



# Antibiotic susceptibility profile of *Pediococcus* spp. from diverse sources

Varsha Singla<sup>1</sup> · Surajit Mandal<sup>1</sup> · Poonam Sharma<sup>1</sup> · Santosh Anand<sup>1</sup> · Sudhir Kumar Tomar<sup>1</sup>

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## Abstract

The aim of the present study was to assess the antibiotic susceptibility profile of *Pediococcus* strains from diverse sources. From a total of 115 dairy and non-dairy samples, 40 *Pediococcus* strains were isolated. Their biochemical and molecular characterization confirmed them as *P. pentosaceus* and *P. acidilactici*. All the 40 identified isolates were evaluated for antibiotic susceptibility using disc diffusion assay against a total of 20 antibiotics. The isolates exhibited varied range of responses towards the antibiotics depending on the strain type, source and location of isolation. All the isolates were either sensitive or intermediate resistant to amoxicillin, erythromycin, ceftriaxone, cloxacillin, cefoperazone, penicillin, netillin, gentamycin and chloramphenicol. Resistance towards vancomycin and nalidixic acid was exhibited by most of the isolates. A total of 16 strains belonging to dosa batter ( $n=4$ ;  $n$  = number of isolates), fermented vegetables ( $n=4$ ), fermented grape juice ( $n=4$ ), idly batter ( $n=3$ ) and the only isolate from butter milk exhibited sensitivity/intermediate towards 80–90% of the studied antibiotics. No considerable difference in susceptibility pattern was observed between the two *Pediococcus* species, i.e., *P. pentosaceus* and *P. acidilactici*. Overall, the maximum resistance was exhibited by isolates belonging to silage (Sil-2; 50%) followed by cow milk (PD-41), dosa batter (8-PD) and human isolate (PD-45) which showed resistance towards 40% of studied antibiotics. The susceptibility profiling of *Pediococcus* strains will be helpful in their safer selection for future food and feed applications.

**Keywords** *Pediococcus* · Antibiotics · Resistance · Lactic acid bacteria · Susceptibility · Food safety

## Introduction

Lactic acid bacteria (LAB) are industrially important microorganisms because of long and safe history of their use for the production of numerous fermented and functional foods (Leroy and De Vuyst 2004; Rhee et al. 2011). Beside others, *Pediococcus* is one of the important LAB which divide alternatively in two perpendicular directions in a single plane to form peculiar tetrads. They are characterized as

Gram-positive, catalase negative, non-motile, chemo-organotrophs and homofermentative (Singla et al. 2018). *Pediococcus pentosaceus* and *P. acidilactici* are among the two most widely occurring species in food and dairy environments (Banwo et al. 2013). Several technological aspects such as antimicrobial activity, phytase activity, galactose fermentation ability, exopolysaccharide (EPS) production, and flavor production make these cultures industrially useful microbes. Pediocin, the anti-listerian antimicrobial peptide produced by this particular genus is the most exploited trait allowing this organism to be used as a bio-preservative in food and dairy industry (Papagianni and Anastasiadou 2009). Beyond technological importance, strains of pediococci also act as safe probiotics (Singla et al. 2017). Due to this techno-functionality, pediococci are used either as starter, non-starter or adjunct probiotic cultures in vegetables, meat, sausages, silage, milk and milk products (Gurira and Buys 2005; Papagianni and Anastasiadou 2009; Yuksekdag and Aslim 2010; Garsa et al. 2014; Porto et al. 2017).

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✉ Varsha Singla  
varshagarg9@gmail.com

✉ Surajit Mandal  
mandalndri@rediffmail.com

<sup>1</sup> TFSL, Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana 132-001, India

Resistance to antibiotics is an emerging food safety concern and the potential of bacteria to transfer the antibiotic resistance genes to pathogenic or commensal bacteria cannot be neglected. Hence, antibiotic sensitivity profiling of bacterial strains intended for their use as starter or probiotic cultures is considered as an important part of safety assessment (Sharma et al. 2014, 2016). European Food Safety Authority (EFSA) recommends that bacterial strains harboring transferable antibiotic resistance genes (acquired resistance) should not be used in animal feeds, fermented and probiotic foods for human use (EFSA 2007). *Pediococci* are mainly found to be susceptible to clinically important antibiotics, and no acquired resistance to these antimicrobials has been reported (Danielsen et al. 2007; Klare et al. 2007) and is also for multidrug resistivity (Cao et al. 2016; Gupta and Sharma 2017). Vancomycin resistance is widespread among *Pediococci*, but fortunately, it is considered as intrinsic due to a modified precursor ending in D-Ala-D-lactate (Banwo et al. 2013). Similarly, resistance to aminoglycosides such as kanamycin, gentamicin and streptomycin is also an intrinsic property among *Pediococcus* spp. (Hummel et al. 2007). Penicillin, chloramphenicol and erythromycin are usually active antibiotics against *Pediococcus* spp. (Danielsen et al. 2007). *Pediococci* are generally considered as safe with regard to antibiotic susceptibility, yet each and every newly isolated strain should be well-assessed for antibiotic susceptibility prior to their applications in food and dairy products.

