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Research Article
Identification, Characterization and Genetic Improvement of Bacteriocin Producing Lactic Acid Bacteria

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Abstract

Background and Objective: Bacteriocin produced by lactic acid bacteria has received more scientific attention, this is due to its ability to inhibit wide range of spoilage and pathogenic microorganisms and their potential usefulness as natural substitute for chemical food preservatives. The aim of the present study isolation, identification and improvement of Lactic Acid Bacteria (LAB) that produce bacteriocin from different traditional dairy products in Egypt. **Methodology:** Isolation and identification of LAB isolates based on morphological, biochemical and molecular 16S rDNA gene. To improve LAB bacteriocin production broth and filter mating techniques were used. The RAPD were used to confirm transconjugants. **Results:** Three bacterial isolates showed a high bacteriocin activity and named (I_1, I_2 and I_3). The three bacterial isolate were Gram positive, cocci, asporogenous, non-motile and negative to catalase and oxidase. The biochemical identification using API kit indicated that isolates I_1, I_2 were related to genus *Enterococcus* while isolate (I_3) was related to genus *Pediococcus* (94.4 and 99.5%) respectively. On the other hand, 16S rDNA sequencing showed 99% homology with *Enterococcus faecium* and 97% homology of I_3 with *Pediococcus pentosaceus*. All sequences were deposited in the GenBank nucleotide databases under accession number LC063691.1, LC063692.1 and LC063861.1. Genetic improvement of selected bacterial isolates were carried out using two different techniques of conjugation, filter and broth mating techniques. The transconjugation frequencies of the filter mating technique was higher than (4.6×10^{-5}) the broth mating technique (2.4×10^{-5}). The genetic variability among the transconjugants lines were tested using RAPD analysis and showed 8.25% polymorphism percentages for donor, recipient and tranconjugants lines. **Conclusion:** Results concluded that three bacteriocin producing LAB isolates *Enterococcus faecium* (I_1-I_2) and *Pediococcus pentosaceus* (I_3) with probiotic properties could be used in wide range of different industrial application.

Key words: Lactic acid bacteria, molecular identification, bacteriocin, antimicrobial activity, conjugation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Benefit bacteria such as LAB are a group of Gram positive bacteria non-motile characterized by common morphological, physiological and metabolic traits. They performed an essential role in the manufacture and preservation of numerous fermented food products[1]. The LAB involved in cheese production not only are responsible for acidification but also play relevant roles in the modification of flavor, texture of cheeses and inhibition of spoilage and pathogenic microorganisms[2]. The interest of LAB in the current industrial food manufacturing is mostly focused in genus *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and *Streptococcus* some often found in dairy products[3].

Many studies have been focused on the characterization and identification of LAB which were considered essential for understanding the contributions of LAB to cheese production[4]. The morphological characteristics of LAB are often unreliable, because of similar morphological and nutritional requirements of different species. Actually, most of the available techniques for the identification of LAB are based on molecular biology. The LAB taxonomy has been conducted recently due to the development in nucleic acid hybridization and sequencing techniques. One of the most common and faster techniques used for bacterial classification and identification in dairy products is partial or total sequencing of the 16S rDNA gene[5]. This technique is based on the amount of similarity of sequences between different individuals. Application of 16S rDNA sequence method could be linked to the databases that provide up to 100,000 sequences for the phylogenetic framework[6].

Some of these organisms producing a variety of antimicrobials including lactic acid, hydrogen peroxide and bacteriocins which may be present in foods that are manufactured with them. Bacteriocins are a heterogeneous group of ribosomally synthesized peptides or proteins displaying antimicrobial activity against other bacteria may be one possible way to replace or enhance chemical preservatives. Over the past years, there has been an explosion of basic and applied research on LAB bacteriocins, primarily due to their potential application as biopreservatives in food and food products to inhibit the growth of food borne bacterial pathogens, especially *Listeria monocytogenes*[7].

