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ASSESSMENT OF THE PROPERTIES OF LACTOBACILLI ISOLATES FROM POLISH FERMENTED FOODS AND HUMAN FECES

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ABSTRACT

Background. Lactic acid bacteria (LAB) play an important role in the gastrointestinal tract (GIT) of humans and animals. They control the entrance of undesirable microorganisms and support intestinal defense. As well as typical representatives of *Lactobacillus* genera in GIT, other LAB strains, which come from fermented foods, can sometimes be found. The objective of this study was the investigation of autoaggregation, coaggregation, hydrophobicity, antibiotic resistant and enzymatic activity of LAB isolated from fecal and fermented foods *in vitro*.

Material and methods. Bacterial isolates were analyzed to determine the survival of LAB in conditions prevailing in the GIT, lipolytic and proteolytic activity, resistance to antibiotics, autoaggregation, coaggregation and hydrophobicity. All genetic analyses were conducted with qualitative real-time PCR.

Results. In this study, 70 isolates were investigated as putative probiotic candidates. 35 isolates each were isolated from fermented foods and feces. Isolates were identified as *L. rhamnosus*, *L. plantarum*, *L. casei*, *L. acidophilus*, *L. delbrueckii*, and *L. brevis.* The ability to survive in pH = 3.0 was displayed by 22 out of the 35 isolates from fermented products, whereas 20 isolates survived in MRS with 7% NaCl. Survivability in an environment after the addition of bile salts was confirmed for 26 isolates. All isolates from feces were resistant to pH = 3.0, 1% bile salt, and 7% NaCl. Among the 70 analyzed isolates only 3 showed lipolytic activity, whereas as many as 65 exhibited proteolytic activity. Of the 70 isolates tested, the largest number of isolates was characterized by average values in the hydrophobicity, autoaggregation, and coaggregation tests. All isolates were found to be resistant to vancomycin and metronizadole.

Conclusions. *Lactobacillus* spp. isolates from the analyzed environments were found to be able to survive at low pH values and in the presence of bile salts. The dominant species among the isolates were *Lactobacilus casei* and *Lactobacillus acidophilus.*

Keywords: LAB, fermented food, resistance to antibiotics, probiotic

INTRODUCTION

Probiotics, according to the definition of the WHO/FAO, are live microorganisms which contribute to the health and well-being of their hosts by maintaining or improving their intestinal microbial balance. Desirable characteristics of probiotic strains include the ability to overcome extremely low pH and the detergent effect of bile salts (Giraffa et al., 2010), as well as the ability to adhere to the intestinal mucosa and coaggregate. Probiotic microorganisms mainly include bacteria of the genera *Lactobacillus* and *Bifidobacterium*, which belong to the LAB, classified as non-adhering, G-positive catalase-resistant rods.

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Lactic acid bacteria (LAB) play an important role in the gastrointestinal tract (GIT) of humans and animals. They compete against enteric pathogens and reduce their colonization. As well as typical representatives of *Lactobacillus* genera in GIT, other LAB strains, which come from fermented foods, can sometimes also be found (Giraffa et al., 2010). Adhesion on the surface of epithelial cells is among the criteria for the selection of probiotic strains. The main goal of this paper was the investigation of autoaggregation, coaggregation, hydrophobicity, antibiotic resistance and enzymatic activity of LAB isolated from fecal and fermented foods.

MATERIAL AND METHODS

Material

The experimental material included 70 isolates, taken from sourdough for sour soup and white borscht (8), sauerkraut (3), yoghurt (9), pickled cucumbers (11), buttermilk (4), and fecal samples from healthy people (35). Food samples were purchased at grocery supermarkets. Analyses were carried out with the reference strains *L. rhamnosus* ATCC 7469, *L. casei* PCH 2677, *L. lactis* ATCC 11454, *L. acidophilus A*TCC 4356, *L. acidophilus* ATCC 314, *L. rhamnosus* GG (ATCC 53103), *B. animalis* subs*. lactis* ATCC 27536, and *L. delbrueckii* subsp*. delbrueckii* ATCC 9649.

