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ORIGINAL RESEARCH PAPER

EVALUATION OF POTENTIAL PROBIOTIC CHARACTERS OF *LACTOBACILLUS FERMENTUM*

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Abstract: The present study aims to evaluate the probiotic potential characters isolated from palm wine and different fermented foods. The strains isolated from palm wine and sourdough were *Lactobacillus fermentum* and from cucumber was *Pediococcus acidilactici* which exhibited potential probiotic characters when tested *in vivo*. *L. fermentum* isolated from palm wine exhibited equal tolerance with strains isolated from sourdough and cucumber. Results presented in this study revealed that *L. fermentum* from palm wine could withstand the harsh conditions in the gastro-intestinal tract by tolerating the low *p*H, bile salts, 0.5 % pancreatin and 0.2 % phenol *in vitro* conditions. The potential probiotic characters exhibited by *L. fermentum* can be further investigated *in vivo* and *in vitro* studies to elucidate the health benefits and assess technological performance as novel starters. Along with sourdough and fermented cucumber, palm wine was a good source for the isolation of prominent probiotic strains for the application at industry level.

Keywords: *antibiotic resistance, bile tolerance, hierarchical cluster analysis (HCA), Lactobacillus fermentum, low pH tolerance, palm wine*

INTRODUCTION

Probiotics are defined as live microorganisms (mono or mixed) which are administered in adequate amounts (10^6 - 10^7 Colony forming unit (CFU)/g of food) and confer health benefits in the host [1, 2]. The term probiotic means "for life" was addressed by Lilly and still well [3]. Most frequently used bacterial probiotics includes *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and *Enterococcus*. Current investigation refers that some yeasts are considered as probiotics which have positive influence on health of the host. Recently, *Saccharomyces boulardii* is the nonpathogenic yeast used as probiotic [4].

These probiotic bacteria are considered as alternative food supplements which show positive effect on the host by stimulating the growth of beneficial microflora in the gut and inhibit the amount of pathogens by competing for nutrients. This leads to lowering the risk of gastrointestinal diseases by maintaining the balance in the gut [5]. In market, for probiotic foods there is a considerable interest as a beneficial health promoting functional food. According to the FAO/WHO [6], the probiotic foods are fermented food containing adequate amounts of viable active microbes to reach the intestine and exert equilibrium action on gut microflora. Probiotic bacteria involves in enhancement of bioavailability of nutrients, certain intolerances like lactose and reduction in prevalence of allergies in susceptible individuals. It is reported that they are hypocholesterolemic, anticarcinogenic, antimutagenic, antihypertensive, antiosteoporosis and immunomodulatory effects [7]. Probiotic bacteria reduce the inflammatory bowel diseases, irritable bowel syndrome, colitis, alcoholic liver disease, constipation and risk for liver and colon [8].

Lactic acid bacteria (LAB) are the most promising probiotic bacteria introduced in various dairy food products like fermented milk, yogurt and cheese. Different cheese products are based on *Lactobacillus helviticus* such as mozzarella, asiago, emmental etc. Probiotic yogurt, ayran, koumiss, maasi, functional fermented milk etc are based on *L. rhamnosus* CAN-1, *L. plantarum*, *L. brevis*, *L. paracasei*, *L. bulgaricus*, *S. thermophilus*, *L. fermentum*, *L. acidophilus* [9]. Recently the usage of non-dairy, traditional fermented foods may constitute a good working bench for the development of probiotic functional food [10 – 12]. Among the traditional fermented foods, olives are the best probiotic food used as functional starter culture. The wild type strains are dominant traditionally in fermentations process. In this process tend to have higher metabolic activities and aroma formation which can beneficially affect the quality and safety of food. Food industry developed a new functional probiotic food with the available information provided from traditional fermented foods and scientific research [13].

Majority of studies published till today was about the physiological properties of strains used for probiotic bacteria, originated from human or animal sources are considered as better colonizers and are adapted in the gastro-intestinal tract (GIT) [11, 14 – 18]. In the present study bacterial strains were isolated from different milk products, fermented foods and palm wine. Palm wine was obtained from fermented sap of plants belonging to *Palmae* family. Sap obtained from *Borassus flabelliformis* is clear, colorless, sweet containing 10 - 12 % of sugar and later on microbial fermentation the sugar content was reduced and converted into alcohol. Studies on microbiology of palm wine were focused on isolation and characterization of microbes from fresh and fermented sap by

culturing methods. The microflora identified from palm wine are *L. plantarum*, *L. casei*, *Lactobacilli* sp., *Leuconostoc menesteroids*, *Leuconostoc lactis*, *Bacillus subtillis*, *Enterobacterium bacterium*, *Pediococcus*, *Acetobacter*, *Staphylococcus* [19].
The aim of the present study was to evaluate the potential probiotic charcters of lactic acid bacteria isolated from different fermented products. These strains can be further used in industrial application for the production of various bioactive compound and functional foods.

