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Isolation and Identification of *Pediococcus pentosaceus* from Cow's Milk Curd and Its Use in Grape Juices Fermentation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BVK and KU designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author OVSR managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study affirms the quality of fermented grape juices with the potential probiotic bacterium *Pediococcus pentosaceus*, which can perhaps be used in the preparation of probioticated grape juices.
Study Design: Potential probiotic bacterium was isolated from curd prepared by using cow milk and identified as *Pediococcus pentosaceus* based on 16S rRNA gene sequencing. It was assessed for fermentation of two varieties of grape (white and red) juices.
Place and Duration of Study: The work was carried out in Microbial Biochemistry Laboratory, Department of Biochemistry, S.V. University, Tirupati - 517 502, India; One year.
Methodology: The isolate was identified by 16s rRNA gene sequencing. Both fruit juices were fermented with isolate and Titratable acidity by titration method, Reducing sugars by DNS method, Cell viability by pour plate, TPC by Folin-Ciocalteu method, Antioxidant assay by DPPH method and Antimicrobial activity by Agar well diffusion method.

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Results: The fermented red grape juice showed better fermentation characteristics of pH (3.1) and viable bacterial count (6.5 log CFU/mL) than the fermented white grape juice (3.2 and 6.2 Log CFU/mL) at 72 h fermentation. An increased titratable acidity (0.34%) was observed in fermented red grape juice as compared to the fermented white grape juice (0.27%). The fermented grapes juices possessed higher radical scavenging activity as well as phenolics content than their unfermented juices. The agar-well-diffusion method showed that both the fermented juices were able to inhibit the growth of the selected pathogenic bacteria (*E. coli* MTCC 40 and *B. cereus* MTCC 6840). Sensory evaluation showed that fermented red grape juice was preferred to other fermented and unfermented grapes juices.

Conclusion: Production of these fermented fruit juices on commercial scale may benefit the consumers, especially those intolerant to lactose and allergic to milk-based products. In addition, fermented fruit products are cholesterol free, low-cost healthy beverages and may provide better nutrition and health to the needy population.

Keywords: Fermentation; grape juices; Pediococcus pentosaceus; antioxidants; antimicrobial activity; sensory evaluation.

1. INTRODUCTION

The probiotics have been shown to improve the intestinal microbial balance and the properties of the indigenous microflora. Probiotics have been defined as living microorganisms which upon ingestion in adequate numbers exert positive health effects beyond inherent basic nutrition [1]. The probiotic strains have an ability to produce antimicrobial metabolites (organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins) [2,3]. Bacteriocins are ribosomally synthesized anti-microbial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria (LAB) [4]. Piva & Headon [5] have reported that Pediocin A like bacteriocin was produced by *Pediococcus pentosaceus* FBB61. Adel et al. [6] reported that better growth performance and disease resistance were observed in *Pediococcus pentosaceus* supplemented to white shrimp.

Fruit juice could serve as a good medium for cultivating probiotics [2]. They do not contain any dairy allergens (e.g., lactose), and might help certain segments of the population having allergy to dairy products [7]. Most of the tropical fruits including grapes are important sources of antioxidants, vitamins, minerals, and dietary fibers, and they constitute as a very healthy part of a human diet. Furthermore, red grapes like many other fruits do not contain any dairy allergic proteins and are a healthy choice over dairy products for probiotication.

Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way [7]. There is a genuine interest in the development of fruit juice based functional beverages with probiotics, because they have taste profiles that are appealing to all age groups, and are perceived as healthy and refreshing foods [8]. Furthermore, fruit juices are often supplemented with oxygen scavenging ingredients such as ascorbic acid, thus promoting anaerobic conditions that facilitate probiotication. Fruit juices contain high amounts of sugars that could encourage probiotic growth. Non-dairy substrates that have been used for lactic acid bacteria (LAB) fermentation include soy protein and cereals. In recent years, several researches have reported that fruits and vegetable juices could serve as basal medium for LAB fermentation.