Previous studies (Ruiz-Moyano et al. 2010; Cao et al. 2016) have also reported the susceptibility pattern of *Pediococcus* spp., but a comprehensive evaluation of a large number (40) of *Pediococcus* strains of diverse origins against as many as 20 antibiotics is scarce. Therefore, the present study was focused on isolating and characterizing *Pediococcus* spp. from dairy and non-dairy sources, and assessing their antibiotic susceptibility pattern. Thus, the outcome of the present investigation will enable the selection of *Pediococcus* strains for their future intended applications.

## Materials and methods

### Isolation and characterization of *Pediococcus* spp.

A total of 115 samples comprising fermented batter of dosa, idli, kadhi, jalebi, raabdi, uttapam; pickles; dairy products including cow milk, buffalo milk, cheese, curd and cream were collected from hostels of Indian Council of Agricultural research-National Dairy Research Institute (ICAR-NDRI), Karnal, Institute's Experimental Dairy Plant, local market, household preparations, rural and urban areas of Karnal, and also from Delhi, Hisar and Jind, India. Silage samples were collected from cattle yard, ICAR-NDRI, Karnal; human feces and human milk, from voluntaries

of ICAR-NDRI, Karnal; fermented vegetables (cabbage and carrot) and fermented grape juices were prepared in our laboratory. Samples were collected in sterile sample bottles (HiMedia, Mumbai, India). Serial dilutions of the processed samples were prepared in normal saline (0.85% NaCl). Appropriate dilutions were pour plated using de Mann Rogosa Sharpe (MRS) agar medium and incubated at 37 °C for 48–72 h. Typical smooth, round, grayish-white colonies were randomly picked up, transferred to MRS broth and incubated at 37 °C for 18–24 h. After incubation, all the isolates were examined under microscope (100×) for morphology.

Gram-positive cocci in pairs/tetrads were further subjected to a series of physiological tests. Catalase, pseudocatalase, oxidase, arginine hydrolysis, hetero-fermentation tests, growth at different temperatures (30, 37, 42 and 50 °C), growth at different pH values (4.2, 6.5, 8.5 and 9.6) and growth at different salt concentrations (4, 6.5, 10 and 15% NaCl) were conducted for the preliminary characterization of the isolates. Hetero-fermentation test was performed in MRS broth with immersed inverted Durham's tubes (Muller 1990). For pseudocatalase test, active cultures were streaked on low glucose agar plates and incubated at 37 °C for 48–72 h. Plates were then flooded with 5 mL of 3% hydrogen peroxide (Sigma-Aldrich, USA). The presence of effervescence indicated positive for pseudocatalase activity (Felton et al. 1953). Oxidase test was performed by rubbing the activated cultures on oxidase disc (HiMedia, India). Development of purple blue color indicated a positive reaction. Hydrolysis of arginine was examined by inoculating the active cultures in arginine broth and incubated at 37 °C for 24–48 h. Release of ammonia from arginine was tested by adding 1 mL of Nessler's reagent (HiMedia, India). Appearance of orange-brown to brick-red color was considered positive for arginine hydrolysis (Hitchener et al. 1982). Putative *Pediococcus* isolates based on morphology and physiological examination were biochemically identified by carbohydrate fermentation pattern using API 50CH test kits (bioMerieux, France; Singla et al. 2018).

### Molecular confirmation of isolated *Pediococcus* strains

Biochemically identified *Pediococcus* isolates were further subjected to molecular characterization by polymerase chain reaction (PCR). DNA was extracted following the protocol of Pospiech and Neumann (1995). Extracted DNA was electrophoresed in 0.8% (w/v) agarose gel and was visualized with ultraviolet (UV) illumination. The isolated DNA of each strain was used to perform PCR using genus and species-specific forward and reverse oligonucleotide primers (Table 1) purchased from Sigma-Aldrich (Pfannebecker and Frohlich 2008). First, the genus-specific PCR was

**Table 1** Genus and species-specific primers and PCR cycling conditions for identification of *Pediococcus* spp

Organism	Primers	Primer sequence (5–3')	PCR cycle conditions	Amplicon size (bp)	Reference
Genus <i>Pediococcus</i>	Pedio23S_F	–GAACTCGTGACGTTGAAAAG TGCTGA–	95 °C/5 min, 94 °C/30 s, 69.5 °C/30 s, 72 °C/30 s, 72 °C/5 min, 32 cycles	701	Pfannebecker and Frohlich 2008
	Pedio23S_R	–GCGTCCCTCCATTGTTCAAAC AAG–			
<i>P. pentosaceus</i>	PPE23S_F	–CCAGGTTGAAGGTGCAGT AAAAT–	95 °C/15 min, 94 °C/30 s, 66 °C/1 min, 72 °C/1 min, 72 °C/10 min, 22 cycles	1647	
	P23S_R	–CTGTCTCGCAGTCAAGCTC–			
<i>P. acidilactici</i>	PAC23S_F	–GTTTCGGAGGAGGCGCAA–	95 °C/15 min, 94 °C/30 s, 66 °C/1 min, 72 °C/1 min, 72 °C/10 min, 22 cycles	213	
	P23S_R	–CTGTCTCGCAGTCAAGCTC–			