MATERIALS AND METHODS

Isolation of lactic acid bacteria (LAB)

The strains were isolated from different sources like milk, curd, yoghurt, bovine colostrum, colostrum powder, sourdough, idly batter, dosa batter, kimchi, fermented cucumber and palm wine. Milk, curd, sourdough, idly battere, dosa batter, kimchi and fermented were the homemade samples. Yoghurt, bovine colostrum and colostrum powder were procured from commercial markets, Bangalore, India. Palm wine was collected from local vendors of Vishakapatnam, Andhra Pradesh, India. Samples were serially diluted, plated on De Man, Rogosa and Sharpe (MRS) plates and incubated at 37 °C for 48 h. The colonies were further purified and cultured for morphological and biochemical identification by following Bergey's Manual of Determinative Bacteriology [20, 21]. The strains were further screened for potential probiotic characteristics.

Evaluation of probiotic potential

Effect of pH and bile salts

According to Todorov *et al*. [22] bacterial strains were grown in MRS broth at different *p*H ranging from 2 to 10. Bacterial strains were tested for resistance to bile salts by culturing the strains in MRS broth containing bile salts ranging from 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0 % (w/v). 100 µL (microliters) of inoculum was adjusted to 0.2 optical density (OD) at 600 nm and inoculated in the tested MRS broth and the OD was recorded for 24 to 48 h. Cultures grown on MRS broth without bile and *p*H were considered as controls. All experiments were conducted in triplicates.

Pancreatin and phenol tolerance

The effect of pancreatin on bacterial strains was determined by inoculating 100 µL (OD 0.2) of culture into MRS broth containing 0.5 % of pancreatin and incubated 37 ^{o}C and optical density was measured at 600 nm for 24 - 48 h [23]. Phenol tolerance was determined by Shehata *et al*. [24] with minor modifications. 24 h old culture (100 µL OD 0.2) was inoculated in MRS broth containing 0.2 and 0.5 % of phenol and optical density was measured at 600 nm for 24 to 48 h using UV-Visible Spectrophotometer (UV-1800, SHIMADZU) and the experiments were conducted in triplicates.

Determination of cell surface hydrophobicity properties

Doyle and Rosenberg [25] described the measuring of cell surface hydrophobicity was modified by Todorov *et al*. [26]. 100 µL of culture (OD 0.2) was inoculated in MRS broth and incubated at 37 °C for 24 h. Cells were centrifuged (6700×g, 4 °C, 6 min) and washed with sterile saline solution (*p*H 6) and re-suspended into same solution and optical density was measured at 580 nm. 1.5 mL of suspension was mixed with equal volume of n-hexadecane and vortexed for 2 min. After 30 min of incubation at room temperature two phases were separated, 1 mL of aqueous solution was removed and optical density ($OD_{580\ nm}$) was measured. Experiments were repeated in triplicates.

The percentage of hydrophobicity was measured as per equation (1):

$$\%Hydrophobicity = \frac{(OD_0 - OD_{30})}{OD_0} X100 \quad (1)$$

where OD_0 and OD_{30} refer to the initial OD and OD measured after 30 min, respectively.

Auto-aggregation properties

Bacterial strains were inoculated into MRS broth for 24 h and cells were harvested (7000×g, 10 min, 20 °C) and washed with sterile saline solution at *p*H 7 and re-suspended into same solution and diluted to OD 0.3 ($OD_{600\ nm}$). 1 mL of the suspension was taken and optical density was recorded over 60 min in spectrophotometer at OD 600 nm. After 60 min the suspension was centrifuged at 300×g, 2 min and OD of supernatant was determined at 600 nm [22].

The percentage of hydrophobicity was measured as per equation (2):

$$\%Auto - Aggregation = \frac{(OD_0 - OD_{60})}{OD_0} X100 \quad (2)$$

where OD_0 and OD_{60} refer to the initial *OD* and *OD* measured after 60 min, respectively.

Antibiotic resistance

To determine the antibiotic resistance, bacterial strains 1 % (v/v) were inoculated in MRS plates supplemented with different antibiotics such as penicillin, streptomycin, erythromycin, gentamycin, ampicillin, and chloramphenicol of various concentrations (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 $\mu g \cdot mL^{-1}$ and determined the minimal inhibitory concentration (MIC) by observing the growth of strains in a microplate reader OD at 610 nm after 24 h of incubation at 37 °C [5].

