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Assessment of Potential Probiotic and Starter Properties of *Pediococcus* spp. Isolated from Turkish-Type Fermented Sausages (Sucuk)

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In this study, the metabolic activities of five strains of *Pediococcus* spp., in terms of the quantities they produced of lactic acid, hydrogen peroxide, exopolysaccharides, and proteolytic activity, were determined. Lactic acid levels produced by these strains were found to be in the range of 2.5–5.6 mg/ml. All strains produced hydrogen peroxide. The *P. pentosaceus* Z13P strain produced the maximum amount (0.25 mg/ml) of proteolytic activity. Exopolysaccharide (EPS) production by the *Pediococcus* strains during growth in MRS (de Man, Rogosa, and Sharpe) medium was in the range 25–64 mg/l. The susceptibility of 10 different antibiotics against these strains was also tested. All strains were found to be resistant to amoxicillin, gentamicin, and vancomycin. Antimicrobial effects of the *Pediococcus* spp. on pathogens were also determined by an agar diffusion method. All of the strains were able to inhibit *L. monocytogenes*. The tolerance of the strains to low pH, their resistance to bile salts of strains, and their abilities to autoaggregate and coaggregate with *L. monocytogenes* were also evaluated.

Keywords: *Pediococcus* spp., probiotics, starter, fermented sausages

One of the oldest and most popular meat products in Turkey is a fermented sausage (sucuk) [15]. Fermented sausage is prepared from seasoned, raw meat that is stuffed in casings and is allowed to ferment and mature. Inoculation of the sausage batter with a starter culture composed of selected lactic acid bacteria (LAB) (*i.e.*, homofermentative lactobacilli and/or pediococci) and Gram-positive, catalase-positive cocci (*i.e.*, nonpathogenic, coagulase-negative staphylococci and/or kocuriae) improves the quality and safety of the final product and standardizes the production process [36]. Nonetheless, the use of a starter culture is not common in the production of sucuk in Turkey, and sucuk sample production is done by the traditional method of spontaneous fermentation without added starter culture [15].

LAB used in commercial starter cultures possess numerous metabolic characteristics, such as acidification activity, proteolytic activity, synthesis of bacteriocin, resistance to bacteriophage, and production of exopolysaccharide, which are strain dependent. All of these important activities contribute to the flavor, texture, and frequently the nutritional attributes of the products [30]. These bacteria inhibit spoilage microorganisms and forborne pathogens in the natural flora of meat. The inhibitory activities of LAB are due to its ability to produce different acids and metabolites: These are lactic acid and acetic acid-like organic acids [44], H_2O_2 [55], bacteriocin and bacteriocin-like substances [5], diacetyl, and CO_2 [14].

Probiotics are defined as live microorganisms that contribute to the health and well-being of the hosts by maintaining or improving their intestinal microbial balance [4]. Among these microorganisms, LAB are already used in many probiotic dairy products. To remain and exert probiotic potential within their host, two factors are usually considered. First, probiotic strains must possess the ability to overcome the extremely low pH and the detergent effect of bile salts and arrive at the site of action in a viable physiological state [11]. Second, they should be capable of adhering to the intestinal mucosa and coaggregation. The adherence to intestinal mucosa is indispensable for colonization of probiotics [7]. The most common use of probiotic microorganisms is in fermented dairy products [38] although recently a new idea is developing to use probiotic strains in other foods such as fermented meat products [41]. Many scientists have recently proposed the use of probiotic meat starter cultures for dry fermented sausage manufacture [32, 41]. However, the number of reports related to select probiotic strains [22] or the potential probiotic use of some selected strains [23] in dry fermented sausages are few.

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Technologically interesting strains to use as starters are usually selected from the food products they are going to be employed for. Therefore, to determine the potential to use probiotics in dry fermented sausages, in this study, the features of *Pediococcus* spp., including their lactic acid, H_2O_2, proteolytic activity, EPS (exopolysaccharide) production, acid resistance, bile tolerance, antibiotic resistance, and autoaggregation/coaggregation, were investigated. Inhibition of strains on the tested pathogens was also evaluated. These evaluations were performed as an initial step toward establishing rational criteria for screening and selecting foodborne microorganisms with human probiotic properties.