Grapes have a long and abundant history. During the ancient time, grapes were esteemed for their use in winemaking. Nowadays, according to FAO recommendations, an intake of five portions of fruit and vegetables per day is beneficial in terms of public health [9]. Some fruits (apple, pineapple, cranberry, pomegranate, red grapes and orange) are under study as beverage or food ingredients (mixed beverages, ready-to-eat products) because they are rich source of natural bioactive compounds with proven health properties [10] and defined bactericidal or bacteriostatic capabilities [11]. As grape juices are a relevant source of poly-phenolic compounds, many people are becoming aware of the importance of their consumption in their daily diet. There is an increasing public concern as to developing healthy habits and eating quality food. Some consumers are also taking into account agricultural methods (conventional or organic) when purchasing their food. Organic agriculture, among other practices, does not use pesticides during the cultivation [12].

Grapes are a storehouse of numerous health promoting phyto-nutrients such as poly-phenolic antioxidants, vitamins and minerals [13]. Generally both fresh fruits and their juices are included in our regular diet as they give benefits to all age groups. Juices from these sources are deemed to be advantageous because of their low allergenicity, perceived health benefits and appeal to a wide segment of the population [14]. Grapes juice is a rich source of the antioxidant and flavonoids (catechin, epicatechin, quercetin, and anthocyanins. Oligomeric proanthocyanidin complexes (OPCs) are powerful antioxidants found in grape juice, skin, and seeds). Those are useful to treat a range of health problems related to free radical damage including heart diseases, diabetes and cancers [15].

In the present study, it was envisaged to ferment red and white grapes juices with *Pediococcus pentosaceus* isolate (VJ1) for certain greater benefits. Comparative analytical data of the fermented juices as well as their unfermented ones on aspects of physico-chemical characteristics, efficacy of anti-oxidants, antimicrobial activity and sensory evaluation were collected and reported in the communication.

2. MATERIALS AND METHODS

2.1 Isolation and Cultivation of Lactic Acid Bacteria

The curd prepared from cow milk (1 g) was transferred in to 10 mL of phosphate buffer (pH 6.8) solution and diluted by following serial decimal technique using sterile peptone (1%) solution. And 1.0 mL from each of the dilution was placed in a sterile Petri plates containing MRS agar medium and spread uniformly by using a sterile bent glass rod and then incubated at 37°C for 72 h in anaerobic gas pack jar system (Hi-media, India). The colonies were isolated and evaluated by cell morphology, Gram staining and physiological characteristics (Carbohydrate fermentation, catalase and arginine hydrolysis).

2.2 Identification by 16S rRNA Gene Sequencing

Bacterial DNA was isolated according to the procedure described by Hiney et al. [16] with certain modifications. PCR reaction was performed in gradient thermal cycler (Eppendorf, Germany), 16S rRNA gene region was amplified with the universal primers like forward primer - 27F (5-AGAGTTTGATCCTGGCTCAG-3) and reverse primer -907R (5'CCGTCAATTCCTTTGAGTTT-3). The reaction mixture of 50 µL consisted of 5 µL 0f 10× PCR amplification buffer, 200 µM dNTP, 0.5 mM $MgCl_2$, 2 pmol of each primer, 200 ng of DNA template and 1.0 U of Taq DNA polymerase. Amplification was done by initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing temperature of primers was 55°C for 30 seconds and extension at 72°C for 10 min. And 10 µL of the reaction mixture was then analyzed by submarine gel electrophoresis (1.0% agarose) and visualized under Geldoc. PCR products were purified by quick gel extraction kit (QIAGEN India Pvt. Ltd., India). Sequencing was carried out using Sanger dideoxy nucleotide method at NCCS (Pune, India). The sequence was compared by BLAST database, and closely related sequence as given to particular microorganism. The sequence of a strain was submitted to NCBI, GenBank database and accession number was noted for further references. Multiple sequence alignment was performed using ClustalX MEGA4.0 software phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

2.3 Probiotic Characteristics of *Pediococcus pentosaceus*

2.3.1 Acid tolerance

The *Pediococcus pentosaceus* was tested for its ability to tolerate varying concentration of acids. The pH of the de Man, Rogosa and Sharpe (MRS) broth was adjusted to 2.0, 2.5, 4.5 and 6.5 with 1 N HCl. Overnight grown culture of the above was inoculated into MRS broth at different pH and incubated at 37°C. Inoculated culture (1 mL) was taken at 1, 2, 3 and 4 h intervals, and again inoculated into fresh broth tubes having adjusted the pH to 6.8. These broth tubes were incubated at 37°C for 12 h and the cell density was measured at 600 nm using a Spectrophotometer.