Chemometric analysis

The data was collocted from viable count of bacterial strains, after exposure to *p*H 3 for 3 h and bile salts (0.4 %) for 4 h respectively. This data was used as input variables in hierarchical cluster analysis (HCA) based on Euclidian distances to allocate strains into homogeneous groups according to these two characteristics [5]. Prior to analysis, the data was standardized by calculating the relative survival ratio (RSR) using equation (3):

$$RSR = \frac{(\log cfuNo - \log cfuNt)}{\log cfuNo} x100 \quad (3)$$

where N_0 represents the total viable count for LAB before treatment and N_t the total viable count after the treatment at low *p*H or bile salts, respectively.
The data was analyzed using the XLStat software (version 2006.06, Addinsoft, Paris, France), a built-in statistical software package of Excel.

Molecular identification by 16sr RNA sequencing

Approximately 1.5 Kb (kilobase) of bacterial genome was isolated to identify the bacteria by 16S rRNA sequencing method. Genome was isolated by DNA isolation kit (RKN15) and subjected to polymerase chain reaction (PCR) to amplify 16S rRNA coding region by using 27 forward and 1492 reverse primers (5' AGAGTTTGATCMTGGCTCAG 3' and 5' AGAGTTTGATCMTGGCTCAG 3'). The reaction mixture (100 µL) containing 1 µL of extracted bacterial genomic DNA, 400 ng (nanogram) of each forward and reverse primer, 10 µL of 10X PCR assay buffer, 1 µL of 3U Taq polymerase enzyme, 50 mM of $MgCl_2$ and nucleic acid free water was added to makeup the final volume. PCR was performed using the following thermal cycling conditions. Initial denaturation: 94 °C 3 min, 30 cycles at 94 °C 30 s, annealing 60 °C 30 s, extension 72 °C 1 min followed by final extension at 72 °C for 10 min and hold at 4 °C ∞. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl capillary sequencer for DNA analysis from Applied Biosystems. A BLAST (Basic Local Alignment Search Tool) was performed to the query sequence using 16S rDNA sequence with the NCBI (National Center for Biotechnology Information) data base gene bank. The phylogeny analysis was performed to query sequence with the closely related sequence of BLAST results followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequences [27]. The program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model. The program Tree Dyn 198.3 was used for tree rendering [28].

Statistical analysis

All experiments were conducted in triplicates. The data were subjected MS Excel and XL Stat software.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria

The bacterial strains from all the sources, M1, M2, M3 (raw milk), C1, C2, C3 (curd), B1, B2 (buttermilk), Ch1, Ch2 (bovine colostrum), Ch3 (colostrum powder), S1, S2, S3, S4, S5 (sourdough), I1, I2, I3, D1, D2, D3, D4 (idly and dosa batter), K1, K2, K3, K4 (kimchi), Cu1, Cu2, Cu3, Cu4, F (fermented cucumber) and W1, W2 (palm wine) predominant colonies were isolated. Morphologically the colonies were smooth, white, elevated, regular colonies were observed on MRS plate. Microscopically gram+, rod or

cocci shaped bacteria were observed. Biochemically strains exhibited catalase, oxidase, MR-VP (Methyl Red-Voges Proskauer), indole negative and positive for lactose, sucrose, fructose, maltose, arabinose, xylose and ribose fermentation. These strains were further screened for probiotic characters.

Screening for probiotic characters

Effect of pH and bile on growth

The ability to grow and survive at low *p*H and higher concentrations of bile salts is an important characteristics of a strain encountered in the human gastrointestinal tract with potential probiotic activity [22]. From the present study results indicated that strains exposed to low *p*H ranging from 4 to 9 were grew well. At *p*H 2 no growth was observed and however less growth was observed at *p*H 3 and variable results were recorded at *p*H 10. Out of 32, four strains (W2, S1, Cu1 and K1) showed maximum tolerance to low *p*H and bile salts (Figure 1). Similar results were reported by Todorov *et al*. [22] *Lactobacillus plantarum* ST16Pa isolated from papaya. *L. plantarum*, *L. rhamnosus*, *L. pentosus*, *L. paracasei* suppressed growth was observed at *p*H 3 and 4. At low *p*H the growth of the bacterial strains was less. Previous investigation indicated that the bacterial growth was affected by various degrees of *p*H. In human stomach the *p*H ranges from 1.5, during fasting, to 4.5 after a meal, and food ingestion can take upto 4 h. In order for probiotic bacteria to exert their physiological role in gut, the bacteria will need to survive in highly acidic gastric juices [29]. According to Prasad *et al*. [16] while consumption of the probiotic strains were buffered with food and carrier molecules and are not exposed to HCl concentration in the stomach. These results were in agreement with *Lactobacilli* exposed to low *p*H (2 and 3) obtained from various sources originated from food, animal and human [17, 30 – 33]. In *Lactobacilli* the acid tolerance is due to occurrence of constant gradient between extracellular and cytoplasmic *p*H. In gram + organisms F_oF_1 – ATP-ase mechanism protects under acidic conditions and it generates proton motive force through proton expulsion [34]. F_oF_1 – ATP-ase is a multiple subunit enzyme consists of F_1catalytic portion and F_o an integral membrane portion. The F_1 subunit involved in ATP hydrolysis consists of α, β, γ and ε subunits, whereas F_1 consists of a, b & c subunits which acts as membrane channel for translocation of protons [35]. When extracellular *p*H lowers, the F_oF_1 – ATP-ase increases the intracellular *p*H and it is regulated at the level of transcription [36].

