8 with *E. faecalis* KGPMF 49; B. *E. coli* KGPMF 17 with *E. faecalis* KGPMF 49; C. *E. coli* KGPMF 17; D. *K. ornithinolytica* KGPMF 8; E. *E. faecalis* KGPMF 49.

photographed using a fluorescent microscope (Eclipse Ti, Nikon, Austria; magnification, 400×). Intestinal epithelium in pure TBS and in PBS served as sterile controls. Pigs have been demonstrated to have genetic and physiological similarities to human beings, which predestines them to be an experimental animal model, particularly with regard to mucosal physiology [19]. This is the reason for using the pig epithelium in this research.

Statistical analysis

All data are presented as means ± standard deviations, and Microsoft Excel (Redmond, Washington DC, USA) was used for calculations. The paired t-test was used for statistically processing the results of adhesion to solvents (IBM SPSS Statistics 20).

RESULTS

Co-aggregation ability

In this paper, we examined the co-aggregation ability of enterobacteria with *E. faecalis* KGPMF 49, with all bacteria isolated from the same cheese. The results indicated that co-aggregation was not observed for *K. oxytoca* KGPMF 1, *K. ornithinolytica* KGPMF 9, *K. pneumoniae* KGPMF 10, *K. pneumoniae* KGPMF 13 or *E. coli* KGPMF 24 after 2 hr of incubation.

When we compared the percentage of co-aggregation between *K. pneumoniae* ATCC 70063 and *E. faecalis* KGPMF 49, it was found that *K. ornithinolytica* KGPMF 8 showed the highest percentage of co-aggregation with *E. faecalis* KGPMF 49 (32.3%). The results are shown in Table 1. *K. oxytoca* KGPMF 2 demonstrated a percentage of co-aggregation of 16.7%. *S. marcescens* biogp 1 KGPMF 19 showed no co-aggregation (0%), while *S. odorifera* KGPMF 18 showed a percentage of co-aggregation of 28%. *E. coli* KGPMF 14 and *E. coli* KGPMF 17 (Fig. 1) demonstrated higher percentages of co-aggregation with *E. faecalis* KGPMF 49 than *E. coli* ATCC 25922 (0%) and an *E. coli* clinical isolate (8%). The highest measured percentage of co-aggregation was observed for *E. coli* KGPMF 17 and *E. faecalis* KGPMF 49 (28%; Table 2). All measurements were performed in triplicate. Gram staining controls are shown in Fig. 1.

Hydrophobicity of bacteria

In this paper, the hydrophobicity of bacteria was examined. The bacterial species showed different degrees of hydrophobicity. Hydrophobicity was dependent on the type of solvent. The highest adhesion ability was observed in chloroform, followed by ethyl acetate, while the lowest adhesion was observed in xylene (chloroform < ethyl acetate < xylene). Only bacterial adhesion to xylene demonstrated cell surface hydrophobicity or hydrophilicity. Based on comparison of the adhesion ability in the presence of all three solvents, it can be concluded that all isolates, except *S. odorifera*, showed statistically significant adhesion ability in relation to chloroform ($p<0.05$).

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Table 1. The adhesion ability of enterobacteria to solvents

Species	Chloroform	Ethyl acetate	Xylene
K. oxytoca KGPMF 1	17.18[1] ± 0.90[a]	/	9.01 ± 0.50[b]
K. oxytoca KGPMF 2	19.77 ± 1.20[a]	10.82 ± 0.25[b]	/
K. ornithinolytica KGPMF 8	20.55 ± 2.50[a]	/	1.70 ± 0.31[b]
K. ornithinolytica KGPMF 9	44.44 ± 1.21[a]	6.72 ± 0.33[b]	/
K. pneumoniae KGPMF 10	32.73 ± 1.03	/	/
K. pneumoniae KGPMF 13	43.13 ± 0.59[a]	13.10 ± 0.59[b]	/
S. odorifera KGPMF 18	9.95 ± 0.50[a]	27.19 ± 0.45[b]	/
S. marcescens biogp 1 KGPMF 19	17.83 ± 0.71[a]	2.91 ± 0.31[b]	/
E. coli KGPMF 14	7.24 ± 0.83[a]	6.05 ± 0.71[a]	/
E. coli KGPMF 17	6.97 ± 0.33[a]	0.51 ± 0.33[b]	/
E. coli KGPMF 22	37.71 ± 0.48	/	/
E. coli KGPMF 24	31.62 ± 0.77	/	/
E. coli (clinical isolate)	32.35 ± 0.91	/	/
E. coli ATCC 25922	15.49 ± 0.50	/	/
K. pneumoniae ATCC 70063	25.49 ± 0.12[a]	10.02 ± 0.25[b]	/