Recently, consumers are aware of functional and better nutritional aspects of the healthy products and this had led to the increasing trends towards healthy eating and development of functional food which meet specific nutritional requirements. In this respect, dairy products could be considered to have a great potential of being a functional food. This emerging situation has increased the isolation of new LAB cultures which are able to produce bioactive compounds and unique probiotic characteristics and used in many potential applications of protective cultures in various food systems[8,9].

Three mechanisms of gene transfer have been identified in microorganisms, transformation, transduction and conjugation in which a gene present on a mobile genetic element (plasmid or conjugative transposon) is transferred to another cell via direct physical contact. Bacterial conjugation is the most commonly used of mechanism and consequently the one that contributes most to the Horizontal Gene Transfer (HGT) marker pool in prokaryotes[10,11].

The LAB organisms have the potential to act as resistance genes conferring resistance to antimicrobials such as tetracycline, erythromycin, streptomycin, chloramphenicol and vancomycin[12]. These resistance genes were found to be located on transferable elements including plasmids and conjugative transposons. The most common laboratory techniques used to assess HGT *in vitro* include: Plate, filter and broth mating protocols. While there is general acceptance that higher transfer frequencies occur when using solid-phase mating mediums since conjugation requires mating cells to be in close contact with each other[13]. This study aimed to isolation, characterization and improvement of LAB having a high bacteriocin production ability.

MATERIALS AND METHODS

LAB isolates: Ten LAB isolates were purified from Egyption traditional dairy products according to the method adopted by Lavanya *et al.*[14].

Indicator strains: Eight bacterial indicator strains were used for study the bacteriocin antimicrobial activity included *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 19404, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus* ATCC 33018, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 7644 and *Lactococcus lactis* ATCC 11454.

Morphological and physiological identification of LAB isolates: Identification of LAB isolates were performed by examination for cell morphology, Gram staining, catalase activity, oxidase, optimum pH, temperatures and salt tolerance. Profile matching method based on Bergey's manual of systematic bacteriology were used for characterization and identification of LAB isolates[15].

Antimicrobial activity: Detection of antimicrobial activity was carried out using disc diffusion method according to Tejero-Sarinena *et al.*[16]. Each assay was performed in triplicate.

Quantification of bacteriocin activity: Arbitrary Units (AU mL^{-1}) of bacteriocin activities were calculated according to Yamamoto *et al.*[17].

Carbohydrate fermentation profile of LAB isolates: The API 50 CHL and API 20 strips kit (API system, Biomerieux, France) were used to identify the LAB isolates based on carbohydrate fermentation profile according to Adebayo-Tayo and Onilude[18] and Maqsood *et al.*[19]. The results were analyzed by API soft ware (API systems, Biomerieux, France).

Extraction of genomic DNA: Extraction of genomic DNA was carried out using Qiagen kit (Qiagen Sciences, Maryland, USA) according to the manufacturer's instruction manual.

PCR amplification of 16S rDNA gene: The PCR reactions were done to amplify the 16S rDNA gene from LAB isolates. The 16S rDNA region was amplified by using the universal primer set; 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), according to Weisburg *et al.*[20]. Reaction was analyzed on 1% (w/v) agarose gel.

Conjugation procedure: The two different conjugating techniques, filter and broth mating were carried out according to Gevers *et al.*[21].

Random Amplified Polymorphic DNA (RAPD): The RAPD-PCR technique was done using 13 oligonucleotide primers (Table 1). The PCR was performed according to Plengvidhya *et al.*[22] in a total of 25 µL reaction volume and amplification was programmed to 40 cycles after an initial denaturation cycle for 2 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an extension step at 72°C for 2 min, followed by extension for 10 min at 72°C in the final cycle.

Computer software: The phylogenetic tree for different LAB were deigned according to the blast result in GenBank database (www.ncbi.nlm.nih.gov).