Isolation of genomic DNA and methods of genetic analysis

Bacterial DNA was isolated from 1 mL of 20-h bacterial cultures of LAB isolates grown at 37°C on the MRS Broth (Scharlau), using a commercial Genomic Mini AX Bacteria kit (A&A Biotechnology). The qualitative analysis of the isolated genetic material was conducted with a spectrophotometer (NanoDrop 1000, NanoDrop Technologies). The measurement was performed three times for each sample of isolated DNA. At the same time, DNA purity was checked by analyzing the absorbance ratios at 260/280 and 260/230. DNA in all samples was diluted in TE buffer to 20 ng/μL and stored until exact analyses at a temperature of –80°C. Genetic analyses included determination of the affiliation of the analyzed isolates to particular species. Starters used for species identification and conditions of particular reactions are provided in Table 1 and Table 2. All analyses were carried out in a LightCycler 480 thermocycler

Table 1. List of primers used in this study

Target	Amplicon size (bp)	Primer	Primer sequence 5’ 3’	Reference
L. acidophilus	85	F	GAA AGA GCC CAA ACC AAG TGA TT	Haarman and Knol, 2006
		R	CTT CCC AGA TAA TTC AAC TAT CGC TTA	
L. casei	132	F	CTA TAA GTA AGC TTT GAT CCG GAG ATT T	
		R	CTT CCT GCG GGT ACT GAG ATG T	
L. plantarum	144	F	TGG ATC ACC TCC TTT CTA AGG AAT	
		R	TGT TCT CGG TTT CAT TAT GAA AAA ATA	
L. rhamnosus	97	F	CGG CTG GAT CAC CTC CTT T	
		R	GCT TGA GGG TAA TCC CCT CAA	
L. delbrueckii	94	F	CAC TTG TAC GTT GAA AAC TGA ATA TCT TAA*a*	
		R	CGA ACT CTC TCG GTC GCT TT	
L. brevis	1340	F	CTTGCACTGATTTTAACA	Guarneri et al., 2001
		R	GGGCGGTGTGTACAAGGC	

Table 2. Qualitative real-time PCR conditions used in the identification LAB

Target microorganism	Components of reaction	Primer hybridization temperature °C
L. acidophilus	LightCycler 480 SYBR Green I Master, 5pM each primers	59
L. casei	LightCycler 480 SYBR Green I Master, 5pM each primers	59
L. plantarum	LightCycler 480 SYBR Green I Master, 5pM each primers	58
L. rhamnosus	LightCycler 480 SYBR Green I Master, 5pM each primers	59
L. delbrueckii	LightCycler 480 SYBR Green I Master, 5pM each primers	58
L. brevis	LightCycler 480 SYBR Green I Master, 5pM each primers	56

(Roche) with the producer's reagents (LightCycler 480 STBR Green I Master).

Survival of LAB in conditions prevailing in the GIT

The material from the culture on MRS Agar (Scharlau) was suspended in MRS Broth (Scharlau) with the addition of oxbile (Oxoid) and 7% NaCl (Sigma). A solution of 1% oxbile in MRS Broth was prepared for the study. The MRS Broth was adjusted to pH = 3 using 0.1 N HCl. The inocula were incubated under anaerobic conditions for 3 h at 37°C. In order to determine isolate survivability under the aforementioned conditions, after incubation the material was seeded onto MRS Agar (Scharlau) and incubated for 48 h at 37°C under anaerobic conditions. The growth on MRS Agar was indicative of isolates' resistance to the above-mentioned conditions. The negative control was the same medium without inoculate. The positive control was a bacterial culture conducted in MRS medium at pH 6.5.

Lipolytic and proteolytic activity

Determination of proteolytic activity was carried out on a medium with skim milk powder according to the method of Taheri et al. (2009) and Moslehishad et al. (2013). Lipolytic activity was determined on a medium with Tween 80 and calcium chloride (peptone K 10 g/L, sodium chloride 5 g/L, calcium chloride 0.1 g/L, agar-agar 15 g/L, Tween 80, 10% aqueous solution 100 cm^3/L, deionized water 1000 cm^3). The pH of the medium for lipolytic activity was adjusted to 7.4 with solutions of 1M NaOH and 1M HCl. *Lactobacillus* isolates were streaked onto the medium using a needle. The cultures were incubated under the conditions described above. After incubation, the presence of a clear zone around the colony was observed to determine the proteolytic activity and the turbidity zone for lipolytic activity.