2.3.2 Bile tolerance

Bile salt tolerance is potentially a probiotic property of LAB cultures. In this experiment, MRS broth containing 0.3% of bile salt (Taurocholic acid) was inoculated with *Pediococcus pentosaceus* and incubated at 37ºC for 24 h. The control comprised of MRS broth

without bile salt. Bacterial growth was monitored by measuring OD at 600 nm after overnight incubation.

2.3.3 Determination of the resistance to low pH in the presence of pepsin

Fresh 24 h culture of strain was centrifuged for 15 min at 5,000 x g. The resulting sludge biomass is washed twice with phosphate buffer saline (PBS) and re-suspended to the initial volume with PBS. The cell suspension (0.2 cm) is incubated with 5 cm^3 buffer solution with pH 2 containing 0.5% NaCl and pepsin (at a concentration of 3.2 g/dm^3) (Sigma, 2,500 - 3,500 U/mg protein). For cell viability determination the samples were drawn at different time intervals (1, 2 and 3 h) [17].

2.4 Preparation and Processing of Grape Juices

The grapes (red and white) were purchased from a local market in Tirupati. The selected fruits were washed thoroughl with running tap water, rinsed with distilled water and blotted dry. The seeds are separated manually from the pulp and then juice were extracted by hand pressing and straining the above prepared material through double fold muslin cloth. After pulping, 0.1 g/L potassium metabisulphate was added as a preservative and kept in a freezer at -4°C for further use.

2.4.1 Extraction of juice by enzyme treatment

Red and white grape juices were treated with 0.1% (w/v) of commercial pectinase enzyme and incubated at 40ºC for 2 h. The activity of enzyme reaction was stopped by heating at 100ºC for 10 min. The juice was extracted by passing through cheese cloth [18] and samples were stored at 4°C. The juice samples were subjected to analysis of total acidity, pH and total soluble solid (TSS) contents. Total sugars were estimated by Anthrone method [19].

2.5 Inoculum Preparation

The isolated culture was maintained in MRS agar stabs as pure cultures. It was grown in two successive MRS broth cultures at 37ºC for 24 h. The activated culture was again inoculated into MRS broth incubated at 37ºC for 24 h and this was used as the inoculum.

2.6 Fermentation of Fruit Juices

Fermentation experiments were conducted in 500 mL Erlenmeyer flasks containing 250 mL of pasteurized (at 85ºC for 15 min) grape (white and red) juices, individually. All samples were inoculated with a 24 h old culture (10^6 CFU/mL) and incubated at 37ºC for 72 h. After probiotification the fermented juices were stored at 4ºC for further analysis.

2.7 Physico-chemical Analysis

Samples were collected periodically during fermentation, and they were analyzed for various parameters. The pH was measured by a microprocessor based pH meter (Eutech, Japan), which was pre-calibrated with buffers of pH 4.0 and 7.0. Titratable acidity of fermented juices was determined by titrating with standard 0.1N NaOH, that was previously standardized with standard oxalic acid, and the values were expressed as tartaric acid equivalents. TSS were determined by using Hand Refractometer (0-30) (Erma, Japan) and values were expressed in terms of °Bx (Brix). Reducing sugars were estimated by spectrophotometric method using 3, 5 dinitro-salicylic acid [20]. The viability of the bacterial culture in fermented grape juices was determined by using pour plate method. The cell growth was assessed by counting the bacterial population after 24, 48 and 72 h of probiotication of grape juices on MRS agar. Plates containing 25-250 colonies were examined and colony forming units (CFU) counted and recorded as CFU per mL [21].

2.8 Antioxidant Activity

The assay was carried out according to the method described earlier by Vijaya Kumar et al. [22] with some modifications. A stock solution was prepared by dissolving 24 mg of 1,1-diphenyl, 2-picrylhydrazyl (DPPH) in 100 mL methanol and then stored at -20°C. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1±0.02 units at 517 nm using a Spectrophotometer. Different volumes of various fruit juices (50-200 µL) were allowed to react with DPPH solution (final volume 4 mL) and were shaken vigorously and allowed to stand for 30 min in dark at room temperature. Methanol was used as a blank. BHT (butylated hydroxytoluene) was used as a standard. Radical-scavenging activity (% inhibition) was calculated using the following equation.