[1]The results are presented as a % of adhesion to solvent; / adhesion was not detected; different letters in the same row means statistical significance ($p<0.05$).

Table 2. Co-aggregation ability of enterobacteria with *Enterococcus faecalis* KGPMF 49

Species	*Enterococcus faecalis* KGPMF 49		
	0 h	2 h	%
K. oxytoca KGPMF 1	0.23[1]± 0.02	0.25 ± 0.04	/
K. oxytoca KGPMF 2	0.24 ± 0.07	0.20 ± 0.01	16.7 ± 0.25
K. ornithinolytica KGPMF 8	0.22 ± 0.03	0.15 ± 0.02	32.3 ± 0.33
K. ornithinolytica KGPMF 9	0.26 ± 0.02	0.31 ± 0.02	/
K. pneumoniae KGPMF 10	0.23 ± 0.03	0.23 ± 0.08	/
K. pneumoniae KGPMF 13	0.21 ± 0.03	0.20 ± 0.01	/
S. odorifera KGPMF 18	0.29 ± 0.00	0.21 ± 0.07	28 ± 0.14
S. marcescens biogp 1 KGPMF19	0.20 ± 0.03	0.20 ± 0.01	/
E. coli KGPMF 14	0.30 ± 0.02	0.25 ± 0.01	16.7 ± 0.50
E. coli KGPMF 17	0.25 ± 0.05	0.18 ± 0.05	28 ± 0.20
E. coli KGPMF 22	0.23 ± 0.03	0.21 ± 0.02	8.7 ± 0.33
E. coli KGPMF 24	0.25 ± 0.03	0.27 ± 0.03	/
E. coli (clinical isolate)	0.25 ± 0.05	0.23 ± 0.04	8 ± 0.20
E. coli ATCC 25922	0 ± 0.00	0 ± 0.00	/
K. pneumoniae ATCC 70063	0.31 ± 0.06	0.21 ± 0.02	32.2 ± 0.25

[1]Absorbance measured at 600 nm; / Co-aggregation was not detected.

The highest bacterial adhesion ability was found in the presence of chloroform (*K. ornithinolytica* KGPMF 9, 44.44%; *K. pneumoniae* KGPMF 10, 32.73%; *K. pneumoniae* KGPMF 13, 43.13%). The adhesion ability of *K. pneumoniae* ATCC 70063 was 25.49%. According to the results, *Klebsiella* spp. are better electron donors and, at the same time, poor recipients of electrons because they showed a higher degree of adhesion in the presence of chloroform. Adhesion in the presence of ethyl acetate was detected with *K. oxytoca* KGPMF 2, *K. ornithinolytica* KGPMF 9 and *K. pneumoniae* KGPMF 13. These bacteria can also be electron recipients. In the presence of xylene, a small percentage of adhesion was detected only with *K. oxytoca* KGPMF 1 and *K. ornithinolytica* KGPMF 8. Based on the results, it can be concluded that the isolates had a low degree of hydrophobicity.

The adhesion abilities of *E. coli* strains from the cheese, a clinical isolate and standard strains to solvents were also determined. The highest adhesion abilities were observed for *E. coli* KGPMF 22 and *E. coli* KGPMF 24 in the presence of chloroform. The *E. coli* clinical isolate showed a similar percentage of adhesion. A lower percentage of adhesion was detected for *E. coli* (KGPMF 14, 17) in the presence of ethyl acetate. This result suggested that this strain could also be an electron recipient. The results show that *E. coli* from cheese is a better electron donor and, at the same time, a poor recipient of electrons because it shows a higher degree of adhesion to chloroform. Since no adhesion was observed in the presence of xylene, *E. coli* has a low degree of hydrophobicity.

