



Mucosa-adherent *Pediococcus Pentosaceus* I44 isolated from healthy human and effect of oleic acid on its probiotic properties

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ABSTRACT

With the aim of selectively isolating and understanding the cultivable lactic acid bacteria that are autochthonous to human gut, biopsy samples were collected from the terminal ileum and caecum of healthy volunteers who underwent routine colonoscopy procedure. The use of tissue samples (over stool samples) provides for a better expression of the autochthonous bacterial population of the human gut. The strains that were stable after many rounds of sub-culture were identified and studied further. One such ileal isolate, a Gram-positive, catalase-negative cocci was identified to be *Pediococcus pentosaceus* I44. It was studied for its gastric tolerance, bile tolerance and surface properties, and reported here. The effect of tween-80, oleic acid, extra virgin olive oil and sesame oil on the aforementioned properties of *P. pentosaceus* I44 was also analysed. The isolate was able to survive well in simulated gastric fluid of pH 3.0 followed by treatment in simulated intestinal fluid with a survival percentage of around 70%. It was, however, unable to withstand pH 2.0 even in the presence of supplements. It showed good tolerance to bile stress (1% w/v), and its cell surface was found to be hydrophilic. *P. pentosaceus* I44 showed good aggregation of 87% after 24 h, with oleic acid having a significant effect on the isolate's aggregation potential. *P. pentosaceus* I44 is one of the few mucosa-bound cultivable bacteria that are adapted for survival in human colon. Studying and analysing such isolates might provide useful insights into their role in metabolism and health.

1. Introduction

Gut microbiota is a collective term for the dynamic community of microorganisms that have colonised the gastrointestinal (GI) tract and have co-evolved with the host. They are known to be involved in diverse functions ranging from regulating endocrine function to influencing host immunity (Thursby and Juge, 2017). Studying the composition and functionality of the healthy gut microbiota is therefore essential to understand their role in health and disease. There are very few studies carried out till date on the gut community of Indian population. Besides, such studies have used stool samples as source for analysis (Green et al., 2006; Momozawa et al., 2011). Stool basically comprises of microbes present along the length of GI tract including those found in food, the luminal population and the shed population from mucosa (Eckburg et al., 2005; Grice and Segre, 2012). Faecal bacterial flora differ quantitatively and qualitatively from the mucosa-adherent community (Green et al., 2006). Moreover it is the permanent gut inhabitants that seem to play an important role in host health and metabolism

(Zoetendal et al., 2002).

Isolates obtained from biopsy samples of patients who had undergone bowel preparation are indeed those bacteria that are tightly bound to mucosa, and not just transient passers-by (Green et al., 2006). Though a number of genome studies are available, a combination of culture-based studies and next generation sequencing techniques is required for a complete understanding of these permanent inhabitants of the human gut.

It was first reported by Whitehill et al. (1947) that oleic acid (cis-9-octadecenoic acid) was required for the growth of Lactobacilli obtained from caecum of rats and the organism could be maintained *in vitro* on synthetic media only in presence of sodium oleate. MRS media used for cultivation of Lactobacilli includes tween 80 (0.1% w/v), which supports the growth of many lactic acid bacteria (LAB) (De Man et al., 1960). Tween 80 (poly-oxyethylene sorbitan mono-oleate or poly-sorbate 80), a non-ionic, water-soluble surfactant consisting of up to 90% oleic acid (as its lipophilic part), is used as an exogenous source of oleic acid. It was of interest to investigate whether oleic acid had any

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Table 1

Selection criteria for subjects.

Subject Specifications Inclusion criteria	Exclusion criteria
Age: 18–60	Diabetes mellitus
Gender: Either	Hypo or hyperthyroid state
Case selection	Medication
1 Fissure/haemorrhoids with bleeding requiring full length colonoscopy	1 Antibiotics/probiotics within 2 weeks of colonoscopy
2 Cancer surveillance	2 Anti-hypertensives, laxatives, antidepressants etc.
3 Patients request for colonoscopy as part of “health check”	

effect on the probiotic properties of the gut isolates. Similarly, it would be of relevance to study the effects of dietary components like olive oil and sesame oil (which contain oleic acid) on these isolates. Olive oil is composed of 55–83% oleic acid while, sesame oil, commonly used in Indian cooking, consists of linoleic (35-50%) and oleic (35-50%) acids (Pathak et al., 2014).

The main objective of this work was to understand the various properties of the beneficial lactic acid bacteria that are truly associated with the gut, and not those transient members that are found in the faeces. Further, it was of interest to understand if oleic acid and compounds containing it (like tween-80, olive oil and sesame oil) had any influence on the properties of the gut isolate.

Since the host and gut microbiota have co-evolved, both depend on each other for various metabolic functions and nutritional requirements (Gerritsen et al., 2011). Thus, the gut microbiota plays an important role in human health. There are many studies that indicate changes in gut microbial composition under healthy and diseased conditions (Bull and Plummer, 2014; Durack and Lynch, 2019; Shreiner et al., 2015). Altered or low gut microbial diversity has been observed in a number of intestinal diseases (ulcerative colitis, coeliac disease, Crohn’s disease, colon cancer), extra-intestinal diseases (asthma, atopic dermatitis, autism, rheumatoid arthritis, fibromyalgia) and lifestyle disorders (diabetes, obesity) (Clemente et al., 2012; Gerritsen et al., 2011).