performed. After the confirmation of *Pediococcus* genus, PCR was carried out for species level identification. The sequences of primers and the corresponding PCR cycles are given in Table 1. PCR was performed in thermocycler (Eppendorf Master cycler, Germany) and the amplified products were separated on 1–2% (w/v) agarose gel depending on the product size in electrophoretic unit (Thermo Scientific, Schwerte, Germany). Subsequently, gels were examined using gel Doc system (Alpha Innotech Corp, Santa Clara, C, USA) and photographed using AlphaView SA software.

### Antibiotic sensitivity assay

Disc diffusion method was used to obtain antibiograms of isolated *Pediococcus* strains following the modified standard Kirby–Bauer procedure (Rojo-Bezarez et al. 2006). Antibiotic susceptibility pattern of isolated pediococci was assessed using commercially available antibiotic discs (HiMedia, Mumbai, India) having 20 antibiotics including amikacin (30 µg), ampicillin (10 µg), amoxicillin (10 µg), cefadroxil (30 µg), cefoperazone (75 µg), ceftazidime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cloxacillin (1 µg), co-trimoxazole (25 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (10 µg), netillin (10 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), penicillin (10 units), tobramycin (10 µg) and vancomycin (30 µg). MRS agar plates were prepared and overlaid with 50 mL of MRS soft agar tempered at 45 °C and seeded with 100 µL of active *Pediococcus* cultures (cell density @ 10<sup>8</sup> cfu/mL). Plates were allowed to stand at room temperature for 15–20 min and then the antibiotic discs were dispensed onto the seeded agar plates under aseptic conditions. Inhibition zone diameters (mm) were measured using antibiotic zone scale after incubation at 37 °C for 24 h. Results were expressed in terms of resistant (R), intermediate resistant (IR) or susceptible (S) with zone diameter of ≤14, 15–19

and ≥20 mm, respectively, as per recommended standards given by Clinical and Laboratory Standards Institute (CLSI 2012) described by Charteris et al. (2000).

## Results and discussion

### Isolation and identification of *Pediococcus* spp.

A total 40 *Pediococcus* strains were isolated from 115 different dairy and non-dairy samples (Table 2). The sample-wise distribution of pediococci in our study were as follows: dosa batter ( $n = 14$ ;  $n =$  number of *Pediococcus* isolates), idli batter ( $n = 7$ ), fermented vegetables (cabbage:  $n = 5$ ; carrot:  $n = 2$ ), fermented grape juice ( $n = 4$ ), pickle ( $n = 2$ ), silage ( $n = 2$ ), cow milk ( $n = 1$ ), buffalo milk ( $n = 1$ ), butter milk ( $n = 1$ ) and human feces ( $n = 1$ ). Out of 40 isolates, 36 belonged to non-dairy sources, while 3 were from dairy environment and one from human fecal sample (Table 2). Pediococci mainly inhabit fermentable sugar-rich niches such as plant materials and fermented food products including idli, dosa, fermented vegetable, pickle, silage, kimchi, etc. (Cai et al. 1999; Kleinschmit and Kung 2006; Vidhyasagar and Jeevaratnam 2012; Belhadj et al. 2014; Monika et al. 2017; Narayanan et al. 2017). The relatively lesser occurrence of pediococci in milk and milk products like in our study may be due to their poor growth in milk (Somkuti and Steinberg 2010). Isolation of few pediococci has also been reported from human sources (Uymaz et al. 2009; Osmanaoglu et al. 2010).

All the isolates were Gram-positive cocci occurring in pairs and tetrads, catalase and oxidase negative and homofermentative, displaying the feature of pediococci (Table 2; Simpson and Taguchi 1995). As pediococci are the only LAB that divide alternatively in two perpendicular directions in a single plane to form tetrads, morphological

**Table 2** Characterization of *Pediococcus* isolates

S. no.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudocatalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (°C)			Growth at different pH			Growth at different salt concentrations (%)			Identified isolates	
									30	37	42	50	4.2	6.5	8.5	9.6	4		6.5
1	PD-1	Dosa batter, girls' hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
2	PD-2	Dosa batter, girls' hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
3	PD-3	Dosa batter, boys' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
4	PD-4	Dosa batter, boys' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
5	PD-7	Butter milk, household, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
6	PD-8	Idli batter, boys' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
7	PD-10	Idli batter, girls' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
8	PD-13	Pickle, household, Hisar	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
9	PD-14	Dosa batter, local market, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
10	PD-16	Pickle, household, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>

Table 2 (continued)