Materials and Methods

Isolation and Identification of *Pediococcus* spp. from Sucuk

Pediococcus spp. were isolated from 15 different Turkish sucuk samples. Tomato Juice agar (Oxoid, Basingstoke, U.K.) was used for isolation of pediococci [50]. In preparation for experiments, the cells were cultured in MRS broth (Oxoid, Basingstoke, U.K.) at 30°C. Initial characterization of isolates included colony and cell morphology. Gram-positive and catalase-negative isolates were stored at −80°C in MRS broth with glycerol added [70/30 (v/v); Merck]. The isolates were then characterized by their growth at various temperatures (10°C, 45°C, and 50°C), tolerance to different concentrations of salt (4%, 6.5%, and 18% NaCl), and production of gas from glucose and hydrolysis of arginine [27]. Using the API 50 CH galleries (bioMerieux, Marcy-l'Etoile, France), the ability of the isolates to ferment carbohydrates was studied. Results were recorded after passage of 24 and 48 h at 30°C. Species were determined tentatively through the use of APILAB PLUS (Version 3.2.2., bioMerieux) and standard taxonomic descriptions from Wood and Holzapfel [52]. According to the results of the identification tests, altogether five strains of the 35 lactic acid bacteria (LAB) isolates obtained from the Turkish-type fermented sausage (sucuk) samples were identified as *Pediococcus* spp.

Determination of Metabolic Properties of *Pediococcus* spp. Strains

The acid-producing ability of the strains was determined using a method described by Demirci and Gunduz [17]. Measurements of titratable acidity, expressed as g lactic acid/ml, were recorded at 42 h.

Hydrogen peroxide was determined spectrophotometrically according to Patrick and Wagner [40]. Measurements were obtained after a 24-h incubation period in skim milk, and the production was monitored at OD_{350}. H_2O_2 was quantified using a H_2O_2 standard curve, employing concentrations ranging from 1 to 10 μg/ml.

Proteolytic activity of the strains was determined by measuring the absorbance in 10% (w/v) skim milk at 630 nm with a spectrophotometer (Hitachi U-1800) at the conclusion of incubation at 30°C for 42 h [12, 37]. The results are expressed in mg tyrosine/ml, in compliance with a standard curve.

The EPS production of *Pediococcus* strains was determined after they were incubated until OD_{600} of 1.04 and 1.50 (the end of the exponential growth phase, about 18 h) at 30°C. The cultures were boiled at 100°C for 10 min. After cooling, they were treated with 17% (v/v) of 85% trichloracetic acid solution and centrifuged [24]. Cells and protein were removed by centrifugation. Ethanol was used to precipitate the EPS, which was then recovered by centrifugation at 4°C at 18,000 ×*g* for 20 min. Total EPS (expressed as mg/l) was estimated in each sample by the phenol-sulfuric acid method [19] using glucose as the standard [48].

Acid Resistance and Bile Tolerance

Cultures were grown in MRS broth at 30°C for 24 h. A 100-μl aliquot of bacterial culture (8.9–9.1 log CFU/ml) was inoculated into 10 ml of sterile phosphate-buffered saline (PBS: NaCl 9 g/l, $Na_2HPO_4 \cdot 2H_2O$ 9 g/l, KH_2PO_4 1.5 g/l, pH 6.2). One experimental series contained saline tubes with the following pH values: 1.0, 1.5, 2.0, 2.5, 3.0, and 6.2 (control) (adjusted using 10 M HCl). Samples were incubated for 2 h at 30°C. Cells were serially diluted 10-fold in physiological saline solution and the residual viable population was determined by plate counting on MRS agar after 24 h incubation at 30°C. The survival rate was calculated as log values of colony-forming units per ml (CFU/ml) and as a percentage of the colonies grown on MRS agar compared with the initial bacterial concentration.

To determine the bile tolerance of strains, each strain was grown in MRS broth at 30°C for 24 h. A 0.25-ml aliquot of bacterial suspension (8.9–9.1 log CFU/ml) was inoculated into 5 ml of sterile MRS broth (7.4–7.6 log CFU/ml after inoculation). After incubation, cells were harvested by centrifugation (10,000 ×*g*, 10 min), washed three times, and resuspended in PBS. An aliquot of 100 μl of bacterial suspension was initially added into 900 μl of bile salts of concentrations 0.15%, 0.3%, 2% and another without bile (control), which was a standardized mixture of bile salts prepared from ox bile (Sigma), and incubated at 30°C for 2 h. Survival cell counts were then determined by plating, as described previously [20].