The highest percentage of *S. odorifera* adhesion (27.19%) was measured in the presence of ethyl acetate. *S. odorifera* is a better recipient electron than donor because it shows greater adhesion in the presence of ethyl acetate. *S. marcescens* shows the highest adhesion in the presence of chloroform and the lowest adhesion in the presence of ethyl acetate. *S. marcescens* from cheese is a better electron donor and, at the same time, a poor recipient of electrons because it shows a higher degree of adhesion to chloroform. No adhesion ability was observed in the presence of xylene. The results are presented as percentages of adhesion

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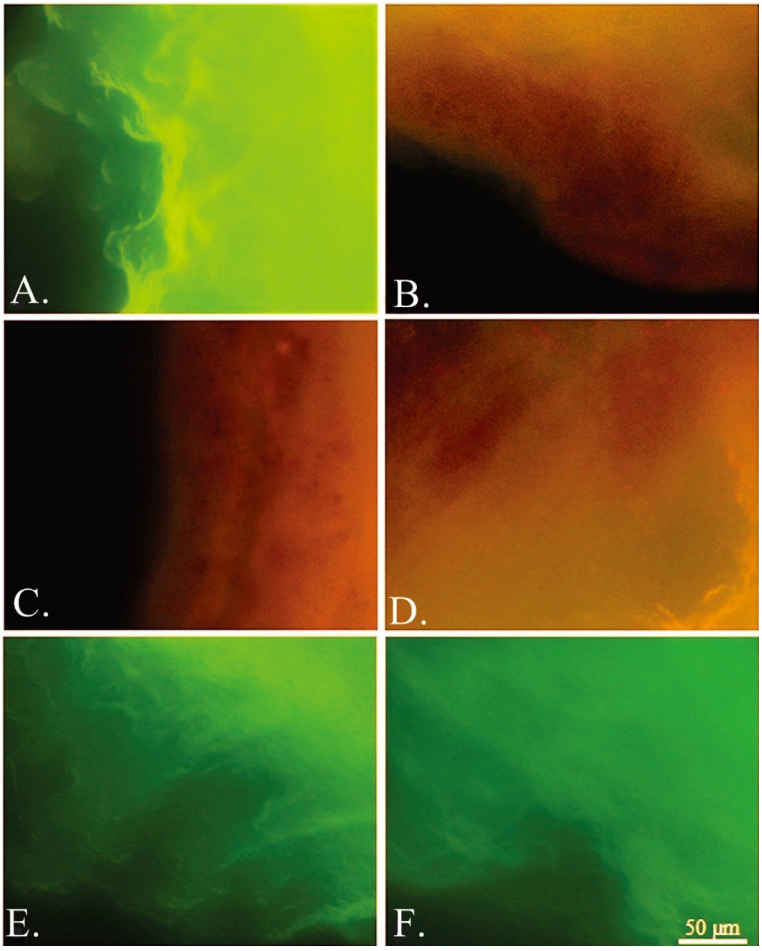

Fig. 2. Adhesion of enterobacteria to pig intestinal epithelium: A. *E. coli* KGPMF 22; B. *S. marcescens* biogp 1 KGPMF 19; C. *K. ornithinolytica* KGPMF 9; D. *K. pneumoniae* KGPMF 13; E. Sterility control-PBS; F. Sterility control-TSB.

in the presence of a solvent in Table 2. Since no adhesion was observed in the presence of xylene, the species have a low degree of hydrophobicity. All measurements were made in triplicate.