RAPD analysis: The analysis RAPD and phylogenetic tree for transconjugants, donor and recipient was generated using SPSS 16 (SPPS Inc., Chicago, Il, USA) and Genomes.urv.cat/UPGMA/index.php?entrada.

Table 1: RAPD primers name and sequence

Primer name	Sequence (5′-3′)	Primer name	Sequence (5′-3′)
OPA-09	GGGTAACGCC	OPD-03	GTCGCCGTCA
OPA-11	CAATCGCCGT	OPD-04	TCTGGTGAGG
OPA-13	CAGCACCCAC	OPD-06	ACCTGAACGG
OPA-18	AGGTGACCGT	OPD-07	TTGGCACGGG
OPB-06	TGCTCTGCCC	OPD-14	GAACGAGGGT
OPD-01	ACCGCGAAGG		
OPD-02	GGACCCAACC		

Statistical analysis: Numerical data were obtained from three independent experiments and these data carried out with MS-excel and the data presented as means. The significant differences between means were analyzed using one way ANOVA with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Isolation and characterization of LAB: Ten local LAB isolates were purified from milk and traditional dairy product samples from Egypt and named as I_1-I_{10}. Ten bacterial isolates were pre-characterized based on their phenotypic and morphological characterizations. The colony morphology of the isolates was tested at different growth media. Three specific media were used for isolation for specific bacterial genus; MRS for used for isolation *Lactobacillus* species[23], (KAA) for streptococci (Enterococci) in foodstuffs[24] and M17 for lactic streptococci[25].

Table 2 summarizes the morphological characterization of ten LAB isolates. These isolates were grouped on the basis of Gram stain reaction. The bacterial isolates were Gram positive, catalase negative and cocci except I_7 and I_8 rods. A similar observation was reported by Khalid[26] who also noted that LAB were Gram positive without spore, with negative catalase, microaerophilic, resistant to acid and could be fermentation. Also, Holzapfel *et al.*[27] found that LAB were bacteria in rod or coccus shapes, with negative catalase, non motile, homo fermentative or hetero fermentative and growing in low acid condition.

Antimicrobial spectrum of crude bacteriocin from LAB isolates: The production of antimicrobial compound such as bacteriocin were more effective criteria used to characterize probiotics. The antimicrobial activity of the ten bacterial isolates were tested (Table 3) all the isolates give variable degree of inhibition zone and activity on different tested strains specially I_1, I_2 and I_3 against Gram positive bacteria, *Listeria monocytogenes* and *Staphylococcus aureus* as target propose. The bacterial isolates (I_1, I_2 and I_3) were the highest bacteriocin activity (320, 640 and 2560 AU mL^{-1}) and large clear inhibition zone (20, 22 and 25 mm), while I_6 and I_9

Table 2: Morphological characterization of the ten bacterial isolates

Bacterial isolate No.	Sources	Cell morphology	Arrangement	Gram staining	Catalase reaction	Growth media 1	2	3	Incubation temperature (°C)
I_1	Kariesh cheese	Cocci	Single to clusters	+ve	-	+	+	-	40
I_2	Yoghurt	Cocci	Single to clusters	+ve	-		+	+	40
I_3	Kariesh cheese	Cocci	Single to pairs	+ve	-	-	-	+	30
I_4	Cow milk	Cocci	Single to pairs	+ve	-	+	-	-	30
I_5	Cow milk	Cocci	Single to pairs	+ve	-	+	-	-	40
I_6	Feta cheese	Cocci	Single to pairs	+ve	-	+	-	-	30
I_7	Yoghurt	Rods	Single to short chain	+ve	-	-	-	+	30
I_8	Feta cheese	Rods	Long rods	+ve	-	-	-	+	30
I_9	Kariesh cheese	Cocci	Single to clusters	+ve	-	+	-	-	40
I_{10}	Ras cheese	Cocci	Single to pairs	+ve	-	+	-	-	30