Resistance to Antibiotics

Susceptibility testing was based on the agar overlay disc diffusion test described by Charteris *et al.* [10]. *Pediococcus* spp. strains were grown overnight in MRS broth at 30°C (0.5 McFarland, after inoculation). The following petri plates containing 15 ml of cysteine–MRS agar were overlaid with 100 μl of an active culture at 30°C. Antibiotic discs (Oxoid) were placed on the inoculated plates under sterile conditions. After incubation for 24 h at 30°C, the diameter of the inhibition zone was measured with calipers. All isolates were screened for their susceptibility to amoxicillin (25 μg), penicillin G (10 units), ampicillin (10 μg), tetracycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), vancomycin (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), and lincomycin (2 μg). Breakpoints for the interpretation of inhibition zone were those defined by the Clinical and Laboratory Standards Institute [13].

Antimicrobial Activity

Antimicrobial effects of all strains on *Listeria monocytogenes* ATCC 7644 (American Type Culture Collection), *Escherichia coli* O-157:H7 (Clinical isolate), *Staphylococcus aureus* ATCC 25923, *Micrococcus flavus* ATCC 14452, *Micrococcus luteus* NRLL-B 4375 (Northern Utilization Research and Development Division), and *Yersinia enterocolitica* ATCC 1501 were determined by the agar diffusion method [43]. Test microorganisms and *Pediococcus* spp. strains were propagated twice and then grown for 18–24 h in 10 ml of appropriate growth media. Turbidity of the culture broth was compared

with McFarland tubes to give an estimate of bacterial population (1×10^6 CFU/ml). Activated *Pediococcus* cultures were centrifuged (4,000 $\times g$ for 15 min) and the clear supernatant was sterilized by filtration (0.45 μm), thus yielding cell-free filtrates. Petri dishes were prepared with 20 ml of nutrient agar (Oxoid), previously inoculated with 0.1 ml of a 24-h nutrient broth culture of test bacteria. Once solidified, the dishes were stored for 2 h in a refrigerator. Four wells (ø 6 mm) were then prepared and filled using 100 μl of cell-free filtrate. The inoculated plates were incubated for 24 h at appropriate temperatures, and the diameter of the inhibition zone was measured in millimeters with calipers. The measurements recorded were from the edge of the zone to the edge of the wall.

Aggregation Experiments

Aggregation experiments were performed as described by Ekmekçi *et al.* [49], with some modification. Activated cultures were harvested by centrifugation at 10,000 $\times g$ for 15 min and washed twice in phosphate-buffered saline (PBS) containing the following (expressed in grams per liter): NaCl, 8; KH_2PO_4, 0.34; and K_2HPO_4, 1.21 (pH 6.0). The cultured cells were then resuspended in PBS to give a final optical density of 0.60±0.02 at 600 nm, as measured by a spectrophotometer.

For coaggregation experiments, 2 ml of each *Pediococcus* spp. suspension was mixed with 2 ml of the *L. monocytogenes* ATCC 7644 suspension for at least 10 s on a vortex mixer. After 4 h of incubation at room temperature, the suspensions were measured with a spectrophotometer (600 nm), and coaggregation was expressed as follows:

$$\% \text{ Coaggregation}=[(OD_1+OD_2)-2(OD_3)/(OD_1+OD_2)\times100]$$

where OD_1 is the optical density of strain 1 (*Pediococcus*), OD_2 is the optical density of strain 2 (*L. monocytogenes* ATCC 7644), and OD_3 is the optical density of mixed strains 1 and 2 after 4 h.

Controls for the autoaggregation experiments consisted of 2 ml of each strain, with OD measured at 600 nm after 4 h of incubation at room temperature. The percent autoaggregation was expressed as follows:

$$\% \text{ Autoaggregation}=[(OD_1-OD_2)/(OD_1)\times100]$$

where OD_1 is the first optical density, and OD_2 is the optical density after 4 h. Thereafter, all of the suspensions were examined by inversion light microscopy and scored for aggregation (++, good aggregation; +, partial aggregation).