DPPH free radical scavenging activity (%) = [(A control - A sample) / A control] × 100. Where, A = absorbance at 517 nm.

2.9 Total Phenolic Content (TPC)

TPC content was determined by using Folin-Ciocalteu method as described by Kumar et al. [23]. Briefly, 0.5 mL of appropriately diluted juice samples or standard solutions of gallic acid were pipetted into test tubes along with 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent, and the mixture was allowed to react for 3 min. Then 1 mL of 20% Na_2CO_3 solution was added, mixed well and left to stand for 1 h at room temperature for color development. Absorbance against prepared reagent blank was measured at 750 nm using a Spectrophotometer (MODEL UV-10, Thermo Fisher Scientific, USA) and values were expressed as mg of gallic acid equivalent per 100 mL.

2.10 Antimicrobial Activity of Fermented Juices

The agar-well-diffusion method was used to determine the antimicrobial activity of the fermented fruit juices. A 24 h-old cultures of the pathogenic strains (*Bacillus cereus* MTCC 6840 *Escherichia coli* MTCC 40) were grown individually in Nutrient Broth (NB) medium and then 1.0 ml each of cell suspension was spread over the surface of Muller Hilton agar plates using a sterile spreader. The plates were allowed to dry and a sterile well borer of diameter (5 mm) was used to cut uniform wells in the agar. Each well was filled with 100 µL of cell-free fermented red or white grape juices. After incubation at 37°C for 24 h, the plates were observed for a zone of inhibition (ZOI) around the well. Results were considered positive if the diameter (mm) of the ZOI was greater than 1 mm.

2.11 Sensory Evaluation

The sensory characteristics of the fermented fruit juices were evaluated with a 20-member panel [18]. The preferences for taste, aroma, flavor, color and overall acceptability were determined by 9-point Hedonic scale. Randomized refrigerated (10°C) samples (50 mL) were served in clear tulip shaped glasses coded with a random 3 digit code. The mean intensity scores of all the attributes were calculated and plotted.

2.12 Statistical Analysis

The analysis of the same sample was made in three replications and the results were expressed as mean value ± standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS, version 16.0.

3. RESULTS AND DISCUSSION

In this investigation, fermentation of red and white grape juices was carried out by a newly isolated and identified strain of *Pediococcus pentosaceus,* and characterized the fermented juices for various chemical and biochemical parameters.

The results presented in Table 1 show that the isolated culture is a coccus-shaped, Gram-positive bacterium and has positive response to growth at different pH and temperatures. It formed ammonia by arginine hydrolysis as well as showed negative response to catalase hydrolysis, which are the main characteristic features of a lactic acid producing bacterium. The isolated culture has also shown positive responses to probiotic characteristics such as bile and acid tolerances.

Hence, the identification of *Pediococcus pentosaceus* was confirmed at molecular level by 16S rRNA amplified nucleotide sequences. The PCR products were sequenced at DNA sequencing facility available at National Centre for Cell Sciences, Pune, India. The 481 bp sequence obtained was used for homology search for 16S rRNA sequences available in database using BLAST analysis. Twenty sequences of NCBI database gave 99% similarity. The phylogenetic tree (Fig. 1) was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method by using MEGA 4.0. The bootstrap values were indicated at nodes of the tree. The isolate (VJ1) was found to possess 99.99% similarity with *Pediococcus pentosaceus* strains and it was confirmed as *Pediococcus pentosaceus* VJ1, and was submitted to NCBI Genebank and obtained an accession number (KJ740608).

The growth kinetics at different temperatures (10-45ºC), different pH (3.0-9.6), NaCl (1-10%) concentrations, acid tolerance and bile salt tolerance were observed as positive. The physiological characteristics of carbohydrate fermentation (fructose, dextrose, sucrose, mannose, maltose, xylose, inulin and cellobiose) were found to be positive. The results of carbohydrate fermentation and biochemical characterization patterns were compared with Bergey's manual to identify that the isolate as *Pediococcus pentosaceus*. Its resistance to acid

Table 1. Morphological, physiological and probiotic characteristics of the isolate culture