Hence biopsy samples were obtained from healthy Indian volunteers whose colonoscopy showed no signs of abnormality and a culture-based approach was employed to characterise the isolated bacteria. Although Fakhry et al. (2009) have previously isolated bacteria from ileal epithelium, to the best of our knowledge, the current study on gut bacteria from healthy Indian population is the first of its kind.

2. Materials and methods

2.1. Chemicals and media

The media, chemicals and genomic DNA isolation kit used in this study were purchased from HiMedia, Mumbai, India. Lysozyme and proteinase were obtained from Sigma-Aldrich, USA. Taq DNA Polymerase Master Mix Red was procured from Ampliqon, Denmark. All chemicals used in this study were of analytical grade.

2.2. Sample collection/Biopsy samples

Tissue samples from terminal ileum and caecum were collected from 6 healthy subjects who underwent routine colonoscopy at Gleneagles Global Health City, Chennai, India. The subjects had to satisfy certain inclusion and exclusion criteria (Table 1), so as to ensure that their gut microbiota remained largely unaffected. No special dietary regimen was required to be followed by the subjects. This study was approved by the concerned ethical committees and informed consent was obtained from the subjects. Samples suspended in sterile phosphate-buffered saline (PBS) were transported to lab under ice cold condition within 2 h, and processed after confirming that there were no signs of inflammation in the subjects.

2.3. Isolation of bacteria from tissue samples

The ileal samples were homogenised and enriched in de Man, Rogosa and Sharpe (MRS) broth pH 6.5. The enrichment was done to specifically select lactic acid bacteria. Appropriate dilutions of the enriched broth were pour-plated onto MRS agar plates and incubated under aerobic conditions for 24 h. Representative colonies were picked by making an incision through the agar medium around the colony and dropping it into MRS broth. Pure cultures were obtained by spread-plating, which were sub-cultured many times to select the stable isolates. These isolates were preserved as glycerol stocks in -20°C .

2.4. Morphological and biochemical characterization

The cell morphology of the isolate was observed using field emission gun-based scanning electron microscopy (Model Apreo, Thermo Scientific, USA) operated at 5 kV. Sample preparation involved centrifugation of 1.0 mL of overnight culture at 3830 x g for 15 mins and pellet was diluted suitably in sterile saline solution (0.85% w/v). A drop of it was placed on a glass slide, dried in a desiccator and fixed onto the carbon tape mounted a metal stub. The sample was coated with gold/palladium using sputter coater (Quorum Technologies, UK) for 30 s.

The ability of *Pediococcus pentosaceus* I44 to ferment different carbohydrates was studied by inoculating the overnight culture (1%) into basal MRS medium supplemented with test carbohydrate and phenol red (100 ug/mL). The tubes were incubated at 37 °C for 48 h, and observed for colour change and bubble formation in the inverted Durham tubes. Cellobiose, dulcitol, inositol, lactose, maltose, mannitol, mannose, melibiose, rhamnose, sucrose, trehalose, xylose were used in this study.

2.5. Identification of isolates based on 16S rRNA gene analysis

Genomic DNA was obtained from the isolates using bacterial genomic DNA isolation kit as per manufacturer’s instructions, with a few modifications.

Briefly, bacterial cells were harvested from 4 mL of overnight culture. 250 μL of lysozyme (50 mg/mL) solution prepared in Gram-positive lysis buffer (GPLA buffer, provided in the kit) was added to the cells and incubated at 37 °C for 45 mins, and further steps performed as mentioned in the kit. For better extraction of genomic DNA, a pre-treatment was employed in which 180 μL of lysis buffer containing 50 mg/mL lysozyme, 20 mM tris-HCl (pH 8.0), 2 mM ethylene-diamine-tetra acetic acid (EDTA) and 1.2% triton-X was added to cells and incubated at 37 °C for 45 mins. In addition, they were also subjected to heat shock at 95 °C in a water bath for 15 mins after treatment with proteinase. The 16S rRNA gene was amplified using universal primers 27f (5’ AGAGTTTGATCCTGGCTCAG 3’) and 1492r (5’ ACGGC-TACCTGTACGACTT 3’) which are universal primers - nearly complete amplification of 16S rRNA gene was facilitated by these eubacterial primers (Allen et al., 2001). The PCR conditions were optimized using gradient PCR and following conditions were found to yield sharp bands corresponding to the desired amplicon - initial denaturation at 95 °C for 4 min, denaturation at 95 °C for 1 min, annealing at 60 °C for 45 s, elongation at 72 °C for 2 min and final elongation at 72 °C for 5 min. The PCR reaction was carried out for 30 cycles in a thermal cycler (Mastecycler gradient 5331, Eppendorf, Germany). The amplicons of size 1500 bp were purified and sequenced at AgriGenome Labs Pvt. Ltd. (Cochin, India), and analysed using nucleotide BLAST for identification of the isolates. The results thus obtained were confirmed by getting the isolates identified and deposited at National Centre for Microbial Resource, Pune, India.