Resistance to antibiotics

Susceptibility testing was based on the agar overlay disc diffusion test. Isolates were grown overnight in MRS Agar (Scharlau) at 37°C (0.5 McFarland, after inoculation). Subsequently, petri plates containing 15 mL cystein-MRS Agar were overlaid with 100 µL of an active culture at 37°C. Antibiotic discs (Emapol, Poland) were placed on the inoculated plates in sterile conditions. After 24-h incubation at 37°C, the diameter of the inhibition zone was measured with calipers. All isolates were screened for their susceptibility to Penicilin G (G10), Rifampin (RA 30), Cephalotin (KF 30), Ceftazidine (CAZ 30), Vancomycin (VA 30), Tobramycin (NN 10), Sterptomycin (S 10), Gentamycin (CN 10), Neomycin (N10), Erythromycin (E15), Clindamycin (DA 2), Doxycycline (DO30), Nalidixic Acid (NA30), Ciprofloxacin (CIP 5), Fusidic Acid (FA 10), and Metronidazole (MET 5). The breakpoints for the interpretation of the inhibition zone were those defined by Liasi et al. (2009), Başyiğit et al. (2006), and Danielsen and Wind (2003).

Autoaggregation, coaggregation and hydrophobicity experiments

Lactobacillus spp. isolates were grown in anaerobic conditions for 16 h at 37°C. Activated cultures were harvested by centrifugation at 10,000 × g for 15 min,

washed twice in phosphate-buffered saline (PBS) (NaCl 8 g/L, KH_2PO_4 0.34 g/L, K_2HPO_4 1.21 g/L, pH 6.0) and resuspended in PBS to give a final optical density of 0.60 ±0.02 at 600 nm as measured by a spectrophotometer (BioPhotometer, Eppendorf). Anaerobic autoaggregation experiments were performed as described by Ekmekçi et al. (2009). For coaggregation experiments, 2 mL of each *Lactobacillus* spp. suspension was mixed with 2 mL of the *Salmonella enterica* subsp. *enterica s*erovar Typhimurium ATCC 14028. Experiments were performed as described by Vandevoorde et al. (1992). The BATH (Bacterial Adhesion to Hydrocarbons) method with hexadecane was used to assess hydrophobicity. One ml of the bacterial suspension was added to 1 ml of *n*-hexadecane (Sigma) and vortexed well for 10 s. The two phases were separated after incubation at room temperature for 1 h. The aqueous phase was carefully removed and measured at OD_{600}. The percent autoaggregation/coaggregation and hydrophobicity were expressed as follows:

$$\% \text{ autoaggregation} = [(OD_1 - OD_2) / OD_1 \times 100]$$

where:

OD_1 – first optical density,
OD_2 – optical density after 1 h.

$$\% \text{ coaggregation} = [(OD_1 + OD_2) - 2 (OD_3 / OD_1 + OD_2) \times 100]$$

where:

OD_1 – optical density of isolate 1 (*Lactobacillus* spp.),
OD_2 – optical density of strains 2 (*Salmonella enterica*),
OD_3 – optical density of strain 1 and strain 2.

$$\% \text{ hydrophobicity} = (OD_0 - OD) \times 100 / OD_0$$

where:

OD_0 – the absorbance of LAB cultures prior to the addition of *n*-hexadecane,
OD – the absorbance after the addition of *n*-hexadecane measured in the aqueous phase.

It was assumed that: result > 70% high autoaggregation / coaggregation / hydrophobicity, 20–70% average value of the tested feature, < 20% low value of the tested feature.

Statistical analysis

All experiments were done in three independent assays and mean values are presented. Statistical analysis of the results obtained was carried out using the Statistica 13 program, and p values < 0.05 were considered to be statistically significant.

RESULTS

In this study, 70 isolates were analyzed as putative probiotic candidates. 35 had been isolated from feces and 35 had been isolated from fermented foods. In the group of isolates from feces, 8 belonged to *L. rhamnosus* (22.8%) 13 to *L. casei* (37.1%), 11 to *L. acidophilus* (31.4%), and 3 to *L. delbrueckii* (8.6%). Among the isolates obtained from fermented foods (Table 3), 7 belonged to *L. rhamnosus* (20%), 5 to *L. plantarum* (14.3%), 6 to *L. casei* (17.1%), 8 to *L. acidophilus* (22.9%), 2 to *L. delbrueckii* (5.7%), and 7 to *L. brevis* (20%).