Statistical Analysis

All experiments were done in triplicate, and mean values are presented. Statistical analysis was performed on the data using SPSS 11.0 Bivariate Correlation Analysis (SPSS Inc., Chicago, IL, U.S.A.) with statistical significance determined at $p<0.01/0.05$. The Pearson rank order correlation test was used for comparisons between EPS production and proteolytic activity ($p<0.01$), acid resistance ($p<0.05$), bile tolerance ($p<0.05$), and aggregation ($p<0.05$).

RESULTS AND DISCUSSION

A probiotic is a culture of living microorganisms (mainly lactic acid bacteria or bifidobacteria), which when ingested in certain amounts beneficially influence the health of the host [21]. These bacteria are believed to provide several health benefits to human beings, including improvement of lactose [30], reduction in blood cholesterol [18], prevention of travelers' diarrhea and diarrhea associated with antibiotics [45], stimulation of the immune system [30], and inhibition of invasion and infection by pathogens [25].

A total of 5 strains of 35 lactic acid bacteria isolates obtained from the Turkish-type fermented sausages (sucuk) samples were identified as *Pediococcus pentosaceus* (3 strains), *P. acidilactici* (1), and *P. dextrinicus* (1). All these five strains were tested for their lactic acid production, hydrogen peroxide, proteolytic activity, and EPS production.

Lactic acid bacteria have long been used in the food industry as starter cultures for the manufacture of dairy and meat products. The major by-product of fermentation, lactic acid, is also a commercially valuable product with applications in the food, manufacturing, and pharmaceutical industries [9]. The acid levels produced by *Pediococcus* spp. in skim milk are shown in Table 1. The *P. dextrinicus* Z11P strain produced the highest quantity (5.6 mg/ml), whereas the *P. pentosaceus* Z9P strain produced the lowest (2.5 mg/ml). The production of lactic acid was found to be strain-dependent. Other authors have obtained similar results. Toksoy [47] and Yaman *et al.* [54] reported that the highest and lowest levels of lactic acid produced by *Pediococcus* spp. isolated from sucuk samples ranged between 5.0 and 7.5 mg/ml, and 3.20 and 7.75 mg/ml, respectively.

Hydrogen peroxide is one of the primary metabolites that may be produced by lactic acid bacteria and that may contribute to their antagonistic action. Production of H_2O_2 is deemed beneficial for food preservation and prevention of pathogen growth [55]. The quantity of hydrogen peroxide produced by LAB varies, depending on the strain and the availability of oxygen [28]. The levels of H_2O_2 induced by the *Pediococcus* strains ranged from 2.3 to 8.0 μg/ml. The H_2O_2 production of *P. pentosaceus*

Table 1. Exopolysaccharides production and metabolic activities of *Pediococcus* strains.

Strains	Acid (mg/ml)	Hydrogen peroxide (μg/ml)	Proteolytic activity (mg/ml)	EPS production (mg/l)
P. pentosaceus Z9P	2.5±0.0	2.3±0.0	0.06±0.00	25±0
P. pentosaceus Z12P	2.9±0.1	7.0±0.0	0.17±0.00	44±2
P. pentosaceus Z13P	2.8±0.0	8.0±0.2	0.25±0.01	64±0
P. acidilactici Z10P	4.6±0.1	6.2±0.2	0.13±0.01	35±0
P. dextrinicus Z11P	5.6±0.0	5.3±0.3	0.12±0.02	32±1

Z13P was the highest (Table 1). Toksoy [47] revealed that 26 *Pediococcus* cultures produced small quantities of hydrogen peroxide in skim milk (the maximum being 1.24 μg/ml).

Proteolytic bacteria produce free amino acids that can be a source of metabolic substrate for other microorganisms and result in the production of secondary metabolites including flavored compounds [56]. The results obtained for proteolytic activities indicate that the highest was 0.25 mg/ml (*P. pentosaceus* Z13P) and the lowest proteolytic activity was 0.06 mg/ml (*P. pentosaceus* Z9P) (Table 1). Yaman *et al.* [54] found that in different studies of strains, different values of proteolytic activity were produced; however, proteolytic activity levels were in the range of 0.13 to 0.21 mg/ml.