In the present study maximum growth of bacterial strains was recorded at 0.8 % of bile. Suppressed growth was observed with increased concentration of bile salts (1, 2 and 3 %).

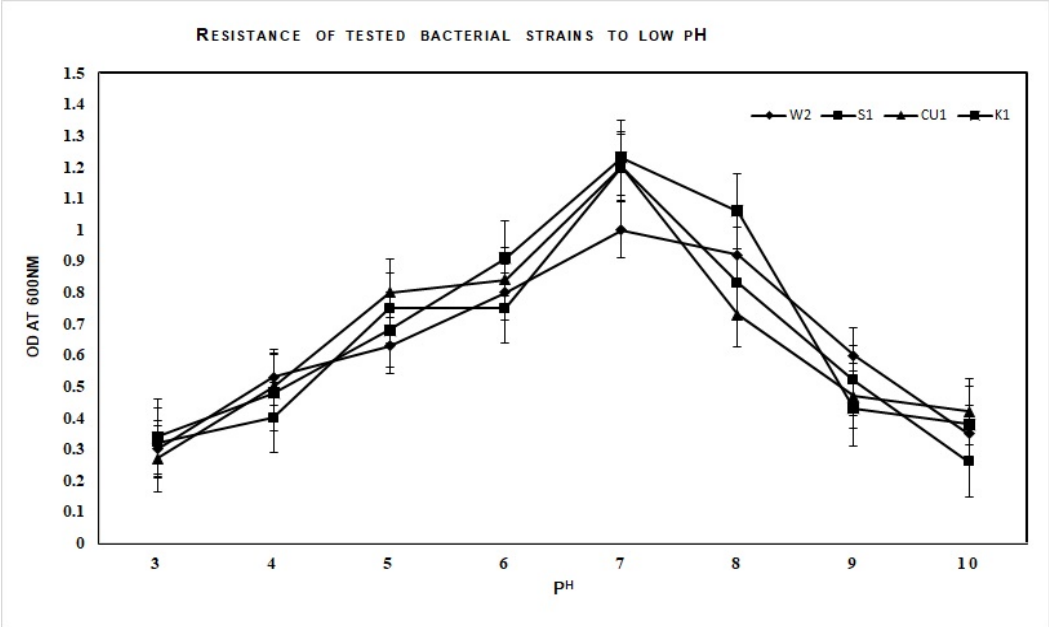

Figure 1. *Growth of the four tested bacterial strains (W2, S1, Cu1 and K1) in MRS broth with different pH 3 - 10*

Among all strains four bacterial strains (S1, Cu1, W2 and K1) showed maximum resistance to bile salts (Figure 2). Bile plays an important and fundamental role in specific and non-specific defense-mechanism in the human gut [37, 38]. Resistance to bile is an important probiotic characteristic to grow, survive under gastrointestinal environment and exert their action. These results were supported by Todorov *et al*. [22], the reduced growth was reported by *L. plantarum* ST194BZ and ST441BZ, *L. paracasei* ST242BZ and ST284BZ at 0.6 % of bile. Arasu *et al*. reported that *L. brevis* p68 isolated from fermented foods exhibited highest tolerance to low *p*H and bile salts [39]. *Lactobacillus* strains were resistant to bile salts, could grow survive and exert their normal physiological action and metabolize in the gastrointestinal tract (GIT). In addition to that *Lactobacilli* protect and promote the resistance to bile salts by some components in the food [40]. The resistance towards several stress factors in bacteria leads to resistance to bile salts and survive under gastrointestinal conditions [41]. The active mechanism involved in counteract bile salt toxicity is through efflux pumps which actively extrudes the bile acids and salts that are accumulated in the cytoplasm [42].