The ability to survive in a pH = 3.0 environment was displayed by 22 of the 35 isolates obtained from fermented products (Table 4). The most numerous were the isolates from pickled cucumbers (72%), followed by isolates from sauerkraut (66.6%), sourdoughs for soup (62.5%), yogurts (55.5%), and buttermilk (50%). Survivability in the environment with the addition of oxbile was confirmed for 26 isolates, including all those isolated from sauerkraut and buttermilk, 81.8% from pickled cucumber, 50% from sourdough, and 66.6% from yoghurt. The ability to survive in MRS broth supplemented with 7% NaCl was demonstrated for 20 of the 35 isolates obtained from foods, including these isolated from pickled cucumbers and sourdoughs (63.6% and 62.5% respectively). The lowest number of isolates able to survive in this environment was represented by those isolated from sauerkraut (33.3%). All isolates from feces were resistant to pH = 3.0, 1% bile salt, and 7% NaCl (Table 5). Statistical analysis of the results showed no significant relationship between the source of the isolates and their ability to survive in the gastrointestinal tract ($p > 0.05$).

Among the 70 isolates analyzed only three showed lipolytic activity. They had been isolated (Table 4) from pickled cucumbers (2 isolates) and buttermilk (1 isolate). Proteolytic activity was displayed by 65 out of the 70 isolates, including all the isolates from sourdoughs

Table 3. Result of identification of isolates

Group of isolates (N)	*L. rhamnosus*	*L. plantarum*	*L. casei*	*L. acidophilus*	*L. delbrueckii*	*L. brevis*
Feces (35)	8	0	13	11	3	0
Sauerkraut (3)	0	2	0	1	0	0
Pickled cucumbers (11)	3	3	0	2	0	3
Sourdough for soup (8)	4	0	0	0	0	4
Yoghurt (9)	0	0	3	4	2	0
Buttermilk (4)	0	0	3	1	0	0
Total	15	5	19	19	5	7

and buttermilk (Table 4). Milk casein was also degraded (Table 5) by more than half of the isolates from feces (94.3%), cucumbers (90.9%), and yoghurts (88.8%). The lowest percentage of isolates displaying this activity (Table 4) was isolated from sauerkraut (33.3%).

Six (8.6%) isolates were moderately susceptible to streptomycin at a concentration of 10 mcg, whereas the other isolates were resistant to this antibiotic (Table 6). They were *L. acidophilus*, *L. plantarum*, *L. brevis* (all isolates from pickled cucumbers), *L. plantarum* (sauerkraut), and two *L. delbrueckii* isolates from feces. In turn, greater differences were observed among the isolates in their sensitivity to ceftazidime. 34 isolates (47.1%) were sensitive, among which 10 isolates from

Table 4. Characterization of isolates LAB from fermented food

Isolate	Sample type	Target microorganism	Survival of LAB in conditions prevailing in the GIT			Enzymatic activity	
			pH = 3	NaCl 7%	Oxbail 1%	lipolitic activity	proteolitic activity
1	2	3	4	5	6	7	8
SK2-4	sauerkraut	*L. acidophilus*	+	+	+	–	+
SK5		*L. plantarum*	+	–	+	–	–
SK8-N1		*L. plantarum*	–	–	+	–	–
PJ1-2	yoghurt	*L. casei*	+	+	+	–	+
PJ11-3		*L. casei*	–	+	+	–	+
PJ13-2		*L. casei*	–	+	+	–	+
PJ15-4		*L. acidophilus*	+	–	+	–	–
PJ17-1		*L. acidophilus*	+	+	+	–	+
PJ18-3		*L. acidophilus*	+	+	–	–	+
PJ19-3		*L. acidophilus*	–	+	–	–	+
PJ20-N1		*L. delbrueckii*	+	+	+	–	+
PJ28-2		*L. delbrueckii*	–	–	–	–	+

Table 4 – cont.