In vitro test for adhesion to the pig intestinal epithelium

The ability of enterobacteria isolated from the cheese to adhere to the pig epithelium was investigated. Adhesion was noted for *K. pneumoniae* KGPMF 13, K. *ornithinolytica* KGPMF 9 and *S. marcescens* biogp 1 KGPMF 19, while *E. coli* KGPMF 22 demonstrated no ability to adhere to the pig epithelium. The results are shown in Fig. 2. Bacteria were selected after observing the adhesion in the presence of chloroform. All bacteria that demonstrated a higher percentage of adhesion in the presence of chloroform could adhere to the pig epithelium, except for *E. coli*.

DISCUSSION

It is well-known that enterobacteria isolated from raw milk and cheese produced from raw milk could be considered potential pathogens [20]. Therefore, it is very important to determine the potential factors affecting the pathogenicity of isolated enterobacteria. In the present study, *S. odorifera* KGPMF 18 and *S. marcescens* biogp 1 KGPMF 19 isolates were demonstrated to have adhesion ability in the presence of three different solvents for the first time.

Furthermore, the present study demonstrated for the first time co-aggregation of enterobacteria with *E. faecalis* KGPMF 49 isolated from cheese. *Enterococcus* species cohabit with enterobacteria in the gastrointestinal tract of healthy people and animals. Our aim was to show that the strains from cheese possessed potential co-aggregation abilities. Based on previous research, co-aggregation of lactobacilli and enterococci and co-aggregation interactions between oral endodontic *E. faecalis* and bacteria isolated from persistent apical periodontitis in monkeys have been studied [4, 21]. According to Ledder *et al.* [22], co-aggregation between human intestinal and oral bacteria is possible. In our paper, the highest measured co-aggregation was observed between *E. faecalis* KGPMF 49 and the following enterobacteria: *K. ornithinolytica* PMFKG 8 (32.3%), *S. odorifera* KGPMF 18 (28%) and *E. coli* KGPMF 17 (28%). The significance of co-aggregation between *E. faecalis* and enterobacteria lies in the fact that these bacteria were isolated from the same cheese [10–12]. If enterobacteria could interact with *E. faecalis*, there is a reasonable possibility that it would interact with another LAB. The authors assumed that low pH influenced by the presence of LAB could inhibit a larger number of enterobacteria and have

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an impact on cheese safety [23]. Therefore, co-aggregation is a significant parameter.

Hydrophobicity of microorganisms affects their adhesion to various abiotic and biotic surfaces. Hydrophobic microorganisms possess the ability to form biofilms [24]. *K. pneumoniae* demonstrated the ability to form a biofilm [25]. Bacterial biofilms, which are detected on abiotic and biotic surfaces, may serve as one of the potential sources of cheese contamination. It is known that bacterial biofilms are more resistant to planktonic bacteria and may survive the processes of thermal treatment and disinfection (washing in detergents). Bacterial biofilms may be a bacteria reservoir for months, which may affect the texture and aroma of cheese [26]. *K. pneumoniae* was found in milk samples and milk products, and the isolates were hydrophobic [27]. In our paper, the highest adhesion to solvents was observed in the following order: chloroform < ethyl acetate < xylene. When chloroform was used as a solvent, bacteria from *Klebsiella* genus demonstrated the highest percentage of adhesion. It is important to emphasize that only bacterial adhesion to xylene demonstrated cell surface hydrophobicity or hydrophilicity, while bacterial adhesion in the presence of chloroform and ethyl acetate were regarded as indicators of electron donor ability and electron acceptor ability.

In a previous study, the results of adhesion to hexadecane indicated that 9 of 22 tested *E. coli* strains were moderately hydrophobic, with about 50% of the cells bound to the solvent. Generally, O157:H7 strains possessed lower adhesive properties to beef muscle than other serotypes. No correlation was found between *E. coli* cell surface charge, hydrophobicity, and adhesion to beef muscle [28]. In the present study, *E. coli* strains demonstrated different percentages of adhesion to tested solvents. *E. coli* KGPMF 14 demonstrated lower adhesion ability than *E. coli* (clinical isolates). *E. coli* KGPMF 22 exhibited a higher percentage of hydrophobic adhesion (chloroform as solvent). *E. coli* KGPMF 17 and *E. coli* KGPMF 24 demonstrated lower adhesion than controls. According to a rapid latex agglutination test, described by Mladenović *et al.* [11, 12], *E. coli* KGPMF 14 and *E. coli* KGPMF 22 have agglutination ability. *E. coli* KGPMF 17 and *E. coli* KGPMF 24 do not have this ability.