1: MRS, 2: KAA and 3: MI7

Table 3: Bacteriocin activity of LAB isolates and inhibition zone

No. of producer isolates	Bacteriocin activity (AU mL^{-1})	Average zone of inhibition (mm)
1_1	320	22
I_2	640	20
I_3	2560	25
I_4	80	15
I_5	160	13
I_6	20	11
I_7	320	15
I_8	40	11
I_9	20	10
I_{10}	20	15

Table 4: Morphological and physiological characterization of three isolates that highly bacteriocin activity

Test	Isolate I_1	Isolate I_2	Isolate I_3
Gram staining	Gram positive	Gram positive	Gram positive
Oxidase	-	-	-
Catalase	-	-	-
Spore formation	-	-	-
Motility	-	-	-
Optimum growth temperature (°C)	30-45	30-45	30-40
Optimum growth pH	6-9	6-9	6-8
Growth at NaCl (% w/v)			
0	+	+	+
1	+	+	+
2	+	+	+
4	+	+	+
6	+	+	+
8	+	+	-
10	+	+	-
12	-	-	-

were the lowest bacteriocin activity (20 AU mL^{-1}) with hazy clear zone. Figure 1 show the inhibition zone of (I_1, I_2, I_3 and I_4) against *Staphylococcus aureus*.

These results agreement with Buntin *et al.*[28] who reported that three strains of lactic acid bacteria, *P. pentosaceus* APa4, *P. pentosaceus* Ala1 and *Enterococcus faecium* ARa1 were able to inhibit growth of *S. aureus*, *Salmonella* sp., *E. coli* and *L. monocytogenes* by agar well diffusion method. Bacteriocins produced by lactic acid

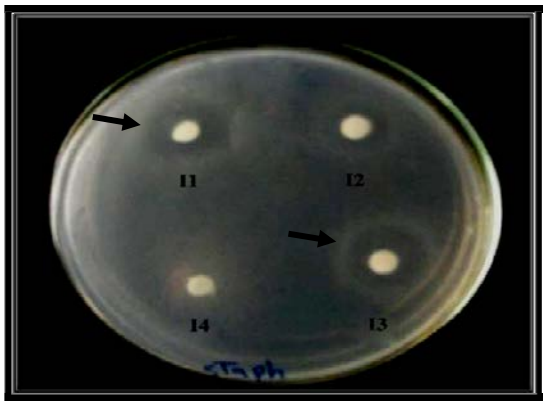

Fig. 1: *Staphyloccus* ATCC 25923 clear zone for different bacterial isolates (I_1, I_2, I_3 and I_4) on agar plate

bacteria can inhibit growth of Gram positive bacteria more than Gram negative bacteria because the outer membranes of Gram positive bacteria that play as barrier for bacteriocins to penetrate into their cell membranes[28]. Also, Bassyouni *et al.*[29] and Du Toit *et al.*[30] conferred that bacteriocins produced by the *E. faecium* strains showing a broader spectrum of activity, against indicator strains of *Enterococcus* spp., *Listeria* spp., *Clostridium* spp. and *Propionibacterium* spp., compared with those from *E. faecalis* strains showed a narrow spectrum of activity, mainly against other *Enterococcus* spp. Another study were isolated LAB from raw fruits and vegetables inhibited *E. coli* isolated from human sources[31]. Because of the high production of bacteriocin by the I_1, I_2 and I_3 isolates the three bacterial isolates (I_1, I_2 and I_3) were identified at the morphological, biochemical and molecular level.