1	2	3	4	5	6	7	8
Z1-2	sourdough for soup	*L. brevis*	–	+	+	–	+
Z1-4		*L. brevis*	–	+	–	–	+
Z1-6		*L. brevis*	+	+	+	–	+
Z2-1		*L. rhamnosus*	+	–	+	–	+
Z2-2		*L. rhamnosus*	+	–	+	–	+
Z2-3		*L. rhamnosus*	+	–	–	–	+
Z2-4		*L. rhamnosus*	+	+	–	–	+
Z2-5		*L. brevis*	–	+	–	–	+
M1-1	buttermilk	*L. acidophilus*	+	–	+	–	+
M1-4		*L. casei*	+	–	+	+	+
M2-7		*L. casei*	–	–	+	–	+
M2-10		*L. casei*	–	–	+	–	+
S4-4B	pickled cucumbers	*L. acidophilus*	+	+	+	–	+
S6-4		*L. acidophilus*		+	+	–	+
S7-1		*L. rhamnosus*	+	–	+	–	+
S7-3B		*L. rhamnosus*	+	+	–	–	+
S8-4B		*L. rhamnosus*	+	+	+	–	+
S9-N1		*L. plantarum*	–	+	–	–	+
S10-4		*L. plantarum*	+	+	+	+	+
S11-2		*L. plantarum*	–	–	+	–	+
S15-2		*L. brevis*	+	+	+	+	+
S15-4		*L. brevis*	+	–	+	–	+
S18-2		*L. brevis*	+	–	+	–	+
Number of positive results			22	20	26	3	32

+ positive result, – negative result.

food displayed moderately susceptibility (14.3%). Resistance was reported for 26 isolates (38.6%). The highest percentages of resistant isolates were determined for VA30, S10, N10, NA30, CIP10, FA10, and MET 5. Among all isolates tested, 64 (91.4%) were resistant to neomycin, 69 (98.6%) to nalidixic acid, 66 (94.3%) to ciprofloxacin, and 53 (75.7%) to fusidic acid. All analyzed isolates were resistant to vancomycin and metronidazole (Table 6). No correlation was found between the multi-resistance of isolates and the environment they had been isolated from ($p > 0.05$).

The study results indicate great differences in isolate hydrophobicity. After 1-h incubation, 30 of the 70 analyzed isolates were characterized by a medium value of this parameter (20–70%). They were mainly *L. acidophilus* and *L. casei*. The remaining 40 isolates (Table 7) displayed little adherence to hexadecane (not higher than 20%). In the autoaggregation test, no isolate achieved a high value. 21 isolates (*L. acidophilus* (8 isolate), *L. casei* (6), *L. rhamnosus* (4), *L. delbrueckii* (2)) exhibited medium values after 1 h and 49 isolates exhibited low values. No high ability to coaggregate with *Salmonella enterica* was demonstrated for any isolate. A considerable number of isolates (59) were characterized be a moderate coaggregation ability. Low coaggregation ability after 1 h of incubation was presented by 11 isolates (five from yoghurts and six from feces). Statistical analysis of the results did

Table 5. Characterization of isolates LAB from feces

Isolate	Target microorganism	Survival of LAB in conditions prevailing in the GIT			Enzymatic activity	
		pH = 3	NaCl 7%	Oxbail 1%	lipolitic activity	proteolitic activity
K1-5	*L. rhamnosus*	+	+	+	–	+
K1-107	*L. rhamnosus*	+	+	+	–	+
K2-2	*L. rhamnosus*	+	+	+	–	+
K2-4	*L. rhamnosus*	+	+	+	–	+
K2-4B	*L. rhamnosus*	+	+	+	–	–
K3-1	*L. rhamnosus*	+	+	+	–	+
K4-3	*L. rhamnosus*	+	+	+	–	+
K4-5	*L. rhamnosus*	+	+	+	–	+
K5-1	*L. casei*	+	+	+	–	+
K5-2	*L. casei*	+	+	+	–	+
K5-3	*L. casei*	+	+	+	–	+
K5-4	*L. casei*	+	+	+	–	+
K5-101	*L. casei*	+	+	+	–	+
K5-102	*L. casei*	+	+	+	–	+
K6-102	*L. casei*	+	+	+	–	+
K7-1	*L. casei*	+	+	+	–	+
K7-3	*L. casei*	+	+	+	–	+
K7-4	*L. casei*	+	+	+	–	+
K8-2	*L. casei*	+	+	+	–	+
K8-4	*L. casei*	+	+	+	–	+
K8-5	*L. casei*	+	+	+	–	+
K9-1	*L. acidophilus*	+	+	+	–	–
K9-2	*L. acidophilus*	+	+	+	–	+
K9-3	*L. acidophilus*	+	+	+	–	+
K9-4	*L. acidophilus*	+	+	+	–	+
K9-6	*L. acidophilus*	+	+	+	–	+
K9-8	*L. acidophilus*	+	+	+	–	+
K9-9	*L. acidophilus*	+	+	+	–	+
K9-11	*L. acidophilus*	+	+	+	–	+
K9-12	*L. acidophilus*	+	+	+	–	+
K10-1	*L. acidophilus*	+	+	+	–	+
K10-2	*L. acidophilus*	+	+	+	–	+
K16-2	*L. delbrueckii*	+	+	+	–	+
K17-2B	*L. delbrueckii*	+	+	+	–	+
K18-5B	*L. delbrueckii*	+	+	+	–	+
Number of positive results		35	35	35	0	33

+ positive result, – negative result.