All the five strains of *Pediococcus* isolated from Turkish-type fermented sausages (sucuk) were found to produce exopolysaccharides in MRS medium. The levels of EPS produced by the five strains are shown in Table 1. Cultures identified to be of the same species isolated did not exhibit similar exopolysaccharide production characteristics. For example, whereas the *P. pentosaceus* Z13P strain produced the highest quantity of EPS (64 mg/l), the *P. pentosaceus* Z9P strain produced the lowest (25 mg/l). Walling *et al.* [51] reported that EPS produced by *P. damnosus* IOEB8801 was 102 mg/l. Aslim *et al.* [6] indicated that the EPS produced by lactobacilli strains during growth in MRS medium ranged between 21 and 211 mg/l, whereas that produced by streptococci strains during growth in M17 medium was between 16 and 114 mg/l. Our results revealed that *Pediococcus* strains produce comparatively lower quantities of EPS, and in this respect varied from the findings of other researchers who found higher values.

Moreover, both the type and concentration of nitrogen sources in the growth medium are known to influence the quantity of EPS produced by LAB [6]. However, the strain displays a very low proteolytic activity, which is probably the main reason for its slow growth and hence low EPS production. These enzymes are also very important for the development of flavors in fermented products. Moreover, proteolysis can influence rheological parameters and viscosity [42]. In this study, the relationship between EPS production and proteolytic activity was found to be fairly consistent; that is, the high proteolytic activity is consistent with high EPS production. *P. pentosaceus* Z13P produced 64 mg of EPS/l in MRS, having a proteolytic activity of 0.25 mg/ml. Our results showed that the correlations observed between the quantity of EPS produced by the strains and their proteolytic activities were significant ($p<0.01$). Pailin *et al.* [39] and Aslim *et al.* [6] showed that there are strong positive correlations between the capsule sizes of EPS and the proteolytic activities of both *Streptococcus* and *Lactobacillus* cultures.

The viability of probiotic bacteria is the most important parameter in order to provide therapeutic functions. A number of factors have been claimed to affect the viability of probiotic bacteria in fermented foods, including low pH value and bile salts. For their use as potential probiotics, lactic acid bacteria strains have to be screened for their capacity of transit tolerance to the low pH. Low pH is known to be an effective barrier to the entry of bacteria into the intestinal tract [11].

The effects of pH (1.0–3.0) on the *Pediococcus* strains were tested and the number of viable cells and survival percentage at each pH value was determined (Table 2). For all strains, there was a decrease in the number of viable cells after 1 h of incubation at pH values of 3.0 and 2.5. The viable counts of *P. pentosaceus* Z12P and Z13P strains were higher, whereas those of *P. pentosaceus* Z9P, *P. acidilactici* Z10P, and *P. dextrinicus* Z11P were lower at pH value of 3.0. Whereas the *P. pentosaceus* Z12P and Z13P strains reduced viability at pH values of 1.0 and 1.5, *P. pentosaceus* Z9P, *P. acidilactici* Z10P, and *P. dextrinicus* Z11P lost their viability at pH values of 1.0 and 1.5. Erkkilä and Petäjä [20] found that the strains of *Pediococcus acidilactici* (P2), *Lactobacillus curvatus* (RM10), and *P. pentosaceus* (FF) proved to be most acid-tolerant. Little or no effect was seen at the pH value of 3.0 with these strains.

Table 2. *Pediococcus* strains, incubated at pH values ranging from 6.2 to 1.0, and the number of viable cells (log CFU/ml) and survival percentage at each pH value.