Characteristics	*Pediococcus pentosaceus* VJ1
Grams staining	+
Cell morphology	Coccus
Catalase	-
Arginine hydrolysis	+
Growth at temperature 10–45ºC	+
Growth at pH range pH 3.0–4.0 and pH 5.0–9.6	+
Growth at NaCl concentration 1–10	+
Fermentation of xylose, maltose, fructose, dextrose, sucrose, mannose, inulin, cellobiose.	+
Acid tolerance	+
Acidifying activity	+
Bile salt tolerance	+

+ Indicates growth/turbidity

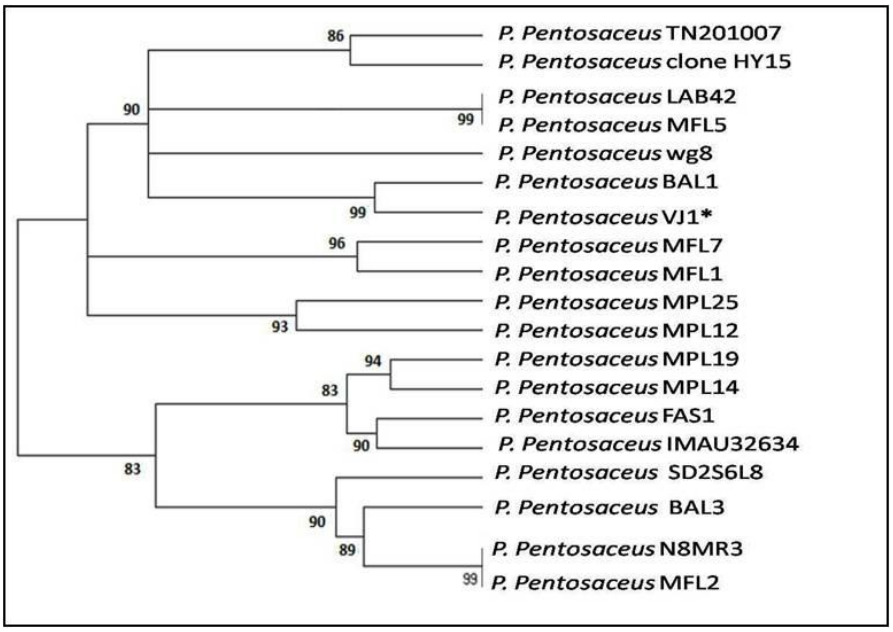

Fig. 1. Phylogenetic tree of *Pediococcus pentosaceus* VJ1 (indicated by Asterisk *)

conditions and observed that 78% survival after treatment of cells in the presence of pepsin(pH 2.0) for 1 h, and the viability decreased significantly beyond 3 h (data not shown). Survival at pH 3 is a significant feature, as ingestion of the probiotic with food is subjected to a raise in pH to 3 in the stomach [24].

The survival of the strain at pH 6.4-6.7 was also tested using 0.3% of bile salt (Taurocholic acid). The results showed that 10^5 CFU/mL survived for 14 h at 37°C and then significantly decreased by 24 h. Bile salt hydrolase (BSH) activity of 1 and 2% for 3 h with a viable count of 10^4–10^3 CFU/mL was reported earlier [24]. Growth of the strain in bile salts is an important criterion for intestinal survival accessibility. The above results support that the strain is tolerant to both environments, i.e., acidic conditions (pH 2.0 – 3.0) and in the presence of bile. In addition it also had an antimicrobial activity against some pathogenic bacteria. These characteristics of the strain may imply that it is a probiotic bacterium.

Pediococcus pentosaceus VJ1 is catalase negative and hydrogen peroxide is unstable at room temperature. Therefore, it is less likely that either acid or hydrogen peroxide was responsible for the antimicrobial potential of *Pediococcus pentosaceus* VJ1. The predominant microbial member in *Kimchi,* a Korean fermented pickle,

has been reported to be *Pediococcus* sp. in the early phase of fermentation [25]. It was also reported to be a producer of bacteriocin which may be of use as a preservative in food industry. Fruit and vegetable juices served as an alternative carrier or medium for probiotic drink production [2].