2.6. Probiotic properties and effect of oleic acid on the properties

In order to evaluate the effect of oleic acid on the below mentioned properties of the isolate, tween 80, oleic acid, extra virgin olive oil

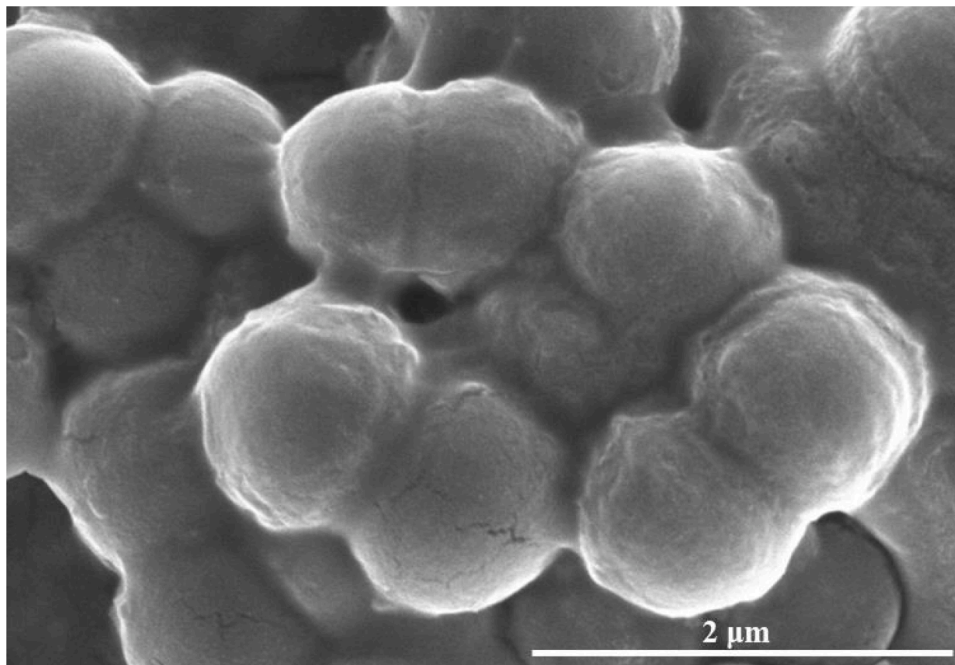


Fig. 1. Scanning electron microscope image of the isolate *P. pentosaceus* I44 isolate from ileal tissue.

(obtained locally) and sesame oil (obtained locally) were incorporated at a concentration of 0.1% (v/v) in MRS media.

2.6.1. Tolerance to simulated gastro-intestinal conditions

Simulated gastric fluid (SGF) was prepared freshly by suspending pepsin (3 g/L) in sterile PBS (0.01 M) and adjusting the pH to 2.0 or 3.0 with concentrated HCl. Simulated intestinal fluid (SIF) was prepared freshly by adding pancreatin (1 g/L), ox bile (3 g/L), NaCl (6.5 g/L), NaHCO₃ (1.386 g/L), KCl (0.835 g/L), CaCl₂ (0.22 g/L) in deionised water and adjusting the pH to 8.0 (Priya et al., 2011).

Tolerance of the cells to SGF and SIF were carried out as per Charteris et al. (1998) with slight modifications. 1.0 mL of exponentially growing cells was centrifuged at 3830 x g for 5 min, washed twice with and suspended in 0.01 M PBS (pH 7.4). To 2.0 mL of this cell suspension, 1.0 mL of SGF (pH 2.0 or 3.0) was added, vortexed gently for 5–10 s and incubated at 37 °C. Aliquots (0.1 mL) were drawn after 0 min, 90 min and 180 min and plated on MRS agar plates for determination of the total viable count.

After 180 min of incubation in SGF, the contents were centrifuged at 3830 x g for 5 min, washed twice with and suspended in 0.2 mL of 0.01 M PBS (pH 7.4). To this, 1.0 mL of SIF was added and incubated at 37 °C. Aliquots were plated at 0 min and 240 min to determine the cell viability. The difference in viable colony counts before and after incubation has been reported.

2.6.2. Bile tolerance

The ability of the strain to survive in the presence of bile salts was assessed by supplementing MRS media with 0.5% and 1.0% (w/v) ox bile. Overnight culture with OD₆₀₀ set to 1.0 was used to inoculate the bile-MRS media and aliquots were plated on MRS agar at 0 h, 4 h and 24 h. The colony forming units were counted after 24 h of incubation at 37 °C.

2.6.3. Surface properties

2.6.3.1. Cell aggregation. Auto-aggregation assay was performed according to Kos et al. (2003). Bacterial culture (24 h) was pelleted by centrifugation at 8243 x g for 15 min at 4 °C. The cells were washed twice with and suspended in 0.01 M PBS (pH 7.4). The OD₆₀₀ of the cell

suspension was set to 0.6 (OD_{initial}), and incubated at 37 °C. Without disturbing, the topmost layer was pipetted carefully at the end of 1 h, 2 h, 3 h and 4 h and OD₆₀₀ measured (OD_{final}). The percentage of auto-aggregation was calculated as per the formula

$$\% \text{ Aggregation} = ((\text{OD}_{\text{initial}} - \text{OD}_{\text{final}}) / \text{OD}_{\text{initial}}) \times 100$$

2.6.3.2. Cell surface hydrophobicity. Cell surface hydrophobicity was determined as per Iyer et al. (2010), with a few modifications. Briefly, overnight active culture was harvested by centrifugation at 8243 x g for 10 min at 4 °C, washed twice and re-suspended in 0.05 M phosphate urea magnesium (PUM) buffer of pH 7.2. To 3 mL of cell suspension, whose OD at 600 nm was set to approx. 0.5 (OD_{initial}), toluene (0.6 mL) was added slowly along the sides and allowed to stand for 10 min at 37 °C. It was then vortexed for 2 min and incubated at 37 °C for 1 h thereby allowing phase separation. The aqueous layer was carefully pipetted out and OD was measured at 600 nm (OD_{final}). The cell surface hydrophobicity was measured using the following formula

$$\% \text{ Hydrophobicity} = ((\text{OD}_{\text{initial}} - \text{OD}_{\text{final}}) / \text{OD}_{\text{initial}}) \times 100$$

2.7. Statistical analysis

All experiments were performed in triplicates and the data are represented as mean ± standard deviation (SD). The statistical significance and standard deviation were calculated using Microsoft Excel (Version 15.0; Microsoft Corp., USA). Analysis of variance was done using one-way ANOVA method. Any significant differences between means were analysed using Tukey multiple comparison test.