S. no.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudocatalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (°C)			Growth at different pH			Growth at different salt concentrations (%)			Identified isolates	
									30	37	42	50	4.2	6.5	8.5	9.6	4		6.5
11	PD-18	Dosa batter, local market, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
12	PD-19	Dosa batter, local market Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
13	PD-20	Dosa batter, girls' hostel, NDRI Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
14	PD-21	Dosa batter, local market, Jind	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
15	PD-22	Dosa batter, local market, Jind	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
16	PD-24	Idli batter, local market, Karnal	Gram-positive Cocci in P&T	-	+	(weakly)	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
17	PD-25	Dosa batter, local market, Delhi	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
18	PD-26	Idli batter, local market, Hisar	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
19	PD-27	Idli batter, local market, Hisar	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
20	PD-28	Fermented vegetable (cabbage) prepared in lab NDRI, Karnal	Gram-positive Cocci in P&T	-	+	(weakly)	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>

Table 2 (continued)

S. no.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudocatalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (°C)			Growth at different pH			Growth at different salt concentrations (%)			Identified isolates	
									30	37	42	50	4.2	6.5	8.5	9.6	4		6.5
21	PD-29	Fermented vegetable (cabbage) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
22	PD-30	Fermented vegetable (cabbage) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
23	PD-31	Fermented vegetable (cabbage) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
24	PD-32	Fermented vegetable (carrot) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
25	PD-33	Fermented vegetable (carrot) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
26	PD-34	Fermented vegetable (cabbage) prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>

Table 2 (continued)

S. no.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudocatalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (°C)					Growth at different pH concentrations (%)					Identified isolates					
									30	37	42	50	4.2	6.5	8.5	9.6	4	6.5		10	15			
27	PD-35	Fermented grape juice, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
28	PD-36	Fermented grape juice, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
29	PD-37	Fermented grape juice, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
30	PD-38	Dosa batter, local market, Delhi	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
31	PD-39	Fermented grape juice, prepared in lab, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
32	PD-41	Cow milk, cattle yard Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
33	PD-44	Buffalo milk, local market, Karnal	Gram-positive Cocci in P&T	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
34	PD-45	Human feces, volunteers from NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. acidilactici</i>
35	8-PD	Dosa batter, boys' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>

Table 2 (continued)

S. no.	Isolates	Source of isolation	Microscopic observation	Catalase test	Pseudocatalase test	Oxidase test	Gas production from glucose	Arginine hydrolysis	Growth at different temperatures (°C)			Growth at different pH			Growth at different salt concentrations (%)			Identified isolates	
									30	37	42	50	4.2	6.5	8.5	9.6	4		6.5
36	14-PD	Dosa batter, boys' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
37	25-PD	Idli batter, girls' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
38	42-PD	Idli batter, girls' hostel, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
39	Sil-1	Silage, cattle yard, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>
40	Sil-2	Silage, cattle yard, NDRI, Karnal	Gram-positive Cocci in P&T	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	<i>P. pentosaceus</i>

\*P&T Pairs and Tetrads



characterization plays a significant role in their easy identification at very early stage of isolation procedure (Singla et al. 2017). *Pediococcus* spp. exhibit variations in physiological and biochemical characteristics which can be used for their identification purpose (Papagianni and Anastasiadou 2009). All the strains were positive for arginine hydrolysis. Ten isolates (PD-2, PD-3, PD-4, PD-25, PD-44, 14-PD, 25-PD, 42-PD, Sil-1 and Sil-2) were pseudocatalase positive; while other two isolates (PD-24 and PD-28) exhibited weak reaction (Table 2). Strain specific pseudocatalase activity in low carbohydrate media has been reported in pediococci (Korasapati 1998). All the isolates showed appreciable growth at 30, 37 and 42 °C temperature, while only one isolate (PD-45) could grow at 50 °C. All the strains were capable to grow at pH 4.2, 6.5 and 8.5, and 4 and 6.5% NaCl, but not at pH 9.6, and 10% NaCl (Table 2). The isolates were further characterized for sugar fermentation using the analytical profile index (API). Thirty-nine isolates were biochemically (APi 50CH kit) identified as *P. pentosaceus* and one (PD-45) belonged to *P. acidilactici* (Table S1; Singla et al. 2018). *Pediococcus pentosaceus* and *P. acidilactici* are the two most common and industrially important species (Danielsen et al. 2007). Phylogenetically, they are very closely related to each other. Physiologically, fermentation of maltose and growth at 50 °C is the key differentiating characteristics among these species which is in agreement with the present investigation, where only *P. acidilactici* (PD-45; human fecal isolate) could grow at 50 °C and not able to ferment maltose (Table 2; Holzapfel et al. 2006).

All the *Pediococcus* isolates yielded 701 bp amplicon while performing genus-specific PCR (Fig. S1). Species identity was also confirmed at molecular level using species-specific primers. Thirty-nine isolates resulted into 1647 bp product, while one isolate (PD-45) yielded 213 bp amplicon (Fig. S2; Pfannebecker and Frohlich 2008), which confirmed their identity as *P. pentosaceus* and *P. acidilactici*, respectively, as similar with biochemical identification (Table 2; Fig. S2). All the 40 identified cultures were deposited in National Collection of Dairy cultures (NCDC)-ICAR NDRI, Karnal having accession numbers NCDC 867–906.