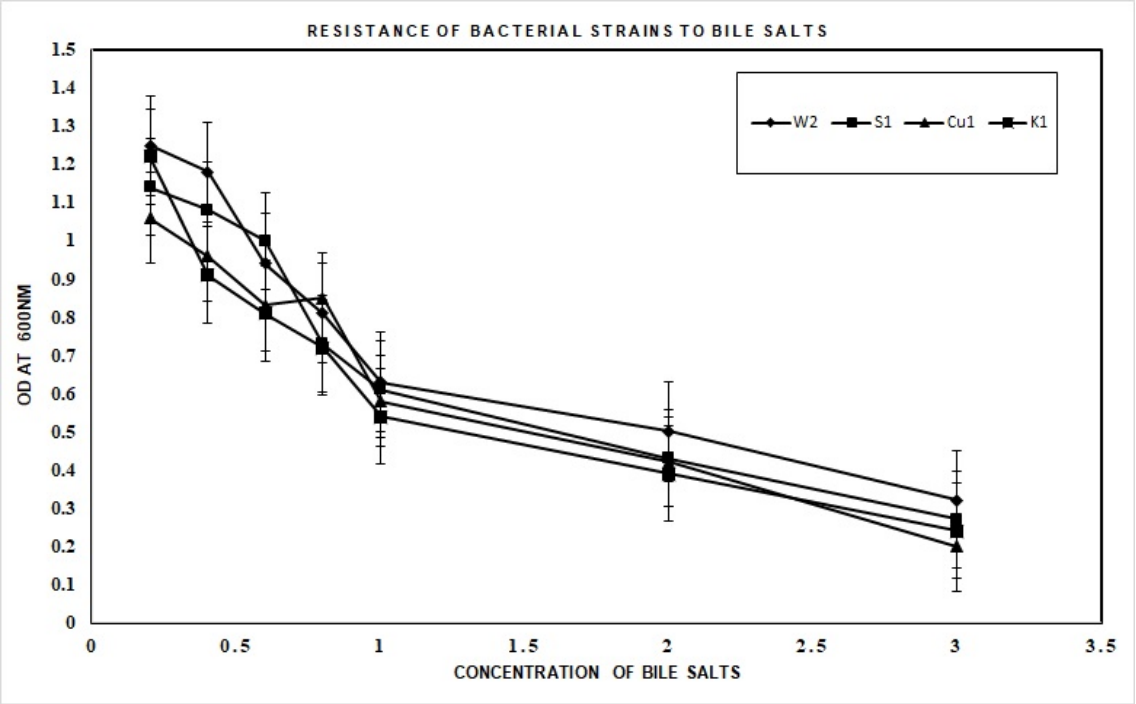

Figure 2. *Growth of the four tested bacterial strains (W2, S1, Cu1 and K1) in MRS broth supplemented with 0.2 - 3 % of bile salts*

Effect of pancreatin and phenol tolerance

The potential probiotic *Lactobacilli* are screened for pancreatin tolerance to survive under GIT environment. All tested bacterial strains exhibited resistance at 0.5 % of pancreatin. Khagwal *et al*. [23] reported that NKC903, NKC17(3b), NKC6L2 and NKC18(5C) exhibited maximum growth at 48hr of incubation at 0.5 % of pancreatin. In the current study tested bacterial strains showed different degrees of sensitivity towards 0.2 and 0.5 % of phenol. All tested bacterial strains showed resistance to 0.2 % of phenol and at 0.5 % of phenol concentration reduced growth of bacterial strains was observed. Results were in supported by Shehata *et al*. [24] isolates B037 and RM39 from boza and rayed milk showed 95 and 94 % of tolerance to 0.2 % of phenol concentration. At 0.5 % of phenol concentration 15 - 21 % of relative growth was reported. Phenols have bacteriostatic property. Phenols are the toxic metabolites produced during digestion process [43]. It is produced by some aromatic amino acids from dietary and endogenous proteins undergo deamination by gut bacteria [44].

Hydrophobicity and auto-aggregation properties

Maximum percentage of hydrophobicity was observed in all tested bacterial strains. Percentage of hydrophobicity ranges in between 30- 60 % for all strains). The highest percentage was observed in w1, S1, Cu1 and K1 (50, 45, 60 and 50 %) tested bacterial strains (Figure 3). Todorov *et al*. reported similar results that *L. rhamnosus* GG, *L. rhamnosus* ST461BZ, *L. rhamnosus* ST462BZ, *L. plantarum* ST664BZ showed 50 - 80 % of hydrophobicity [22]. Bacterial strains with highest hydrophobicity property forms strong interaction with intestinal mucosal cells. Bacterial cells show non-specific interaction with mucosal cells of the human intestine, but probiotic strains showing specific interaction with mucosal cells of intestine and exert physiological functions.