3. Results and discussion

3.1. Isolation and identification of bacteria

With the aim of studying autochthonous lactic acid bacteria, the selection process and media were specifically designed to eliminate all other groups of bacteria. Although the samples were collected from 6 subjects, the isolates from only 3 were stable after repeated sub-culturing *in vitro*.

Table 2
Carbohydrate fermentation profile of *P. pentosaceus* isolates.

Sl. No	Carbohydrate	CRAG3 (Shukla and Goyal, 2014)	(Vidhyasagar and Jeevaratnam, 2012)					LS5 (Cai et al., 1999)	Pep1 (Osmanagaoglu et al., 2001)	Q6 (Xu et al., 2018)	I44 [this study]	
			VJ13	VJ31	VJ35	VJ41	VJ49					VJ56
1	Cellobiose	+	-	+	+	±	-	-	+	NA	+	+
2	Dulcitol	-	-	-	-	-	-	-	NA	NA	NA	-
3	Inositol	-	-	-	-	-	-	-	NA	NA	NA	-
4	Lactose	-	-	-	-	-	-	-	+	NA	-	-
5	Maltose	+	-	+	+	+	-	-	+	+	+	+
6	Mannitol	+	-	-	-	-	-	-	-	-	+	-
7	Mannose	+	+	+	+	+	+	+	+	NA	+	+
8	Melibiose	-	-	+	+	-	-	-	-	NA	-	+
9	Rhamnose	+	-	-	-	-	-	-	+	-	+	-
10	Sucrose	+	-	-	-	±	-	±	-	-	NA	+
11	Trehalose	+	+	+	-	+	-	-	+	+	+	+
12	Xylose	-	-	-	-	-	-	-	-	+	+	-

(+) positive; (-) negative; (±) weak growth; (NA) not included in study.

Table 3
Percentage survivability of *P. pentosaceus* I44 after treatment with SGF (pH 3.0 for 180 min) and subsequent treatment with SIF for 180 min (as indicated by final% viability).

Sl	Media	% survival in SGF	Final % survival
1	MRS	68.69 ± 0.91	68.54 ± 1.19
2	MRS-Tween80	76.49 ± 0.86	75.19 ± 1.23
3	MRS-Oleic acid	75.76 ± 1.42	73.91 ± 0.64
4	MRS-Olive oil	78.11 ± 0.31	75.63 ± 0.67
5	MRS-Sesame oil	77.15 ± 1.55	76.49 ± 2.32

% survival = ((cfu/mL after treatment) / (initial cfu/mL)) * 100.

Enrichment was done under both aerobic and anaerobic conditions. Though anaerobic enrichment gave higher colony number, colony diversity did not vary between aerobic and anaerobic enrichments. Hence, we have reported only aerobic enrichment. Since all the isolates were able to grow under both aerobic and anaerobic conditions, all the later experiments were carried out under aerobic conditions. This is in concordance with Marteau et al. (2001) who showed that facultative anaerobes dominate in the caecum.

Of the isolates obtained, *Pediococcus pentosaceus* I44 was found to be stable after many rounds of subculture, and is the focus of this study.

This isolate has been deposited at National Centre for Microbial Resource (NCMR), India with accession number MCC4200.

The genus *Pediococcus* belongs to the family *Lactobacillaceae* in the order *Lactobacillales*, with five species belonging to the genus *Pediococcus* - *Pediococcus acidilactici*, *Pediococcus damnosus*, *Pediococcus dextrinicus*, *Pediococcus parvulus*, and *Pediococcus pentosaceus* (Barros et al., 2001). Most studies concentrate on *Pediococcus* obtained from fermented food sources like cheese, pickles, kimchi (Cavicchioli et al., 2017; Jang et al., 2015; Jonganurakkun et al., 2008), from saliva (Kuvatanasuchati et al., 2012), from vagina (Borges et al., 2013) or from stool samples (Lv et al., 2014). However it has been reported that *P. pentosaceus* associated with human gut differ from those found in food (Lv et al., 2014).

P. pentosaceus I44 is Gram-positive, non-motile and catalase-negative. It was able to grow under both aerobic and anaerobic conditions. The colony was entire, smooth, round, creamy white, butyrous and around 1.0 – 2.0 mm in diameter. The bacterial cells were observed to be cocci-shaped with a diameter ranging from 0.8 to 0.9 µm (Fig. 1), usually found in pairs or clusters. The pH of the growth media dropped from pH 6.5 to pH 4.0–4.5 after growth of this isolate. The drop in pH is, in fact, a good indication of growth in the case of lactic acid bacteria. Of the 12 sugars tested for utilization, *P. pentosaceus* I44 produced acid with cellobiose, maltose, mannose, melibiose, sucrose and trehalose. It did