3.1 Physico-chemical Analysis of Fermented Grape Juices

According to Young Yoon et al. [26] fruit juices could serve as a good medium for cultivating probiotics. Recently Vijaya Kumar et al. [18] reported mango and sapota and other fruit juices as good media. It was observed that grapes (white and red) juices, without any nutrients, served as good culture media and matrix for the growth of *Pediococcus pentosaceus* VJ1. Physico-chemical properties like TSS, pH, titratable acidity and total sugars of red and white grape juices were shown in Table 2. The total sugars and TSS were high in red grape juice when compared with white grape juice. But, white grape juice has lower titratable acidity than red grape juice. pH of red grape juice is 3.6 as compared to 3.5 in white grape juice. However, Sheehan et al. [14] reported low pH of fruit juices in the pH range 2.5-3.7 caused the bacteria sensitive to stressful conditions that decreased their growth, and resulted in extensive differences with respect to the acid resistance property of *Lactobacillus* and *Bifidobacterium* in orange, pineapple and cranberry juices.

Grape juices fermented with *Pediococcus pentosaceus* VJ1 showed a decrease in pH, increase in acidity, and an improvement in the utilization of sugars at different time intervals (24, 48 and 72 h) as shown in Table 3. The pH was decreased in both the fermented fruit juices after 24 h due to carbohydrate fermentation that lead to increased acidity due to formation of organic acids. However, no significant change was noticed at 72 h. as there was slight decrease in cell viability due to decrease in concentration of reducing sugars and an increase in titratable acidity. Yoon et al. [26] reported significant increase in beet juice titratable acidity due to organic acids formation by *Lb. casei, Lb. plantarum, Lb. acidophilus* and *Lb. delbruckeii*. The bacteria rapidly consumed the substrates (sugars) in the fruit matrix and liberated their products in to the medium. The results were in good agreement with earlier reports [14] as some probiotic strains were found to have the capability to grow in fruit matrices. It has been proposed that cell viability is a factor which depends on the substrate, the oxygen content, status of nutrients and the final acidity of the matrix used. Shah [27] and Yoon et al. [28] also observed that rapid sugar utilization implied good bacterial growth, decrease in sugar concentration and pH and increased acidity in fermented fruit juices.

The grape juices supported the growth of *Pediococcus pentosaceus* and shown better viable count (survival rate) in both red and white grape juices (6.5 and 6.2 $\times 10^8$ CFU/mL, respectively) at 72 h of fermentation. Higher cell viability was observed in fermented red grape juice than in fermented white grape juice. Tezcan et al. [29] reported a rapid utilization of sugars (19-13% of initial concentration) especially more with glucose and fructose by *Lb. plantarum* than the others three lactic cultures i.e., *Lb. delbruckii, Lb. paracasei* and *Lb. acidophilus.* Similar results were reported by other authors on carbohydrate fermentation that varied depending on type of substrate consumed and even on fermentation time [30]. Also it was reported that glucose is a primary energy source for *Lactobacillus*. Therefore, glucose has been introduced as the most important sugar source for the growth of probiotic lactobacilli [31].

3.2 Antioxidant Activity and Total Phenolic Content (TPC)

The antioxidant activity of the fruit juices was quantitatively measured by using DPPH method and the results are shown in Fig. 2. The fruit juices were shown better scavenging activity before fermentation and after fermentation. The fermented fruit juices have slightly higher scavenging activity than non fermented fruit juices. The total polyphenolic content of fermented grape juices at different time period are shown in Fig. 3. The fermented red grape juice has higher content of total phenolics than fermented white grape juice at various time intervals (24, 48 and 72 h). Similarly, the fermented white grape juice also has shown increased total polyphenolic content progressively with increase in fermentation time.

3.3 Antimicrobial Activity of Fermented Grape Juices

The antimicrobial activities of fermented red and white grape juices were given in Table 4. Tetracycline has shown higher inhibitory activity on both *E. coli* and *B. cereus* (12 ± 1.74 and 14 ±

0.86 mm, respectively) than that of both fermented grape juices. Fermented red grape juice significantly inhibited the growth of *E. coli* (9±0.1 mm, ZOI), followed by *B. cereus* (8±0.2). The fermented white grape juice has given higher ZOI against *B. cereus* (8±0.1 mm) than that by *E. coli*, (7±0.2 mm). The phenolics of juices inhibit pathogenic bacterial growth by disturbing the proton gradient across the membrane or by increasing the membrane permeability due to the formation of pores or pits on the membrane. Ham et al. [32] reported that garlic fermented by *Pediococcus pentosaceus* KACC 91419 had inhibitory activity against antibiotic-resistant pathogens.