Adherence to mucosal cells was mediated by cell surface proteins and lipoteichoic acids [45]. Mucus binding proteins (MUB) are the bacterial adhesions which are responsible for attachment to mucus layer produced by *Lactobacillus reuteri* [46, 47]. The other reports on the binding of *L. reuteri* and *L. fermentum* to the mucus layer are mediated by mucus adhesion promoting proteins like MapA [15]. Aggregation is also an important probiotic character, those two bacterial cells co exits each other which helps in biofilm formation. Results from this experiment some bacterial strains reported aggregation properties in 30 - 50 % respectively. In some cells aggregation property was not observed. Similar results were reported by Todorov *et al*. [26] that *L. plantarum* ST16a showed 37.05 % of aggregation.

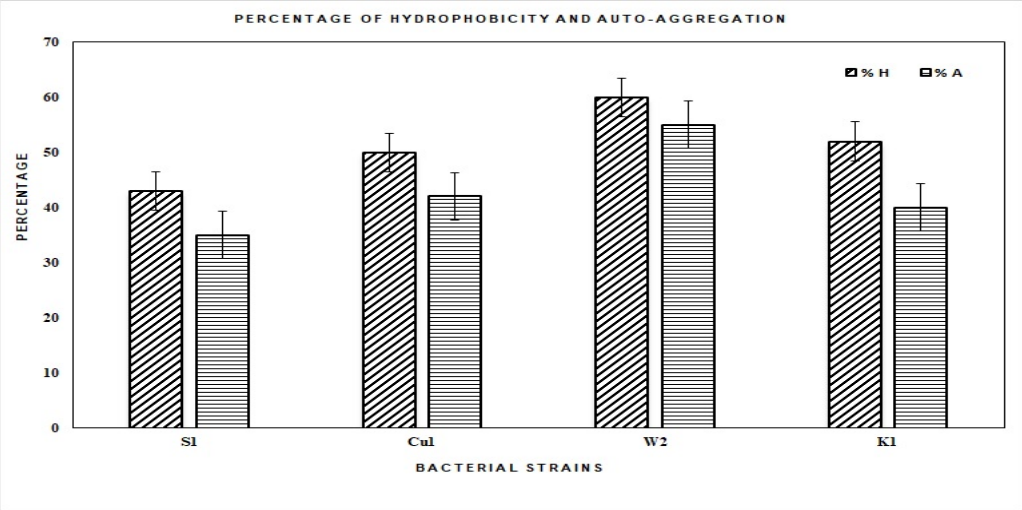

Figure 3. *Hydrophobicity and auto-aggregation property of bacterial strains*

Antibiotic resistance

Table 1 show minimal inhibitory concentrations (MIC) of tested bacterial strains to different groups of antibiotics: cell wall inhibitors (penicillin, ampicillin) and protein synthesis inhibitors (streptomycin, tetracycline, gentamycin, tetracycline, erythromycin and chloramphenicol) were studied. Bacterial strains were resistant when higher MICs values were compared with MIC break points established by European Food Safety Authority [48]. Bacterial isolates tested in this study were characterized as resistant strain to kanamycin, vancomycin, streptomycin, and chloramphenicol. All bacterial strains were vancomycin resistant which were supported by previous report [49, 50]. Zhou *et al*. also reported that *Pediococcus*, *Lueconostoc* and *L. rhamnosus* were resistant to vancomycin, kanamycin and tetracycline [51].

Charteris *et al*. reported that antibiotic resistant strains were involved in antibiotic induced diarrhea [52]. On other hand negative consequences like antibiotic resistance was transferred to intestinal pathogens [53]. According to previous study the resistance to aminoglycoside antibiotics (streptomycin, kanamycin and gentamycin) is considered to be intrinsic in the *Lactobacillus* genus and which are not mediated by drug uptake due to absence of cytochrome electron transport chain. The vancomycin resistance of *Lactobacillus* was due presence of D-Ala-D-lactate in the peptidoglycan layer instead of dipeptide D-Ala-D-Ala [50, 54, 55]. The genus of *Lactobacillus* that is responsible for vancomycin resistance is sited on the chromosomal, and is not easily transferred to other species [4].