Table 6. Antibiotic resistance of isolates LAB

Antibiotic	Concentration, mcg	N of strains sensitive	N of strains moderately susceptible	N of strains resistant
Penicilin	10	60	9	1
Ceftazidine	30	34	10	26
Cephalotin	30	64	4	2
Vancomycin	30	0	0	70
Sterptomycin	10	0	6	64
Neomycin	10	5	1	64
Gentamycin	10	26	18	26
Erythromycin	15	63	3	4
Rifampin	30	69	0	1
Clindamycin	2	57	3	10
Doxycycline	30	64	4	2
Nalidixic acid	30	1	0	69
Ciprofloxacin	5	1	3	66
Fusidic acid	10	9	8	53
Metronidazole	5	0	0	70

not show any significant relationship between autoaggregation/coagregation/hydrophobicity and the source of the isolates ($p > 0.05$).

DISCUSSION

Differences in the qualitative composition of fermented products depend on the material being fermented. *L. plantarum*, *L. brevis*, *P. cerevisiae*, and *L. mesenteroides* were found in pickled cucumbers whereas *L. citreum*, *L. paraplantarum*, *L. corynoformis* were isolated from sauerkraut (Plengvidhya et al., 2007). A greater species diversity is generally observed in fermented dairy products. In this study, species diversity in fermented dairy products was found to be relatively low: only three isolates, *L. casei*, *L. acidophilus*, and *L. delbrueckii*. In previous studies, the isolation and identification of lactic acid bacteria from the gastrointestinal tract has confirmed the diversity of this ecosystem; however, in most studies, only isolates with specific functional properties are subject to genetic identification (Pérez-Sánchez et al., 2011). Therefore, these results do not reflect the total qualitative composition of microbiota but only provide information about the presence of individual bacteria in the microbiome. In our study, the prevailing species were *L. casei* and *L. acidophilus.*

Enzymatic properties of bacteria are especially desirable in supplements intended for animals, because they may aid the feed digestion process. Previously reported data indicate that the enzymatic properties of LAB are highly diversified. When analyzing LAB isolated from the feces and intestines of piglets, Kim et al. (2007) demonstrated that 81% of the strains displayed proteolytic properties, whereas only 15% of the isolates obtained from feces and 17% of the isolates from intestines exhibited lipolytic activity. Our study also demonstrated a significant preponderance of isolates exhibiting proteolytic activity. These differences are most likely due to the origin of the isolates. Hydrophobicity, autoaggregation, and coaggreation are properties of bacteria which greatly affect their ability to colonize intestines and their protective action against gastrointestinal pathogens. Wang et al. (2010)

Table 7. Autoaggregation, coaggregation and hydrophobicity ability of *Lactobacillus* spp. isolates

Group of isolates (N)	Autoaggregation, %			Coaggregation, %			Hydrophobicity, %		
	>70	20–70	<20	>70	20–70	<20	>70	20–70	<20
Feces (35)	0	15	20	0	29	6	0	15	20
Sauerkraut (3)	0	0	3	0	3	0	0	2	1
Pickled cucumbers (11)	0	0	11	0	11	0	0	5	6
Sourdough for soup (8)	0	2	6	0	8	0	0	5	3
Yoghurt (9)	0	1	8	0	4	5	0	3	6
Buttermilk (4)	0	3	1	0	4	0	0	0	4
Total (70)	0	21	49	0	59	11	0	30	40

>0% – high autoaggregation / coaggregation / hydrophobicity, 20–70% – average value of the tested feature, <20% – low value of the tested feature.

analyzed the autoaggregation of *Bifidobacterium* strains isolated from adult physiological samples. They showed that the value increased in proportion to the incubation time of the strains. Previously published data points to a correlation between the autoaggregation ability and hydrophobicity of strains. Nikolic et al. (2010) demonstrated that *Lactobacillus* strains that were highly capable of autoaggregation were at the same time highly hydrophobic. In our study, 15 isolates from feces characterized by a medium value of aggregation showed the same range of hydrophobicity (Table 7). Studies of other *Lactobacillus* isolates have not confirmed these data.