### Antibiotic susceptibility

All the 40 *Pediococcus* strains were screened for their antibiotic sensitivity using disc diffusion soft agar overlay assay. Based on the inhibition zone, varied range of responses by tested *Pediococcus* cultures from different sources was observed against different antibiotics (Tables 3, 4; Fig. S3). All the strains also showed variations in terms of the degree of resistance towards the same antibiotic (Tables 3, 4). Lactic acid bacteria are generally considered as safe, but due to their high consumption, there is a need to put sufficient safeguards to protect the consumers from any adverse effects.

The safety of these strains is becoming prerequisite with antibiotic resistance as an emerging issue (Hummel et al. 2007). FAO/WHO guidelines have strongly recommended antibiotic susceptibility pattern of every probiotic strain prior to their consumption or applications in food (FAO/WHO 2002).

All the studied 40 isolates irrespective of their source of isolation, location and species were completely sensitive to penicillin, chloramphenicol and erythromycin (Tables 3, 4). Earlier studies also supported that these antibiotics are usually active against *Pediococcus* spp. (Temmerman et al. 2003; Ruiz-Moyano et al. 2010; Ribeiro et al. 2014). Contrary to this, resistance to erythromycin by *Pediococcus* spp. although at very low frequency has also been reported (Danielsen et al. 2007). Uymaz et al. (2009) reported that a human isolate *P. pentosaceus* BH105 was sensitive to penicillin and chloramphenicol while resistant towards erythromycin. Most of the isolates in our study exhibited sensitivity towards cell-wall inhibitor  $\beta$ -lactam group of antibiotics (penicillin: 100%, amoxicillin: 92.5%, cloxacillin: 90%, ampicillin: 67.5%; Table 3) and protein synthesis inhibitors, aminoglycosides (netillin: 90%, tobramycin: 82.5%, gentamicin: 87.5% and amikacin: 72.5%; Table 3). None of the isolate from any source was completely resistant towards amoxicillin and cloxacillin; however, 3 (PD-26: idly batter; PD-41: cow milk; PD-44: buffalo milk) and 4 (PD-1 and PD-19: dosa batter; PD-44: buffalo milk; Sil-2: silage) isolates were intermediate resistant towards these antibiotics, respectively. Isolates belonging to butter milk, fermented vegetables and fermented grape juice were sensitive to ampicillin (Table 3). Sensitivity towards ampicillin (Venkateshwari et al. 2010), amoxicillin (Ribeiro et al. 2014) and penicillin (Mandal et al. 2011) from diverse sources has been well-reported. No isolate was completely resistant towards netillin and gentamycin; however, two isolates from dosa batter (PD-3 and 8-PD), one from silage (Sil-2) and the only isolate from butter milk (PD-7) showed resistance towards tobramycin. Only 12.5% of total isolates (3 from dosa batter: PD-1, PD-4, 8-PD, and both isolates from silage) were resistant towards amikacin (Table 3). Sensitivity towards these antibiotics is well-reported (Venkateshwari et al. 2010; Cao et al. 2016). A low level of resistance was exhibited against tobramycin (10% isolates) and amikacin (12.5% isolates) (Table 3). Although at a low level, the resistance towards aminoglycosides may not be a threat as it is considered intrinsic in LAB including pediococci (Charteris et al. 2001).

The class cephalosporin disrupts the synthesis of peptidoglycan layer of bacterial cell walls. A varied response was exhibited by the isolates towards this group of antibiotics. None of the isolates was completely resistant towards ceftriaxone and cefoperazone. Interestingly, at a very low frequency, intermediate resistivity against these antibiotics