Table 1. Antibiotic resistance of the tested bacterial strains

	MICs [µg·mL^{-1}]							
Strains	**T**	**S**	**C**	**G**	**P**	**A**	**E**	**V**
M1	<2	256	2	2	<2	2	8	64
M2	16	64	2	2	<2	2	<2	8
M3	16	16	2	2	<2	2	<2	8
C1	2	256	8	16	<2	2	<2	4
C2	2	64	16	16	<2	2	<2	32
C3	2	2	32	16	<2	2	<2	16
Ch1	<4	256	<2	2	<2	2	<2	16
Ch2	<2	64	<2	2	<2	2	<2	<4
Ch3	<2	256	<2	2	2	2	<2	<4
F	2^{R}	64^{R}	2	8^{R}	2	2^{R}	<2	<16
B1	2	32	<2	2	2	2	<2	64
B2	2	4	<2	2	2	2	<2	64
B3	2	256	<2	2	2	2	<2	8
S1	2^{R}	64^{R}	16^{R}	8^{R}	<16	2^{R}	4	512
S2	<2	8	2	4	2	4	2	64
S3	<2	8	2	4	4	2	2	8
S4	2	8	2	2	<2	2	<2	32
S5	2	4	2	4	<2	2	<2	8
I1	<2	2	<2	<8	<2	2	<2	32
I2	<2	2	2	<2	2	2	<2	512
I3	<2	2	<2	<2	2	4	<2	32
D1	<2	8	<2	8	2	2	<2	16
D2	<2	32	4	8	4	2	<2	64
D3	<2	8	2	8	4	2	<2	8
K1	2^{R}	32^{R}	8	16	32^{R}	16	4	256
K2	2	16	2	4	16	4	2	64
K3	2	16	2	64	4	4	2	32
K4	2	16	2	64	4	8	2	32
Cu1	2^{R}	64^{R}	<16	16^{R}	64	4^{R}	4	>1024
Cu2	<2	128	<2	32	16	16	2	1024
Cu3	<2	16	<2	16	16	8	<2	<512
Cu4	<2	16	<2	16	2	8	<2	512
w1	<2	2	2	16	32	16	2	>1024
w2	2R	64R	4	8R	4	2R	2	>10242

T - Tetracycline, S - Streptomycin, C - Chloramphenicol, G - Gentamycin, P - Penicillin, A - Ampicillin, E - Erythromycin, V - Vancomycin. MIC - Minimal inhibitory concentration. R - Resistant according to the EFSA's break points (EFSA, 2008)

Chemometric analysis

By considering the viable count of tested bacterial cells and calculating the relative survival ratio (RSR) HCA grouped the strains into three clusters as shown in Figure 4. The strains grouped in the lower cluster (group 1) are 13 which exhibited the maximum resistance to low *p*H and bile salts. In this group 1, S1, K1, Cu1 and W2 are located. In the (group 3) upper cluster, eight strains were presented which shows minor or no resistance to low *p*H and bile salts. 11 strains which were grouped in the middle cluster (group 2) exhibited intermediate performance.

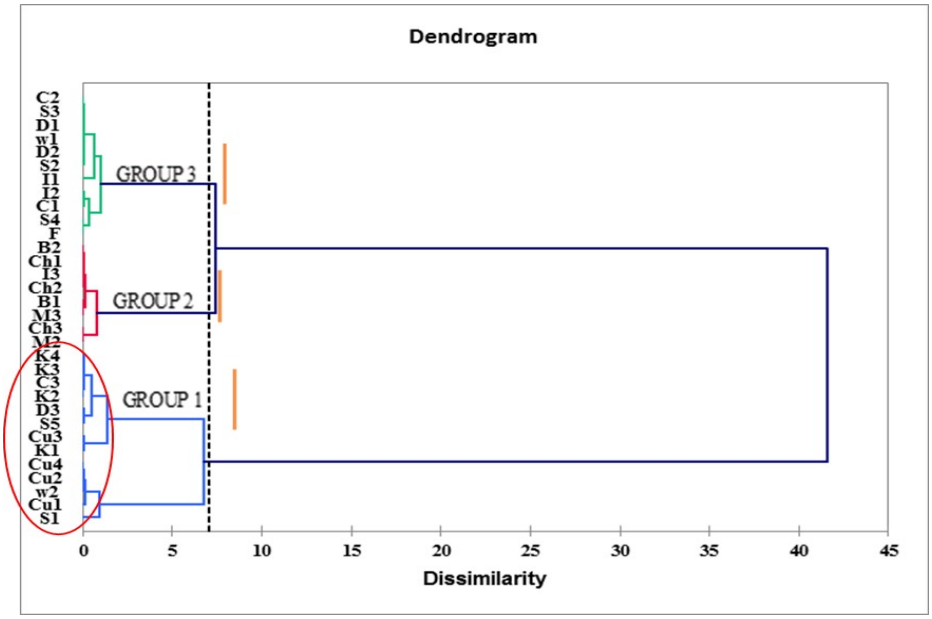

Figure 4. *Results of the hierarchical cluster analysis for the grouping of 34 tested bacterial strains into 3 clusters based on the resistance to low pH and bile salts*