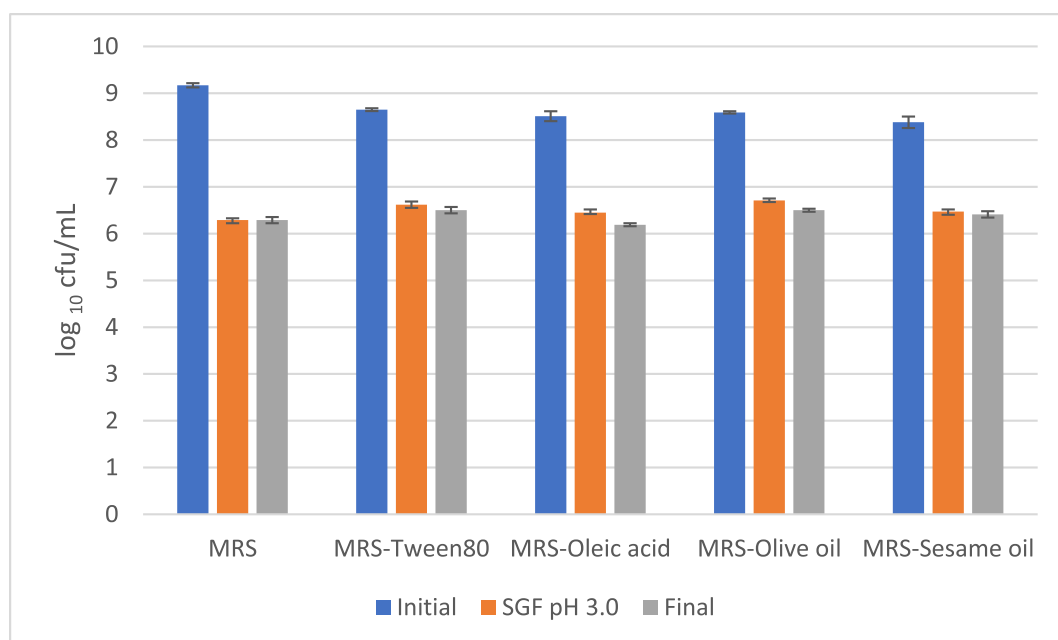


Fig. 2. Viability of *P. pentosaceus* I44 cells after treatment with SGF (pH 3.0 for 180 mins) and subsequently with SIF (as represented by "final").

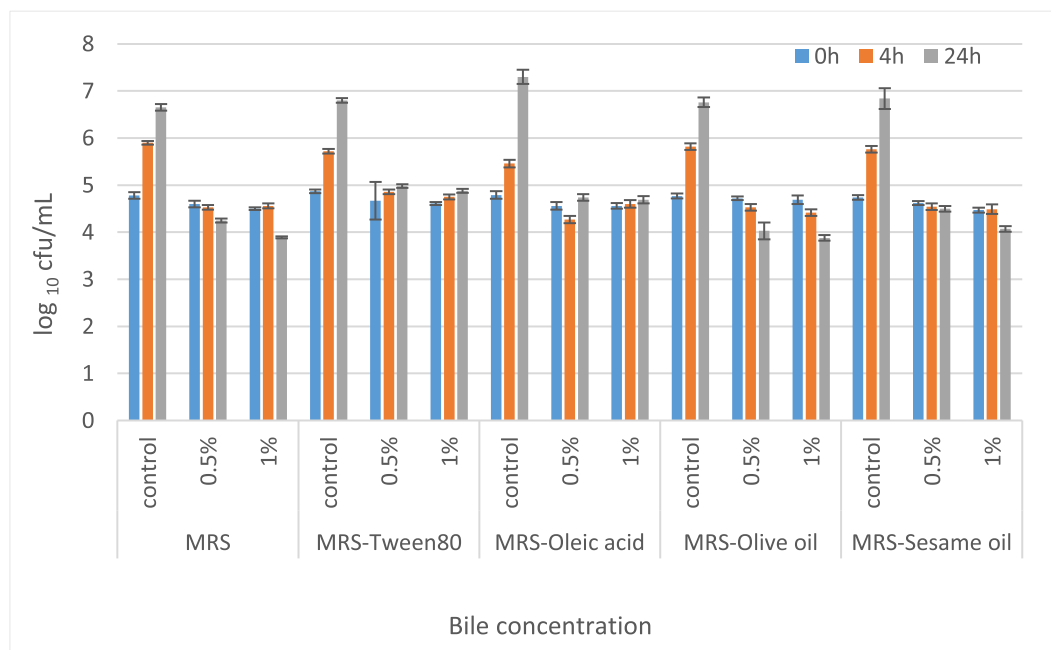


Fig. 3. Bile tolerance of *P. pentosaceus* I44 in MRS broth and in MRS supplemented with oleic acid-containing compounds.

not show growth with lactose, xylose, rhamnose, mannitol, inositol and dulcitol. From Table 2, which gives a compilation of the sugar fermentation profiles of various *P. pentosaceus* isolates, it is clear that it was unable to utilise dulcitol, inositol, and lactose. However, it must be noted that all the isolates in Table 2 except *P. pentosaceus* I44 were isolated from fermented food or fodder.

3.2. Tolerance to simulated gastro-intestinal conditions

The ability of *P. pentosaceus* I44 to tolerate gastric treatment was expressed as percentage survival (Table 3). Around 68% of the cells were able to survive in simulated gastric fluid (pH 3.0) followed by treatment in simulated intestinal fluid, while none were able to survive in pH 2.0. These results are in agreement with Osmanagaoglu et al. (2010) and Shukla and Goyal (2014), who have reported that *Pediococcus* was unable to survive harsh acidic conditions of pH 2.0. Fig. 2 shows the effect of the supplements on the colony forming units upon treatment with SGF (pH 3.0) followed by SIF, for a time period of 180 min. It was observed that percentage survival was higher in the presence of oleic acid and its supplements, and this effect was found to be statistically significant. This observation is in agreement with Corcoran et al. (2007) who showed that tween 80 improved tolerance in simulated gastric juice and subsequent survival rates of *Lactobacillus* species.