Morphological and physiological characterization: Table 4 summarizes the morphological and physiological characterization of three important isolates (I_1, I_2 and I_3). The three isolates were cell shape cocci, non-motility,

Gram positive, which not forming spores and negative catalase and oxidase. The bacterial isolates I_1 and I_2 grow between 30-45°C but isolate I_3 grow at 30-40°C. These isolates grow at various concentrations of NaCl ranging from 2-10% (w/v). On the other hand, isolate I_3 was grow at 6% (w/v) NaCl. The selected LAB isolates were tolerate to acidic pH and resist to high concentration of NaCl which important and crucial criteria for potential probiotic and important starter culture in dairy product. These results agreement with Axelsson[32] who determined that LAB were belong to Gram positive group, with negative catalase, coccus or round shaped without spore and non-motile without respiration, but can grow on aerobic or microaerotolerant conditions and can produce lactate acid as main metabolic product from carbohydrate fermentation. The API 20 strep and API CHL 50 kits were used for biochemical identification of I_1, I_2 and I_3 bacterial isolates (Table 5).

The results indicated that isolates classified as follows: I_1-I_2 were *Enterococcus faecium* with 94.4% identical rate and I_3 *Pediococcus pentosaceus* with 99.5% identical rate. These results were preliminary identification of isolates by standard biochemical and morphological testes.

Molecular characterization: Universal primers designed for the amplification of 16S rDNA used to amplify ~1.5 kb size fragment in all of the selected isolates I_1, I_2 and 1_3 in Fig. 2. Amplicons of 16S rDNA were column purified and sequenced for ~1.5 kb fragment using a set of primers. The 16S rDNA sequences were aligned using blast algorithm (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and compared with the published sequences of 16S rDNA gene of different LAB strains deposited in NCBI databases.

The molecular identify were confirmed to be I_1 (99%) similarity with *Enterococcus faecium* strain L3-23, I_2 (98%) similarity with *Enterococcus faecium* strain gp 34, I_3 (97%) similarity with *Pediococcus pentosaceus* strain LAB2. Figure 3-5 show the phylogenetic tree of isolates and neighbor joining according to blast results. All sequences were deposited in the GenBank nucleotide databases under accession number LC063691.1, LC063692.1 and LC063861.1. These results indicated that the importance of using molecular methods for typing newly isolates microorganisms, phenotypic and genotypic identifications are part of the first step in the selection of potential probiotic bacteria[33].

The identification of LAB based on the morphological, physiological and biochemical characteristics are often considered as unreliable, since different species may have similar morphological and nutritional requirements[6]. The phenotypic characterization based on sugar fermentation profile may be used as a presumptive identification, it may not provide reliable identification, but genotype-based methods such as 16S rDNA are robust to identify bacteria as a complement or alternative to phenotypic methods[20]. Genotype methods are independent from variation of growth conditions, if species-specific primers or probes are available; these offer a very fast way to detect the target organism.

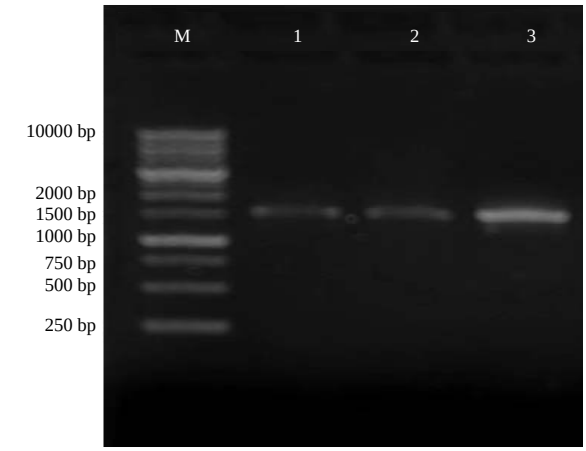

Fig. 2: PCR-amplification of 16S rDNA gene using 8F and 1492R primers, Lane M: Gene ruler DNA ladder 1 kb, Lane 1: 16s rDNA gene fragment of isolate I_1, Lane 2: 16s rDNA gene fragment of isolate I_2, Lane 3: 16s rDNA gene fragment of isolate I_3