Molecular identification of tested bacterial strains

Considering the above probiotic characters of these organisms (S1, Cu1 and W2) were identified as *Lactobacillus fermentum*, *Pediococcus acidilactici* and *Lactobacillus fermentum* by 16S rRNA sequencing and the accession numbers from NCBI was SUB2927621, SUB2927697 and SUB2927719 and dendrograms were observed in Figures 5, 6 and 7.

In previous study the *L. fermentum* was isolated from different fermented foods such as sayur asin (mustard cabbage leaf), tempe (soya bean), dadih (fermented buffalo milk) and fermented mare milk [56]. Satish Kumar *et al*. [29] reported that *L. fermentum* and *pediococcus acidilactici* are isolated from different traditional Indian fermented foods such as koozhu, pazhaiya soru, adai dosa, kallappam, dhokla, ambali, jilebi, curd, chhurpi, gundruk, sinki, khorisa-tenga. Owusu-Kwarteng *et al*. [57] isolated sixteen strains of *L. fermentum* from West African fermented millet dough possess desirable technological and probiotic properties. These sixteen *L. fermentum* showed above 80 % survival rate after incubation at *p*H 2.5 and 0.3 % of bile salts for 4 h. Suhartatik *et al*. [58] isolated the *Pediococcus acidilactici* from fermented foods such as tape, tempe and fermented vegetables showed *β*-glucosidase activity.

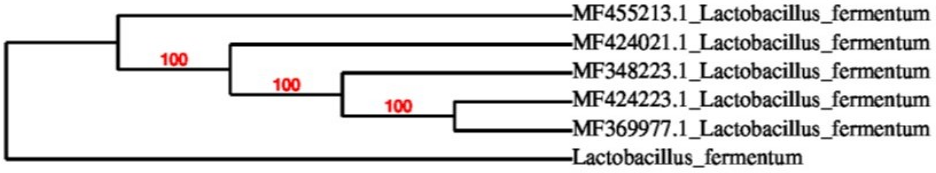

Figure 5. *S1 identified as L. fermentum by 16S rRNA sequencing method isolated from sourdough*

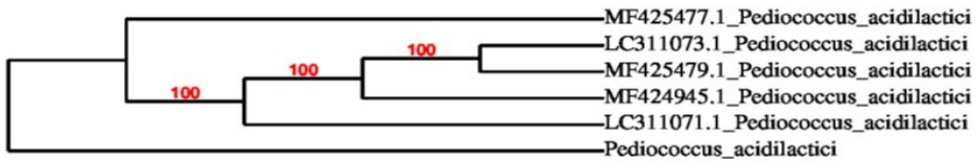

***Figure 6**. Cu1 identified as Pediococcus acidilactici by 16S rRNA sequencing method isolated from fermented cucumber*

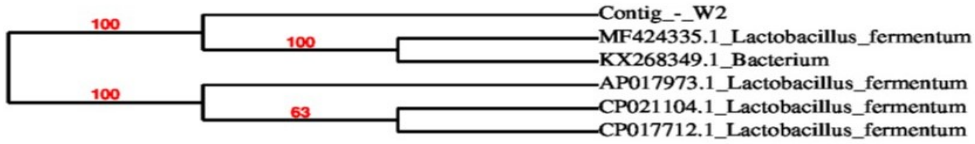

***Figure 7.** W2 identified as L. fermentum by 16S rRNA sequencing method isolated from palm wine*

CONCLUSION

Based on the results reported from early studies, strains *L. fermentum* from sourdough and *Pediococcus acidilactici* exhibited highest probiotic potential characters. In the present study it is reported that *L. fermentum* was isolated from palm wine exhibited potential probiotic characters which was not studied earlier. According to the available literature this is the first report that *L. fermentum* isolated from palm wine which exhibits equal probiotic characters compared with other lactic acid bacteria from different sources. The present investigation resulted that palm wine is a rich source for isolation of probiotic strains with potential application in the food industry. *L. fermentum* exhibited probiotic characters responsible for the survival and resistance in the gastrointestinal tract. So these strains are good candidates for further study, to elucidate their full potential and possible application as novel probiotic starter cultures. Further research is required for application of these strains in industry to bring out the consumable commercial probiotic products.

COMPLIANCE WITH ETHICS REQUIREMENTS

This article does not contain any studies with humans and animal subjects.

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