Tween 80 not only improves the growth of *Lactobacilli* (Johnsson et al., 1995; Partanen et al., 2001; Tan et al., 2012; Terraf et al., 2012), but also protects them from stress of low pH (Broadbent et al., 2014; Corcoran et al., 2007), bile (Li et al., 2011), hydrostatic pressure (Reitermayer et al., 2018), and freeze-drying (Hansen et al., 2015). When grown in presence of tween 80, LAB tend to incorporate oleic acid into their Gram positive membranes, which gets converted to cyclopropane fatty acids (Partanen et al., 2001), a characteristic component in *Lactobacilli* membrane. Cyclopropane fatty acids impart fluidity to the membrane and is responsible for protective effect against stress and adverse conditions (Jacques and Hunt, 1980; Jacques, 1981; Smittle et al., 1972; Suutari and Laakso, 1992). Such protective effect have been observed with tween 80, tween 20, free oleic acid, vaccenic acid (Corcoran et al., 2007) but not with lauric acid, tween 40 and tween 60 (Partanen et al., 2001). This suggests that it is the unsaturated fatty acids that play an important role in the survival and properties of LAB.

Table 4

% Tolerance and % growth of *P. pentosaceus* I44 in MRS media supplemented with 0.5% and 1.0% (w/v) bile over a period of 24 h.

Media	% Tolerance		% Growth		MRS
	0.5%	1.0%	0.5%	1.0%	
MRS	63.9 ± 0.7	58.5 ± 0.3	-7.6 ± 1.3	-13.5 ± 0.3	39.3 ± 3.4
MRS-Tween80	73.2 ± 0.1	71.8 ± 0.5	6.6 ± 1.4	5.9 ± 1.4	39.6 ± 1.1
MRS-Oleic acid	64.9 ± 1.1	64.3 ± 2.1	4.0 ± 0.7	2.9 ± 2.4	52.5 ± 3.6
MRS-Olive oil	59.6 ± 3.2	57.3 ± 1.2	-14.6 ± 3.4	-17.3 ± 0.4	41.7 ± 0.5
MRS-Sesame oil	65.8 ± 1.4	59.5 ± 2.9	-2.7 ± 1.1	-8.9 ± 1.7	44.4 ± 5.8

% tolerance = (growth in presence of bile after 24 h/growth in MRS after 24 h) * 100;

% growth = ((Viable cells at 24 h - Viable cells at 0 h) / (Viable cells at 0 h)) * 100.

3.3. Bile tolerance

P. pentosaceus I44 was able to survive in the presence of 0.5% and 1% bile in MRS (Fig. 3). However, the viable cells decreased by 1 log unit after 4 h in both 0.5% and 1% bile as compared to control. The % tolerance and % growth of *P. pentosaceus* I44 over 24 h in the presence of bile have been recorded in Table 4. As per Gilliland et al. (1984), an organism needs to tolerate 0.3% bile for it to be regarded as bile tolerant. It must be noted that the concentration of bile used in the study was high as compared to physiological conditions. Similar observations of tolerance to bile concentrations of 0.3% and 0.4% by *Pediococcus* strains have been reported previously (Abbasiliasi et al., 2012; Barbosa et al., 2015; Ilavenil et al., 2016; Noohi et al., 2016). The ability of the isolate in this study to show good tolerance to bile stress (of up to 1%) further proves that it is indeed an inhabitant autochthonous to intestine and not a transient member.

Although the isolate was able to withstand bile stress, it can be observed from Fig. 3 that in MRS media containing bile, the viable count decreased with time. This indicates that the organism was unable to grow in presence of bile (% growth being negative in presence of bile).