	Z9P		Z12P		Z13P		Z10P		Z11P	
	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition
Control (6.2)	11.9±0.2		11.4±0.0		11.9±0.0		11.9±0.1		11.3±0.0	
pH 3.0	9.5±0.3	80	10.5±0.1	92	10.5±0.1	88	9.0±0.0	76	9.9±0.1	87
pH 2.5	8.8±0.1	74	8.3±0.0	73	9.1±0.0	76	6.9±0.1	58	6.6±0.0	58
pH 2.0	0.0	0	6.6±0.1	58	8.8±0.0	74	4.4±0.2	37	4.1±0.1	36
pH 1.5	0.0	0	5.9±0.2	52	4.5±0.2	38	0.0	0	4.4±0.0	39
pH 1.0	0.0	0	4.3±0.0	38	3.0±0.1	25	0.0	0	0.0	0

$$\% \text{ Inhibition} = \frac{\text{final(CFU/ml)}}{\text{control(CFU/ml)}} \times 100$$

Table 3. Log CFU/ml and survival percentage of *Pediococcus* spp. after 2 h in PBS containing bile salt (of concentrations 0.15%, 0.3%, and 2%).

	Z9P		Z12P		Z13P		Z10P		Z11P	
	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition	log CFU/ml	% Inhibition
Control[a]	7.0±0.1		8.0±0.2		9.6±0.0		8.8±0.1		6.7±0.0	
0.15%	2.6±0.0	37	6.2±0.0	78	7.4±0.2	77	1.5±0.1	17	2.4±0.2	36
0.3%	1.6±0.1	23	4.4±0.1	55	6.2±0.0	65	0.5±0.0	6	1.6±0.0	24
2%	0.5±0.2	7	3.2±0.0	4	4.3±0.1	45	0.4±0.1	4	0.4±0.1	6

[a]Control did not receive ox bile.

$$\% \text{ Inhibition} = \frac{\text{final(CFU/ml)}}{\text{control(CFU/ml)}} \times 100$$

Our results also showed that the correlations between EPS quantity produced by these strains and their acid resistance were significant ($p<0.05$).

The number of viable cells and survival percentage at different concentrations of bile salts (0.15%, 0.3%, and 2%) were selected. The results are shown in Table 3. These results indicate that bile salts at all three concentrations (0.15%, 0.3%, and 2%) affected the viability of the strains. The *P. pentosaceus* Z13P strain showed a viability of 65% and 45% at bile salts concentrations of 0.3% and 2%, respectively. After exposure to all three bile salts concentrations of 0.15%, 0.3%, and 2%, the greatest viability was found to be in strain Z13P with the highest EPS producing capacity. The low EPS-producing *P. pentosaceus* Z19P strain was also observed to have an inhibitory effect on the survival of cells (7%) when the bile salt concentration was 2%. There was significant ($p<0.05$) correlation between the quantity of EPS produced by the strains and their bile tolerance. Erkkilä and Petäjä [20] reported that the strains of *P. acidilactici* (P2), *L. curvatus* (RM10), and *L. sake* (L2) were the most resistant to bile salts of 0.3% concentration.

In the current study, the *P. pentosaceus* Z13P (25%) strain was shown to survive well at pH value of 1.0. Furthermore, the *P. pentosaceus* Z13P (45%) strain was shown to survive well at the bile salt concentration of 2%. Xanthopoulos *et al.* [53] tested *L. acidophilus, L. gasseri, L. rhamnosus,* and *L. reuteri,* isolated from infant feces, for their ability to tolerate low pH and bile salt. Survival characteristics of the tested strains ranged between 0.01% and 68.3% at pH value of 3.0 and between 10.3% and 57.4% in bile salts of 0.15% concentration. Another study has shown that all cells of *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* lose their viability at a pH value of 2.0 and in bile salt of 0.2% concentration [33]. When we compare our results with these, our strains exhibit greater effectiveness at low pH and bile salt concentration. This finding is very important for selection of probiotic bacteria. We have suggested that the bacterial EPSs play a role in the protection of microbial cells against low pH and bile salts. This aspect has not been investigated so far.

Ingestion of antibiotics often disrupts the healthy balance of residential microflora in the host, causing intestinal disorders. The administration of antibiotic-resistant strains can help retain the normal bacterial ratio in the intestines, or its fast restoration if administered after the antibiotic treatment [31]. The resistance of the five *Pediococcus* strains to 10 antibiotics was tested. All strains were subjected to antibiotic susceptibility testing using the agar disc diffusion method, the results of which are presented in Table 4. The *P. pentosaceus* Z12P and Z13P strains proved to be resistant to the inhibitors of the cell wall synthesis, penicillin G and ampicillin, as well as to the glycopeptide vancomycin. Gotcheva *et al.* [26] reported that vancomycin resistance is an intrinsic property of many LAB. All strains are resistant to vancomycin, amoxicillin, and gentamicin. Tetracycline, erythromycin, clindamycin, lincomycin, and chloramphenicol antibiotics, which inhibit protein synthesis, affected most of the strains. Beyatli *et al.* [8] obtained similar results. Interestingly, however, similar studies by other researchers

Table 4. Antibiotic resistance of *Pediococcus* spp. isolates from sucuk.