Table 2. Physico-chemical analyses of red and white grape juices

Grape juice	Juice yield (mL/kg)	Total soluble solids (Brix %)	Titratable acidity (%)	pH	Total sugars (g/100 ml)
White	500	18±0.81^{b}	0.30±0.10^{b}	3.5±0.10^{a}	14±0.2^{b}
Red	450	24±0.76^{a}	0.15±0.01^{a}	3.6±0.15^{b}	18±0.01^{a}

**Values are mean of three replicates (± Standard Deviation). The experimental values within columns that do not have a common superscript are significantly different (p<0.05) according to Duncan's multiple test range*

Table 3. Physico-chemical analyses of fermented red and white grape juices

Grape juice	Incubation (h)	TSS (Brix)	Titratable acidity (% Oxalic acid)	pH	Reducing Sugars (g/100 ml)	Viable count (× 10^{8} CFU/ml)
White	24	16±0.8^{b}	0.28±0.08^{a}	3.4±0.04^{b}	12±0.03^{b}	7.1±0.04^{a}
	48	14±1.0^{b}	0.30±0.03^{a}	3.3±0.04^{a}	10±0.02^{a}	6.4±0.1^{a}
	72	9±0.81^{a}	0.34±0.02^{b}	3.2±0.08^{a}	6±0.04^{a}	6.2±0.08^{b}
Red	24	23±0.47^{b}	0.22±0.016^{a}	3.5±0.04^{b}	17±0.04^{b}	8.0±0.1^{b}
	48	20±0.81^{a}	0.25±0.08^{a}	3.3±0.04^{a}	14±0.03^{b}	6.8 ±0.09^{a}
	72	16±0.47^{a}	0.27±0.004^{a}	3.1±0.04^{a}	9±0.06^{a}	6.5±0.04^{a}

**Values are mean of three replicates (± Standard Deviation). The experimental values within columns that do not have a common superscript are significantly different (p<0.05) according to Duncan's multiple test range*

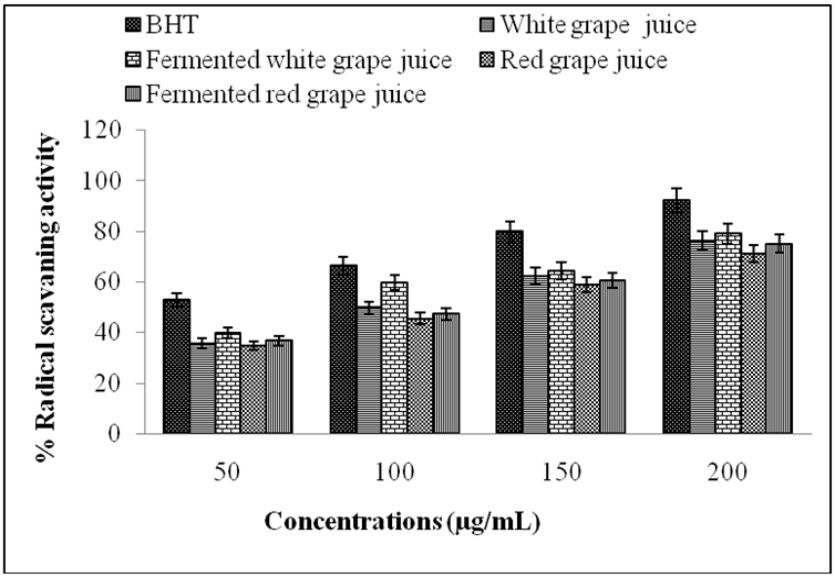

Fig. 2. DPPH radical scavenging activities of control and fermented white and red grape juices (BHT- Butylated hydroxyl toluene)