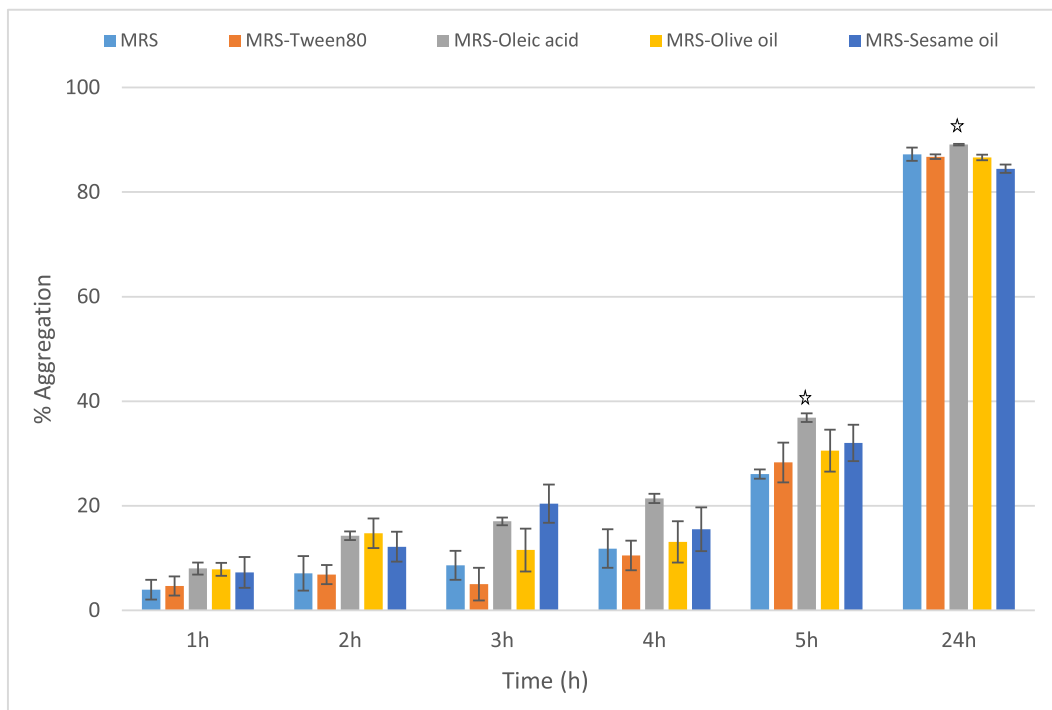


Fig. 4. Auto-aggregation ability of *P. pentosaceus* 144. Star symbol indicates that it is statistically significant ($p < 0.05$) from others.

However, in media containing tween-80, there is a marginal increase in viable count with time in both 0.5% and 1% bile (Table 4), suggesting that tween-80 had a protective effect against bile stress. This is in agreement with Kimoto et al. (2002) who demonstrated that tween 80 increased bile tolerance in Lactococci by a two-step process - by reducing cell permeability and by forming micelles with oxgall, thereby preventing its binding to bacterial cells. Oleic acid was also observed to exhibit such protective effect while no increase in viable cell counts were observed in olive and sesame oils.

3.4. Surface properties

Surface phenomena such as aggregation and adhesion play a major role in the sustained colonization of bacteria to the intestinal mucosa (Terraf et al., 2014), and thus influence the colonization location in the gut and functionality (Deepika and Charalampopoulos, 2010). Hence it is important to study the surface characteristics of commensal bacteria to understand the mechanism behind its colonization.

3.4.1. Cell aggregation

Ability to aggregate is a direct indication of an organism to establish itself with permanence in an ecological niche. In the present study, the

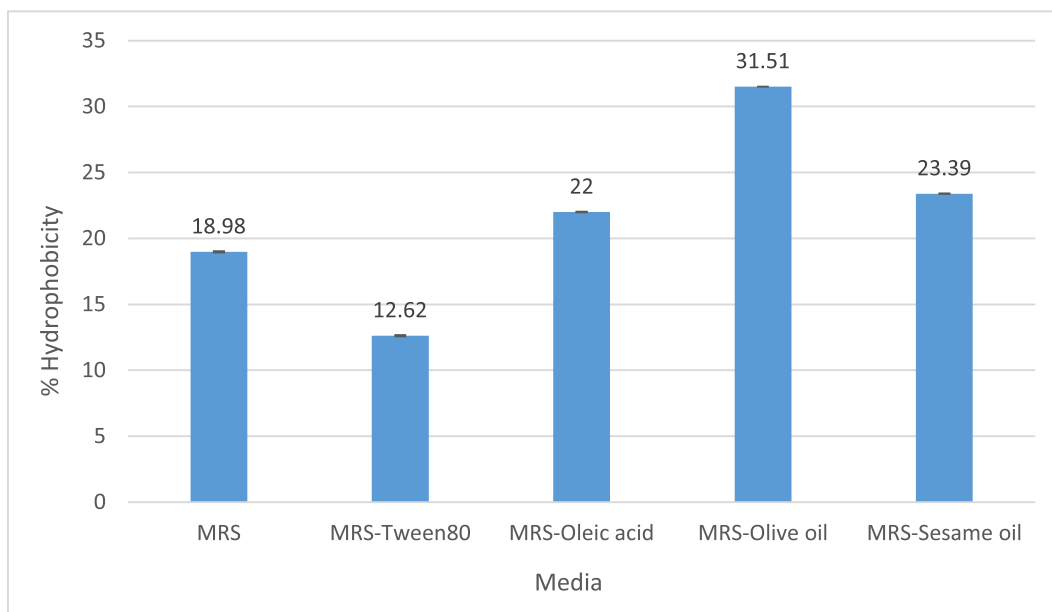


Fig. 5. Cell surface hydrophobicity of *P. pentosaceus* 144.

Table 5
Characteristics of *P. pentosaceus* isolated from various sources.

Organism	Source	Characteristic features				Reference
		Acid tolerance	Bile tolerance (% bile, w/v)	% Auto-aggregation	% Cell surface hydrophobicity	
<i>P. pentosaceus</i> MZF16	Fermented meat	pH 3 for 3h	0.3	88	18	(Zommiti et al., 2018)
<i>P. pentosaceus</i> VJ strains	Idly batter	pH 2 for 2 h and 4h	0.3	89	79	(Vidhyasagar and Jeevaratnam, 2013)
<i>P. pentosaceus</i> OZF	Human breast milk	pH 3 for 3h	1.0	86	34	(Osmanagaoglu et al., 2010)
<i>P. pentosaceus</i> CFR R123	Fermented cereal/pulse preparation	pH 2 for 3h	0.3	–	62.8	(Raghavendra et al., 2010)
<i>P. pentosaceus</i> BH105	Human faeces	pH 3 for 3h	0.3	–	–	(Uymaz et al., 2009)
<i>P. pentosaceus</i> NB-17	Vegetable pickles	pH 3 for 3h	–	–	–	(Jonganurakkun et al., 2008)
<i>P. pentosaceus</i> I44	Ileal biopsy	pH 3 for 3h	1.0	87	19	This study

sedimentation rate of *P. pentosaceus* I44 was studied over a period of 5 h in intervals of 1 h, and at 24 h. There was sudden increase in the aggregation rate after 4 h (Fig. 4). Since the isolate is a permanent inhabitant of intestinal mucosa, it can be said with confidence that the organism has to have a strong aggregation ability. *P. pentosaceus* I44 exhibited an aggregation ability of 26% after 5 h, and 87% after 24 h. It was observed that oleic acid was able to improve the isolate's aggregation ability.