**Table 3** Antibiotic susceptibility patterns of *Pediotococcus* strains

S. no.	Isolates	Antibiotics (concentration)													
		Quinolones				β-lactams				Aminoglycosides					
		Nx (10 µg)	CIP (5 µg)	NA (10 µg)	Amx (10 µg)	Amp (10 µg)	Cox (1 µg)	P (10 units)	NET (10 µg)	TOB (10 µg)	GEN (10 µg)	AK (30 µg)			
1	<i>P. pentosaceus</i> PD-1	0	14	0	22	21	18	22	21	18	16	19	16	18	12
2	<i>P. pentosaceus</i> PD-2	10	20	0	27	15	20	34	15	20	22	27	22	26	21
3	<i>P. pentosaceus</i> PD-3	14	15	0	25	24	22	25	24	22	13	20	13	18	15
4	<i>P. pentosaceus</i> PD-4	12	13	0	24	25	23	26	25	23	15	16	15	15	10
5	<i>P. pentosaceus</i> PD-7	>40	0	0	>40	>40	39	26	>40	39	10	22	10	26	>40
6	<i>P. pentosaceus</i> PD-8	0	15	0	22	20	26	31	20	26	21	24	21	21	17
7	<i>P. pentosaceus</i> PD-10	15	14	0	23	13	24	25	13	24	21	24	21	23	23
8	<i>P. pentosaceus</i> PD-13	11	16	0	22	26	23	28	26	23	27	27	27	26	24
9	<i>P. pentosaceus</i> PD-14	15	15	0	29	11	23	32	11	23	23	28	23	23	20
10	<i>P. pentosaceus</i> PD-16	13	16	0	22	15	22	33	15	22	23	26	23	23	23
11	<i>P. pentosaceus</i> PD-18	19	15	0	23	12	20	33	12	20	23	26	23	27	22
12	<i>P. pentosaceus</i> PD-19	16	15	0	21	26	18	30	26	18	26	29	26	27	23
13	<i>P. pentosaceus</i> PD-20	21	16	0	34	30	24	29	30	24	27	32	27	30	23
14	<i>P. pentosaceus</i> PD-21	31	16	0	38	39	36	38	39	36	20	27	20	26	27
15	<i>P. pentosaceus</i> PD-22	19	22	0	25	13	30	39	13	30	29	33	29	31	27
16	<i>P. pentosaceus</i> PD-24	10	11	0	22	12	22	24	12	22	23	29	23	27	19
17	<i>P. pentosaceus</i> PD-25	17	18	0	21	17	31	40	17	31	27	34	27	30	27
18	<i>P. pentosaceus</i> PD-26	20	16	0	16	12	24	36	12	24	27	31	27	29	23

Table 3 (continued)

S. no.	Isolates	Antibiotics (concentration)												
		Quinolones					β-lactams			Aminoglycosides				
		Nx (10 µg)	CIP (5 µg)	NA (10 µg)	Amx (10 µg)	Amp (10 µg)	Cox (1 µg)	P (10 units)	NET (10 µg)	TOB (10 µg)	GEN (10 µg)	AK (30 µg)		
19	<i>P. pentosaceus</i> PD-27	30	21	0	36	34	33	37	29	20	24	20		
20	<i>P. pentosaceus</i> PD-28	29	10	0	37	36	28	35	27	24	30	23		
21	<i>P. pentosaceus</i> PD-29	20	25	0	35	35	28	31	30	27	28	21		
22	<i>P. pentosaceus</i> PD-30	19	19	0	34	28	24	>40	28	27	26	22		
23	<i>P. pentosaceus</i> PD-31	26	18	0	40	32	28	29	31	27	26	24		
24	<i>P. pentosaceus</i> PD-32	29	14	0	36	31	28	34	29	24	26	23		
25	<i>P. pentosaceus</i> PD-33	24	17	0	30	29	31	31	33	25	31	24		
26	<i>P. pentosaceus</i> PD-34	26	18	0	38	33	37	37	31	31	27	24		
27	<i>P. pentosaceus</i> PD-35	27	17	0	35	34	34	33	30	28	27	25		
28	<i>P. pentosaceus</i> PD-36	33	16	0	35	33	32	>40	26	21	29	21		
29	<i>P. pentosaceus</i> PD-37	30	17	0	35	38	33	37	33	24	28	24		
30	<i>P. pentosaceus</i> PD-38	29	25	0	29	29	34	38	34	26	28	27		
31	<i>P. pentosaceus</i> PD-39	24	18	0	32	30	25	30	29	24	24	21		
32	<i>P. pentosaceus</i> PD-41	0	12	0	18	11	23	34	28	22	27	18		
33	<i>P. pentosaceus</i> PD-44	0	17	0	18	13	18	24	25	20	22	18		
34	<i>P. acidilactici</i> PD-45	10	11	0	25	10	27	37	26	30	23	24		
35	<i>P. pentosaceus</i> 8-PD	11	15	0	23	20	20	24	20	14	16	12		
36	<i>P. pentosaceus</i> 14-PD	19	10	0	28	25	24	34	26	24	24	24		

Table 3 (continued)

S. no.	Isolates	Antibiotics (concentration)											
		Quinolones				β-lactams				Aminoglycosides			
		Nx (10 µg)	CIP (5 µg)	NA (10 µg)	Amx (10 µg)	Amp (10 µg)	Cox (1 µg)	P (10 units)	NET (10 µg)	TOB (10 µg)	GEN (10 µg)	Ak (30 µg)	
37	<i>P. pentosaceus</i> 25-PD	0	14	0	31	28	28	32	29	24	28	22	
38	<i>P. pentosaceus</i> 42-PD	16	18	0	29	29	24	34	25	24	25	19	
39	<i>P. pentosaceus</i> Sil-1	0	16	0	26	22	21	22	19	16	20	14	
40	<i>P. pentosaceus</i> Sil-2	0	0	0	22	19	18	21	19	14	19	11	

Nx norfloxacin, CIP ciprofloxacin, NA nalidixic acid, Amx amoxicillin, Amp ampicillin, Cox cloxacillin, P penicillin, NET netillin, TOB tobramycin, GEN gentamicin, Ak amikacin  
Zero (0) represents no zone of inhibition

was shown by silage isolates only (Table 4). Comparatively, higher resistance towards cefadroxil (52.5% isolates) and ceftazidime (32.5% isolates) was observed. Isolates from the dosa batter exhibited the maximum variations (sensitivity, intermediate resistivity and complete resistance) against cefadroxil and ceftazidime. Similar to our study (PD-45: human fecal isolate), Uymaz et al. (2009) also reported sensitivity towards ceftriaxone, while the human isolate *P. pentosaceus* strain BH105 showed resistance against ceftazidime.