Table 5: Biochemical identification of LAB isolates

Kit	Isolates No.	Similarity (%)	Strain
API 20 Strep	I_1- I_2	94.4	*Enterococcus faecium*
AP CHL 50	I_3	99.5	*Pediococcus pentosaceus*

Table 6: Conjugation between *Lactococcus lactis* sub sp. *lactis* and *Enterococcus faecium* AH2

Mating pair strains	Techniques	Average conjugation frequency
Lactococcus lactis sub sp. *lactis*	Filter mating	4.6×10^{-5}
Enterococcus faecium AH2	Broth mating	2.4×10^{-5}

Traditional genetic improvement of selected isolates via conjugation: Table 6 summarized the mating experiments were carried out in broth and filter mating techniques. The experiments were assessed using *Lactococcus lactis* sub sp. *lactis* (nisin producer) as a donor strain with tetracycline resistance markers, located on transferable genetic elements and *Enterococcus faecium* AH2 (LC063692.1) erythromycin resistance as a recipient strain. The results show low frequency of gene transfer approximately (2.4×10^{-5}) progeny per recipient occurred during broth mating, but (4.6×10^{-5}) progeny per recipient by filter mating, these results indicated

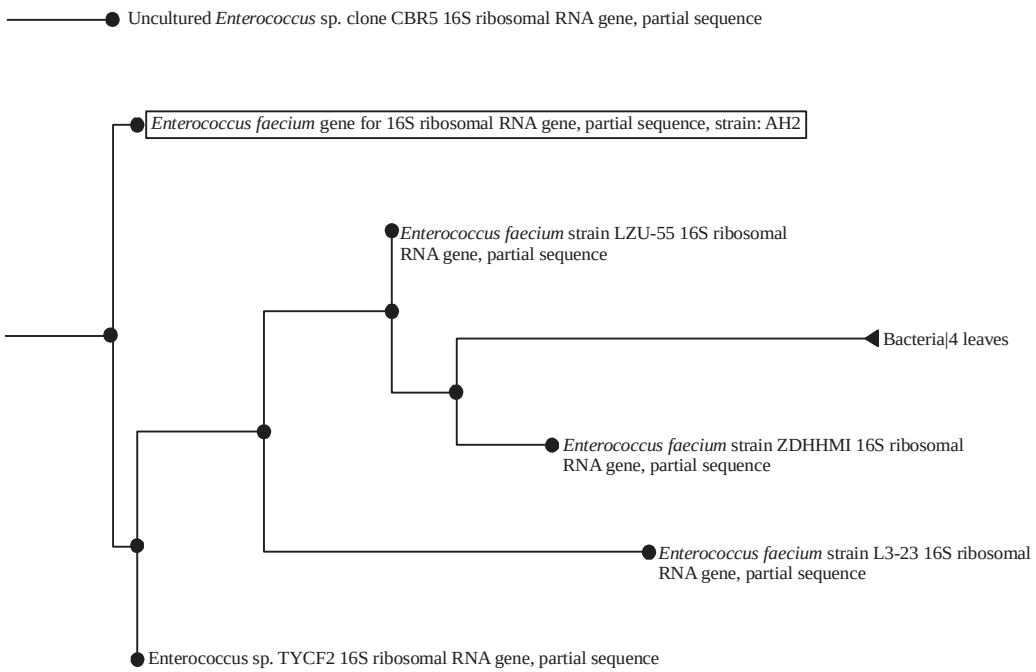

Fig. 3: Phylogenetic tree based on partial 16S rDNA sequences, showing the relationship between isolate (I_1) and other species. The tree was constructed using the neighbor-joining method. *Enterococcus faecium* strain: AH2 (LC063692.1)