Strains	AX	P	AM	TE	E	CN	VA	C	CC	L
P. pentosaceus Z9P	-	+	++	-	+	-	-	++	-	-
P. pentosaceus Z12P	-	-	-	+	++	-	-	++	++	++
P. pentosaceus Z13P	-	-	-	+	+	-	-	++	++	++
P. acidilactici Z10P	-	++	++	++	-	-	-	++	-	-
P. dextrinicus Z11P	-	+	++	++	++	-	-	-	-	-

Susceptibility is expressed as resistant (-), moderately susceptible (+), and susceptible (++).
AX: amoxicillin; P: penicillin; AM: ampicillin; TE: tetracycline; E: erythromycin; CN: gentamicin; VA: vancomycin; C: chloramphenicol; CC: clindamycin; L: lincomycin.

Table 5. Antimicrobial activity of *Pediococcus* spp. strains against pathogenic bacteria.

	Inhibition zone (diameter, mm) against pathogenic bacteria		
Strains	*L. monocytogenes*	*E. coli* O-157: H7	*M. flavus*
Z9P	16.7±0.3	2.6±0.0	-
Z12P	19.0±0.0	-	-
Z13P	10.0±0.0	-	6.3±0.3
Z10P	17.8±0.8	5.0±0.2	-
Z11P	19.4±1.4	-	-

Values are the means±standard deviations of triplicate measurements.
-: No inhibition zone.

have concluded that lactic acid bacteria were sensitive against the above-mentioned antibiotics [2, 46].

An important property of probiotic strains is their antagonistic activity against pathogenic bacteria. Lactic acid bacteria can produce antimicrobial substances capable of inhibiting the growth of pathogenic and spoilage microorganisms. Organic acids, hydrogen peroxide, diacetyl, and bacteriocins are included among these compounds [29]. The strains isolated from Turkish-type fermented sausages (sucuk) were assayed for their ability to produce inhibitory substances against the growth of *L. monocytogenes* ATCC 7644, *E. coli* O-157:H7, *S. aureus* ATCC 25923, *M. flavus*, *M. luteus* NRLL-B 4375, and *Y. enterocolitica* ATCC 1501 (Table 5). All of the *Pediococcus* strains inhibited growth of *L. monocytogenes* ATCC 7644. However, the *Pediococcus* strains did not show antimicrobial activity against *S. aureus* ATCC 25923, *M. luteus* NRLL-B 4375, and *Y. enterocolitica* ATCC 1501. In addition, whereas the growth of *E. coli* O-157:H7 was inhibited by two strains (*P. pentosaceus* Z9P and *P. acidilactici* Z10P), the other strains did not inhibit the growth of *E. coli* O-157:H7. Besides this, whereas the growth of *M. flavus* was inhibited by one strain (*P. pentosaceus* Z13P), the other strains did not exhibit this tendency. Among the *Pediococcus* strains, the greatest antimicrobial activity, of 19.4 mm, was against *L. monocytogenes* ATCC 7644, and the least, of 2.6 mm, was against *E. coli* O-157:H7. Çon *et al.* [15] reported four strains of *Pediococcus* isolated from sucuk. The results of direct inhibition tests indicated that these four strains inhibited the *in vitro* growth of 16 *Listeria* strains.