All the *Pediococcus* strains except the only isolate from butter milk, and PD-36 (fermented grape juice) were resistant towards co-trimoxazole (sulphonamide; inhibitor of nucleic acid synthesis). Mandal et al. (2011) demonstrated the gene location of co-trimoxazole resistance on chromosomes which reflects intrinsic resistance towards this antibiotic. Gupta and Sharma (2017) also reported co-trimoxazole resistance by *P. acidilactici* strain Ch-2. In tune with the previous study (Sukumar and Ghosh 2010), the current study also revealed that all the isolates were resistant towards nalidixic acid, a member of quinolones which interferes with bacterial DNA synthesis. However, 37.5 and 30% isolates showed resistance towards norfloxacin and ciprofloxacin (quinolones), respectively (Table 4). The maximum variability (sensitivity, intermediate resistivity and complete resistance) was displayed by dosa and idly batter isolates towards norfloxacin and ciprofloxacin.

Except an isolate PD-39 (fermented grape juice), all the strains were highly resistant to vancomycin. Vancomycin is the representative of glycopeptide class of antibiotics which is active against most of the Gram-positive bacteria. Vancomycin resistance is of major concern, as vancomycin is considered as one of the last antibiotics in the treatment of multidrug-resistant pathogens (Bernardeau et al. 2008). Reports also favored the widespread vancomycin resistance among LAB, but fortunately, it is non-transferable in case of pediococci (Toomey et al. 2010; Banwo et al. 2013). A high level of glycopeptide resistance has been reported for *Pediococcus* and other LAB (Zarazaga et al. 1999). Intrinsic resistance of LAB to several antibiotics might be considered advantageous as such resistance could be helpful for sustainable utilization of the strains in human intestine to maintain the equilibrium of intestinal microbiota during antibiotic therapy (Ketema et al. 2010).

With regard to antibiotic susceptibility, no considerable/remarkable difference was observed between the two studied *Pediococcus* species, i.e., *P. pentosaceus* and *P. acidilactici* (Tables 3, 4). A total 16 strains belonging to dosa batter (4; PD-20, PD-21, PD-25, PD-38), fermented vegetables (4; PD-30, PD-31, PD-33, PD-34), fermented grape juice (4; PD-35, PD-36, PD-37, PD-39), idly batter (3; PD-8, PD-27, 42-PD) and butter milk (PD-7) exhibited sensitivity/intermediate resistance towards 80–90% of the studied

**Table 4** Antibiotic susceptibility patterns of *Pediotococcus* strains

S. No.	Isolates	Antibiotics (concentration)											Others	
		Cephalosporins												
		CFR (30 µg)	CTR (30 µg)	CPZ (75 µg)	Caz (30 µg)	E (15 µg)	VA (30 µg)	Sulfonamides Cot (25 µg)	Azolidione NIT (300 µg)	C (30 µg)				
1	<i>P. pentosaceus</i> PD-1	0	20	20	17	25	0	0	18	0	0	0	18	30
2	<i>P. pentosaceus</i> PD-2	0	29	27	18	30	10	0	22	0	0	0	22	33
3	<i>P. pentosaceus</i> PD-3	20	25	22	16	26	0	0	16	0	0	0	16	30
4	<i>P. pentosaceus</i> PD-4	14	22	24	17	28	0	0	18	0	0	0	18	31
5	<i>P. pentosaceus</i> PD-7	18	31	40	>40	>40	0	0	22	0	>40	0	22	>40
6	<i>P. pentosaceus</i> PD-8	15	24	31	18	29	0	0	24	0	0	0	24	36
7	<i>P. pentosaceus</i> PD-10	14	25	28	0	28	10	0	21	10	0	0	21	32
8	<i>P. pentosaceus</i> PD-13	0	24	26	21	32	0	0	22	0	0	0	22	40
9	<i>P. pentosaceus</i> PD-14	0	23	26	11	32	0	0	19	0	0	0	19	32
10	<i>P. pentosaceus</i> PD-16	12	24	29	0	32	0	0	20	0	0	0	20	37
11	<i>P. pentosaceus</i> PD-18	0	21	29	0	33	0	0	20	0	0	0	20	35
12	<i>P. pentosaceus</i> PD-19	0	23	23	0	34	0	0	27	0	0	0	27	32
13	<i>P. pentosaceus</i> PD-20	14	27	24	21	34	10	0	19	10	0	0	19	37
14	<i>P. pentosaceus</i> PD-21	18	27	30	28	35	10	0	23	10	0	0	23	36
15	<i>P. pentosaceus</i> PD-22	0	31	35	0	34	10	0	21	10	0	0	21	>40
16	<i>P. pentosaceus</i> PD-24	21	28	29	0	31	10	0	20	10	10	0	20	36
17	<i>P. pentosaceus</i> PD-25	0	30	21	21	40	10	0	20	10	0	0	20	40
18	<i>P. pentosaceus</i> PD-26	19	31	28	0	33	10	0	17	10	0	0	17	39