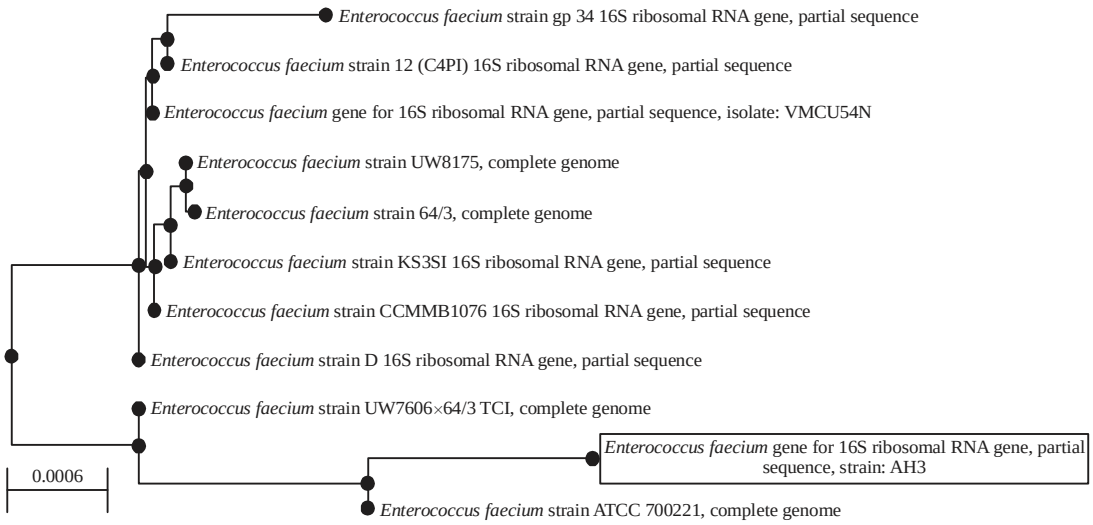

Fig. 4: Phylogenetic tree based on partial 16S rDNA sequences, showing the relationship between isolate 1and other species. The tree was constructed using neighbor-joining method. *Enterococcus faecium*: AH3 (LC063861.1)

that the filter technique facilitates a greater degree of donor-recipient contact than the broth method. These might be due to the concentration of cells in the broth system which considerably less and therefore contact between donor and recipient would be more sporadic. These results agreement with Lampkowska *et al.*[34]. The resulted transconjugants were confirmed using antibiotic selection and activity of bacteriocin (Table 7). The transconjugants T1, T2 and T3 showed increased of bacteriocin activity (1280, 1280 and 2560 AU mL^{-1}), respectively more than the recipient (460 AU mL^{-1}).

These results may be resulted from transfer of some genetic element from donor to recipient strain or genetic recombination lead to increase in bacteriocin production and activity. Moreover, when transposable integrated into cells, the transconjugation has a new genetic makeup or unique