In order to ensure health benefits to a host's body, bacteria should be able to survive in its gastrointestinal tract. Factors which are the most destructive to bacteria in the human gastrointestinal tract include bile juices with a very low pH, a high concentration of sodium chloride, and bile secreted in the duodenum (Giraffa et al., 2010). Tolerance of low pH values and the presence of bile salts is a strain-specific trait, which has been indicated by previously reported data (Sahadeva et al., 2011) and also by results presented in this work. In addition, bacteria survivability in the presence of bile salts is largely dependent on their concentration and on the duration of exposure to their action (Liu et al., 2007; Sahadeva et al., 2011). As expected, all isolates from feces were resistant to conditions occurring in the gastrointestinal tract. Results obtained for the other analyzed isolates confirm that fermented plant products can be sources of beneficial microflora. In recent years, LAB have been intensively studied for their potential to transfer drug resistance genes into other microorganisms, including pathogens. Despite this emphasis on the need to analyze the antibiotic resistance of lactic fermentation bacteria, no uniform standard methods have been established so far for this group of microorganisms. Determination of MIC value has been suggested as a recommendable method for the analysis of LAB sensitivity to antibiotics (Jorgensen and Hindler, 2007). However, many authors still use the disk-diffusion method, especially for screening purposes (El Jeni et al., 2015; Morandi et al., 2015; Saeed et al., 2014), and this method was also employed in this study.

LAB have a relatively wide spectrum of innate resistance to antibiotics, e.g. to vancomycin. This trait has been confirmed for *Lactobacillus* spp., *Pediococcus* spp., and *Leuconostoc* spp. (Pérez-Sánchez et al., 2011). Our study also showed all the analyzed isolates to be resistant to vancomycin at the tested concentration. In addition, *Lactobacillus* spp. are resistant to aminoglycosidic antibiotics. Liu et al. (2009) demonstrated resistance of bacteria from *Lactobacillus* and *Enterococcus* genera to gentamycin and streptomycin. Other authors have reported resistance of *Bifidobacterium* genus bacteria to aminoglycosidic antibiotics (Moubareck et al., 2005; Temmerman et al., 2003).

A similar observation was made in this study. The highest number of isolates were resistant to vancomycin (100%), metronizadole (100%), streptomycin (91.4%) and neomycin (94.3%) whereas significantly fewer isolates (37.1%) were resistant to gentamycin. Previous studies have also reported resistance of *Lactobacillus* spp. to quinolone antibiotics (Hummel et al., 2007), e.g. to nalidixic acid. In our study, only one isolate from buttermilk appeared to be sensitive to this antibiotic.

In turn, LAB are sensitive to cell wall synthesis inhibitors, like penicillins and ampicillins (Danielsen and Wind, 2003), and also to protein synthesis inhibitors, like chloramphenicol, erythromycin, lincomycin, clindamycin or tetracylines (Comunian et al., 2010; Federici et al., 2014; Landeta et al., 2013). This was also confirmed by the results of the present study. Only a few isolates were resistant to antibiotics from the above-mentioned groups. Likewise, Aymerich et al. (2006) demonstrated a low percentage of strains resistant to ampicillin and tetracycline when they analyzed isolates obtained from fermented semi-dried sausages.

To sum up, the antibiotics used in this study turned out to strongly and moderately inhibit the growth of lactic acid bacteria isolates. Innate resistance was reported, which may be perceived as a positive phenomenon (e.g., in persons subjected to therapies with antimicrobial drugs) because it can prevent sterilization of their gastrointestinal tract.

CONCLUSIONS

This study describes selected criteria used for the evaluation of potentially probiotic strains isolated from fermented food products and from feces. The susceptibility of the tested LAB isolates to the tested antibiotics varied and depended on the isolate. All isolates were resistant to vancomycin and metronidazole. Only five LAB isolates showed no proteolytic properties. Lipolytic properties were found in three isolates. Most of the tested isolates showed tolerance of low pH and bile salts, while coaggregation proved to be an eliminating factor due to low and average values. Genetic identification showed the diversity of isolates; the dominant species were *Lactobacilus casei* and *Lactobacillus acidophilus.*

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