Table 4 (continued)

S. No.	Isolates	Antibiotics (concentration)											Others	
		Cephalosporins												
		CFR (30 µg)	CTR (30 µg)	CPZ (75 µg)	Caz (30 µg)	E (15 µg)	VA (30 µg)	Cot (25 µg)	NIT (300 µg)	Azolidione	C (30 µg)			
19	<i>P. pentosaceus</i> PD-27	22	30	32	27	34	0	0	20	38				
20	<i>P. pentosaceus</i> PD-28	10	25	28	25	35	0	10	18	>40				
21	<i>P. pentosaceus</i> PD-29	22	28	31	20	35	0	0	24	>40				
22	<i>P. pentosaceus</i> PD-30	19	24	33	20	32	10	0	19	38				
23	<i>P. pentosaceus</i> PD-31	11	28	28	22	37	0	0	21	36				
24	<i>P. pentosaceus</i> PD-32	10	24	28	22	34	10	0	22	40				
25	<i>P. pentosaceus</i> PD-33	15	28	31	22	34	10	0	31	>40				
26	<i>P. pentosaceus</i> PD-34	14	28	37	28	34	0	0	25	>40				
27	<i>P. pentosaceus</i> PD-35	16	30	33	25	35	10	0	25	38				
28	<i>P. pentosaceus</i> PD-36	22	29	32	27	33	0	19	21	37				
29	<i>P. pentosaceus</i> PD-37	24	29	31	31	37	0	11	21	36				
30	<i>P. pentosaceus</i> PD-38	24	32	32	20	34	0	10	24	39				
31	<i>P. pentosaceus</i> PD-39	18	24	25	21	35	17	0	22	39				
32	<i>P. pentosaceus</i> PD-41	0	26	23	0	31	10	0	16	35				
33	<i>P. pentosaceus</i> PD-44	20	26	23	18	29	0	0	16	29				
34	<i>P. acidilactici</i> PD-45	0	29	26	0	33	0	0	19	40				
35	<i>P. pentosaceus</i> 8-PD	0	20	20	10	30	0	0	17	31				
36	<i>P. pentosaceus</i> 14-PD	0	24	23	18	33	0	0	21	36				

Table 4 (continued)

S. No.	Isolates	Antibiotics (concentration)														
		Cephalosporins					Macrolides		Glycopeptides		Sulfonamides		Azolidione		Others	
		CFR (30 µg)	CTR (30 µg)	CPZ (75 µg)	Caz (30 µg)	E (15 µg)	VA (30 µg)	Cot (25 µg)	NIT (300 µg)	C (30 µg)						
37	<i>P. pentosaceus</i> 25-PD	22	28	30	23	32	0	0	22	37						
38	<i>P. pentosaceus</i> 42-PD	17	30	24	22	30	0	0	23	33						
39	<i>P. pentosaceus</i> Sil-1	20	24	18	12	29	0	0	0	28						
40	<i>P. pentosaceus</i> Sil-2	14	18	17	11	25	0	0	0	30						

CFR cefadroxil, CTR ceftriaxone, CPZ cefoperazone, Caz ceftazidime, E erythromycin, VA vancomycin, Cot Co-Trimoxazole, NIT nitrofurantoin, C chloramphenicol  
Zero (0) represents no zone of inhibition

antibiotics. Among them, six strains (PD-7, PD-8, PD-20, PD-25, PD-31 and PD-34) were sensitive/intermediate resistant towards 80%; while eight strains (PD-21, PD-27, PD-30, PD-33, PD-35, PD-37, PD-38 and 42-PD) to 85%, and two strains (fermented grape juice; PD-36 and PD-39) to as many as 90% of antibiotics (Tables 3, 4). Overall, the maximum resistance was exhibited by isolates belonging to silage (Sil-2; 50%) followed by cow milk (PD-41), dosa batter (8-PD) and human isolate (PD-45) which showed resistance towards 40% of studied antibiotics (Tables 3, 4).

## Conclusions

The increased use of LAB as starter, adjunct and non-starter for their application in food products necessitates their safety check. In this regard, the performance of antimicrobial susceptibility testing might be considered as an essential selection criterion for starter as well as probiotic cultures. The results of the present study demonstrated varied responses of susceptibility among *Pediococcus* isolates depending on the strain type, source and location of isolations as well as tested antibiotics. The fact that *Pediococcus* strains gave different responses to the assayed antibiotics demonstrates the importance of individually testing strains for their intended application. Despite this, from the safety point of view, if a bacterial strain exhibits resistance to antimicrobials by phenotypic methods, it should be further evaluated for the molecular mechanisms.

## Compliance with ethical standards

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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