The hydrophobicity of *S. marcescens* is a relevant factor in adhesion and colonization of various interfaces. Bar-Ness *et al.* [29] investigated the potential role of the presence of serratamolides in *S. marcescens* cells and concluded that they led to a decrease in hydrophobicity, probably due to the blocking of hydrophobic locations on the surface of the cell. In our paper, *S. odorifera* KGPMF 18 showed a higher percentage of adhesion in ethyl acetate, while *S. marcescens* biogp 1 KGPMF 19 demonstrated a lower percentage. Both species exhibited no adhesion in xylene (no hydrophobicity).

The ability of enterobacteria isolated from Sokobanja cheese to adhere to the pig epithelium was investigated for the first time in this study. Livrelli *et al.* [30] were investigated the antibiotic resistance and adhesion ability of *Klebsiella* and *Serratia* clinical isolates. *K. pneumoniae* and enteropathogenic *E. coli* were demonstrated to have adhesion ability, while in the case of *Serratia* spp., no adhesive phenotype was observed, as they produced a strong cytotoxic effect on cells [30]. According to Nataro *et al.* [31], enteropathogenic *E. coli* possessed adhesion ability. In the present study, *K. pneumoniae* and *S. marcescens* biogp 1 KGPMF 19 demonstrated adhesion ability, but *E. coli* did not. Adhesion might be one of the pathogenic factors of enterobacteria.

Pathogenic *E. coli* causes infections in humans and animals. A few adhesins of *E. coli* with various morphological characteristics and receptor specificity have been identified [32]. The hydrophobicity and adhesion ability of *S. marcescens* isolated from the urinary tract were examined in a previous study. A higher percentage of hydrophobicity and the ability to adhere were detected [33]. In the present study, *S. marcescens* biogp 1 KGPMF 19 isolated from cheese demonstrated a percentage of adhesion to solvents of 17.83% and the ability to adhere to the pig epithelium. *K. pneumoniae* KGPMF 13 also showed a percentage of adhesion to solvents of 43.13% and the ability to adhere to the pig epithelium, whereas *E. coli* KGPMF 22, which showed a percentage of adhesion to solvents of 37.71%, demonstrated no ability to adhere to the pig epithelium. Regarding most cases in our research, a higher percentage of adhesion to solvents resulted in better adhesion to the pig epithelium.

The ability of microorganisms to adhere depends on physicochemical, electrostatic and acid-base, interactions. These interactions depend on the substratum and the physicochemical properties of bacterial surfaces, such as hydrophobicity [34] and status as an electron donor/electron acceptor [35]. According to Dias *et al.* [36], if a bacterium has an affinity for chloroform, then it is an electron donor and a weak electron recipient. An affinity for ethyl acetate indicates that the bacterium is a better electron recipient and a poor donor. Doyle [37] demonstrated that hydrophobicity played an important role in microbial infections. According to our investigation, the ability to adhere to the porcine epithelium was strain specific.

CONCLUSION

Bacteria from Sokobanja cheese do not demonstrate the ability to adhere in the presence of xylene. In the presence of chloroform, they exhibit the ability to adhere to the pig epithelium. If the number of enterobacteria increases in cheese and they enter the digestive tract and pass through the stomach acid, there is a possibility of adhesion to the intestines. The probability of this is low, as LAB are dominant and control the number and activity of other bacteria. Our study demonstrated that the cheese from Sokobanja contained bacteria which possessed adhesion ability and could interact with other bacteria. This paper is scientifically significant because the described abilities of bacteria isolated from the Sokobanja cheese were tested for the first time. Enterobacteria from Sokobanja cheese demonstrate a part of the natural microflora of cheese that we consume. Therefore, it is important to know their physiological characteristics and potential interactions with other bacteria that coexist with them in communities.

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