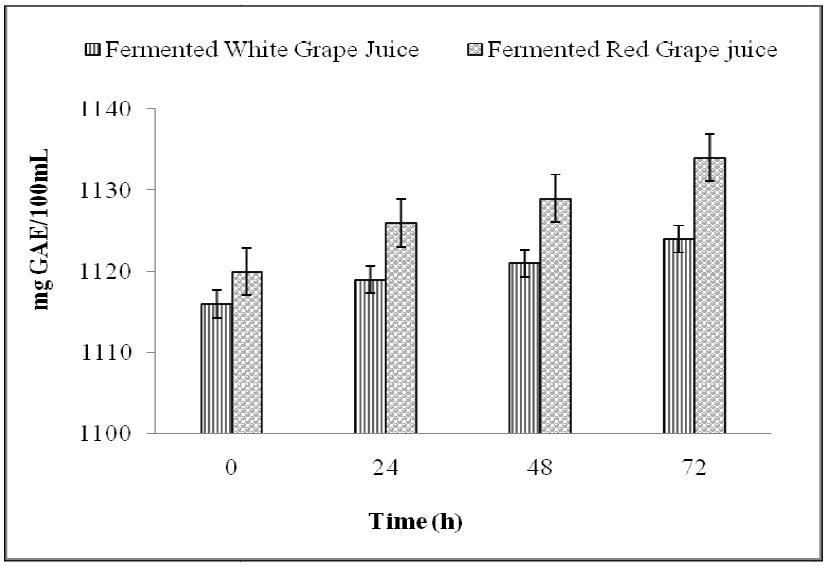

Fig. 3. Total phenolics content of fermented white and red grape juices

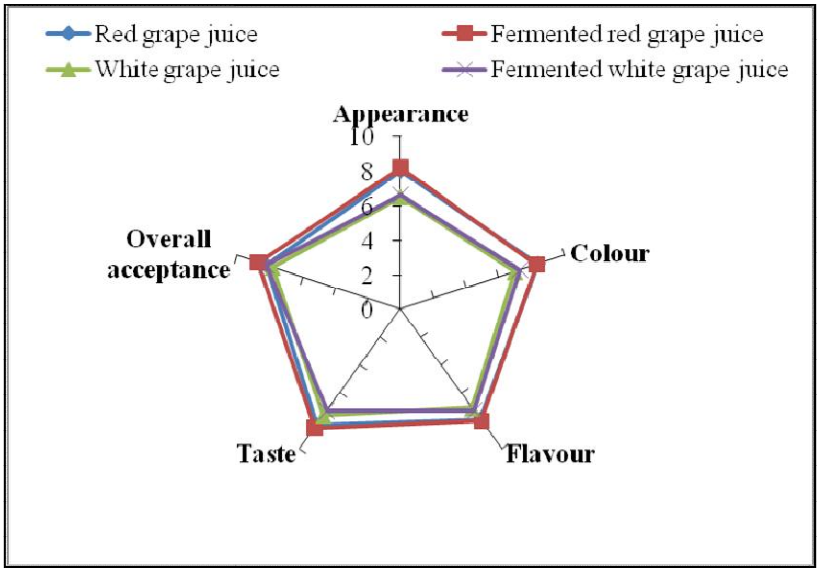

Fig. 4. Sensory evaluation of control and fermented white and red grape juices

Table 4. Antimicrobial activities of fermented red and white grape juices

Sample	Zone of inhibition (mm)	
	E. coli **MTCC40**	***B. cereus*** **MTCC6840**
Fermented red grape juice	9±0.1	8±0.2
Fermented White juice	7±0.2	8±0.1
Tetracycline	12±1.74	14±0.86

**Values are mean of three replicates (± Standard Deviation)*

3.4 Sensorial Analysis

Sensory evaluation results are shown in Fig. 4. These results indicated that both the fermented juices of red and white grapes had good sensory scores when compared to unfermented juices. Only a marginal difference was noticed between the sensory scores of fermented and control juices. The taste, acidity, mouth feel, aroma, flavour, color and overall acceptance were changed in fermented juices. The red grapes fermented juice had better overall organoleptic quality and appearance than the other juices.

4. CONCLUSION

Pediococcus pentosaceus VJ1 was isolated from the curd of cow's milk and found to have probiotics activities such as antibacterial, antioxidant, acid tolerance and bile tolerance. The isolate could grow well in white and red grape juices. Hence, its probiotic properties could be exploited in the preparation of probiotic fruit juices from grapes. Production of these fermented fruit juices on commercial scale may benefit the consumers, especially those intolerant to lactose and allergic to milk-based products. In addition, fermented fruit products are cholesterol free, low-cost healthy beverages and may provide better nutrition and health to the needy population, and the culture also used for growing the shrimp in aqua cultural industries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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