Auto-aggregation refers to clumping of bacterial cells belonging to the same strain. Aggregation potential above 40% is considered good, while the organism is considered to have weak aggregation ability if it is less than 10% (Wang et al., 2010). Zommiti et al. (2018) observed an aggregation ability of 88% after 24 h for *P. pentosaceus* strain isolated from meat, while Vidhyasagar and Jeevaratnam (2013) reported maximum aggregation potential of 89% for *P. pentosaceus* isolated from idly batter. The aggregation potential for *Pediococcus* strains have been observed to vary from 35% to 89% (Abbasiliasi et al., 2017; Ilavenil et al., 2016; Lee et al., 2014).

3.4.2. Cell surface hydrophobicity

Hydrophobicity of microbial cell surface has been shown to play an important role in various biological interactions (Rosenberg et al., 1980), especially in attachment to host tissue which gives the commensals a competitive advantage (Tuo et al., 2013).

In the microbial adhesion to hydrocarbon (MATH) assay, a microbial suspension (in an appropriate buffer) is mixed with small quantity of hydrocarbon (either of hexadecane, octane, xylene or toluene), and is allowed to stand to facilitate separation of the aqueous and organic layers. Decrease in absorbance of the lower aqueous layer (due to the adhesion of microbe to hydrocarbon) is used as qualitative measure of hydrophobicity of microbial cell surface. Bacterial cell surface is said to be hydrophobic if the hydrophobicity index was greater than 70% (Nostro et al., 2004). The percentage of adhesion of *P. pentosaceus* I44 to toluene (an apolar solvent) was around 19% in MRS media indicating that its cell surface was hydrophilic. None of the supplements had an impact on the *Pediococcus* cells to make it hydrophobic (Fig. 5).

High hydrophobicity indicates the presence of glycoproteins on the bacterial surface while hydrophilic nature is due to polysaccharides (Bellon-Fontaine et al., 1996; Greene and Klaenhammer, 1994; Pelletier et al., 1997; Rojas and Conway, 1996). Cell surface hydrophobicity varies with organisms, strains, age of the culture, its surface components and the media composition (García-Cayuela et al., 2014). Different probiotic bacteria have been reported to exhibit varying hydrophobicity index ranging from 14% - 35% for *Bifidobacteria*, 11%–47% for *Lactobacillus* and 10%–79% for *Pediococcus* (Vidhyasagar and Jeevaratnam, 2013; Vinderola and Reinheimer, 2003; Xu et al., 2009; Zommiti et al., 2018).

Fig. 5

Zommiti et al. (2018) observed that the aggregation and hydrophobicity observed in *P. pentosaceus* isolated from meat had no correlation with adhesion to Caco-2 cells. This is in consensus with Vidhyasagar and

Jeevaratnam (2013) who inferred that hydrophobicity is not a necessity for adhesion but can aid in adhesion (Todorov et al., 2008). Many other studies pointed out that there was no significant correlation between hydrophobicity and adhesion under both *in vitro* and *in vivo* conditions (Deepika et al., 2009; Ouwehand et al., 1999; Savage, 1992; Vinderola and Reinheimer, 2003).

It can be inferred from Table 5 that the *P. pentosaceus* I44, like most other *Pediococcus* isolates, exhibited gastric tolerance at pH 3 for 3 h. It was found to have good tolerance to bile concentration of 1%, indicating that it is adapted for survival in the human intestine. Its aggregation percentage was quite low till 4 h, but was seen to increase thereafter exhibiting 87% aggregation at 24 h. The cell surface of this ileal isolate was hydrophilic with a hydrophobicity percentage of 19%. Further, it was observed that oleic acid and oleic-acid containing compounds had a significant effect on the stress tolerance ability and surface properties of the isolate.

4. Conclusion

Understanding the microbiota in healthy individuals provides insights into their functions in the gut. The main focus of this study was to characterise those bacteria that constitute the “probiome”, so that we will be able to sustain and nurture them for better health. This study reports the isolation and basic characterization of a mucosa-adherent lactic acid bacteria from ileal biopsy samples obtained from healthy Indian subject. Very little is known about the genus *Pediococcus* obtained from human sources. Borges et al. (2013), who studied vaginal isolate of *P. pentosaceus* mentions that some of the vaginal lactic acid bacteria may have their origin from the rectum (Petricevic et al., 2012). Such observations help us to appreciate the importance and scope of gut bacteria and the extent of influence they have on human health. Also, this study gives preliminary evidence that oleic acid improved the stress tolerance ability of the isolate. High throughput sequencing along with culture-based analysis of these organisms is required for a thorough understanding of the gut isolates. This organism and the other isolates obtained are being further studied for their adaptations to the human gut.

CRedit authorship contribution statement

Lavanya Vasudevan: Conceptualization, Methodology, Validation, Investigation, Writing – original draft. **Jayanthi V:** Conceptualization, Resources. **Srinivas M:** . **Chandra TS:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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