Aroutcheva *et al.* [3], Annuk *et al.* [1], and Yuksekdag *et al.* [55] have revealed that no correlations were found between bacteriocin activity, lactic acid, and hydrogen peroxide production. They found *Lactobacillus* strains produced H_2O_2 but did not demonstrate any inhibitory effect. Similar results were obtained in this study; the *P. pentosaceus* Z13P strain produced the most H_2O_2 and small quantities of lactic acid. The strain also displayed inhibitory effects against *L. monocytogenes* and *M. flavus* but none against *E. coli, M. luteus*, and *Y. enterocolitica.*

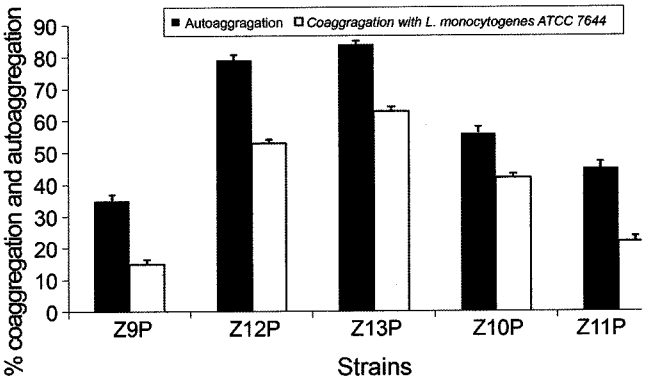

Fig. 1. Aggregation score for *Pediococcus* strains incubated alone (autoaggregation %), and with *L. monocytogenes* ATCC 7644 (coaggregation %).

Probiotic strains are often selected as they normally inhabit intestines of the host and they possess several beneficial properties, such as the hydrophobicity of the bacterial cell surfaces, the presence of substances with capacity to adhere to the epithelium (*e.g.*, polysaccharides, lectins), and the aggregation ability. Autoaggregation of probiotic strains appears necessary for their adhesion to intestinal epithelial cells, with coaggregation presenting a barrier that prevents colonization by pathogenic microorganisms [7]. There are no clear reports about autoaggregation and coaggregation of *Pediococcus* strains.

Fig. 1 presents the results of autoaggregation, and coaggregation of the five *Pediococcus* strains with *L. monocytogenes* ATCC 7644. The autoaggregation abilities of the *Pediococcus* strains ranged between 35% and 84%. The high EPS-producing *P. pentosaceus* Z12P and Z13P strains showed greater autoaggregation (79% and 84%, respectively) than the other strains. Moreover, our results showed that the correlations between the EPS production quantity of the strains and autoaggregation were significant ($p<0.05$). The coaggregation ability of the *Pediococcus* strains with *L. monocytogenes* ATCC 7644 was also evaluated at OD_{600}, and these results were confirmed by light microscopy. The coaggregation scores ranged from good/++ (Z12P and Z13P) to partial/+ (Z9P, Z10P, and Z11P). Our results showed that the correlations between the EPS quantity of the strains produced and coaggregation were significant ($p<0.05$). Studies on the mechanism of autoaggregation in lactobacilli reveal that the proteins present in the culture supernatant and the proteins or lipoproteins and polysaccharides located on the cell surfaces are involved in cell aggregation [7, 34]. Kumar and Anand [35] reported that the adhesion of bacterial cells increased with EPS production. Del Re *et al.* [16] showed that the autoaggregation ability in *Bifidobacterium longum* strains ranged from ≥89 to ≤10% after 2 h of incubation at 37°C. When we compared our results with these findings, autoaggregation phenotypes were identified and defined as

follows: Strongly autoaggregating strains (Z12P and Z13P) showed a high autoaggregation percentage (≥79%), aggregated immediately, and formed a precipitate that resulted in a clear solution. In contrast, strains Z9P and Z11P showed autoaggregation percentages of 35% and 45%, respectively, and their suspension showed both a precipitate and constant turbidity: these results were verified by light microscopy.

The present study describes the metabolic (lactic acid, hydrogen peroxide, EPS, proteolytic activity) and antimicrobial activities and antibiotic resistance of *Pediococcus* strains isolated from Turkish-type fermented sausages (sucuk). We also determined that these *Pediococcus* strains are capable of surviving at low pH values, survive bile salts, and are able to autoaggregate and coaggregate with *L. monocytogenes*, all of which make them potential probiotics. Of course, these strains do require further *in vitro* and *in vivo* investigations in areas such as adhesion to cultured human intestinal epithelial cells. After conducting studies on their specific technological properties in meat fermentation, the strains found suitable may be eventually used as probiotic starter cultures in dry fermented sausage manufacture.

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