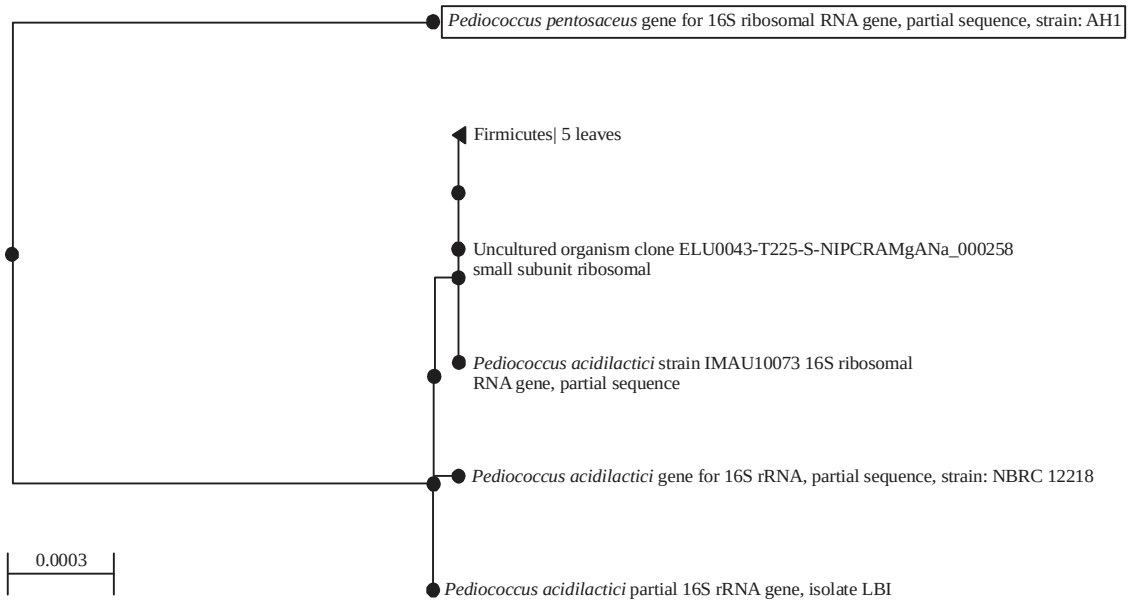

Fig. 5: Phylogenetic tree based on partial 16S rDNA sequences, showing the relationship between isolate (I_3) and other species. The tree was constructed using neighbor-joining method. *Pediococcus pentosaceus*, strain: AH1 (LC063691.1)

Table 7: Activity of bacteriocin (AU mL^{-1}) produced by donor (*Lactococus lactis* sub sp. *lactis*), recipient (*Enterococcus* AH2) and transconjugants strains (T1, T2 and T3)

Strains	Activity of bacteriocin (AU mL^{-1})
Donor	5120
Recipient	460
T1	1280
T2	1280
T3	2560

Table 8: Total number of scorable bands and the polymorphic among strains

Primer name	Total No. of bands	Polymorphic bands	Polymorphism (%)
OPA-09	15	2	13.3
OPD-01	13	2	15.3
OPD-02	13	2	15.3
OPD-03	18	1	5.55
OPD-04	15	0	0.00
OPD-06	13	0	0.00
OPD-07	11	0	0.00
OPD-14	11	2	18.1
Total	109	9	8.25

Table 9: Similarity matrix computed with Jaccard coefficient

	R	D	T3	T2	T1
R	1	0.178	0.500	0.506	0.494
D		1	0.296	0.299	0.298
T3			1	0.787	0.738
T2				1	0.840
T1					1

R: Recipient, D: Donor and T: Transconjugants strains

DNA sequences not completely like either parent, making it possible for the new cells with new characters[35]. In order to study the genetic difference among the transconjugants lines, donor and recipient, DNA samples were subjected to RAPD analysis using 13 selected primers. Eight of the 13 primers produced reproducible PCR products with a clear pattern for each strain and showing informative and easily scrabble RAPD profiles (Fig. 6). A total of 109 bands were detected among the transconjugants lines, donor and recipient (Table 8 and 9). Only 9 of them were polymorphic markers (8.25%). These primers produced multiple band profiles with a number of amplified DNA fragments varying from 11-18. The highest number of bands (18 bands) was generated by using the primer OPD-03, while the lowest two were 11 bands and generated with primer OPD-06 and OPD-07. Donor and recipient strains gave distinct DNA fingerprint patterns, while transconjugants showed patterns matching the corresponding recipient strain. The distance matrix based on RAPD data sets was used to construct a dendrogram (Fig. 7). The results also indicated that the closest relationship between T3, T2 and T1 were closely related to recipient *Enterococcus faecium* AH2 0.500, 0.506 and 0.494 similarity. These results agreement with Toomey *et al.*[36] who used another technique (PFGE) but found the same results.

Resistance to temperature and sodium chloride: The bacterial capability to grow at high temperature and high osmotic is important characteristics of LAB that should be evaluated when selecting strains used as probiotics. Results showed that all transconjugants strains and recipient were

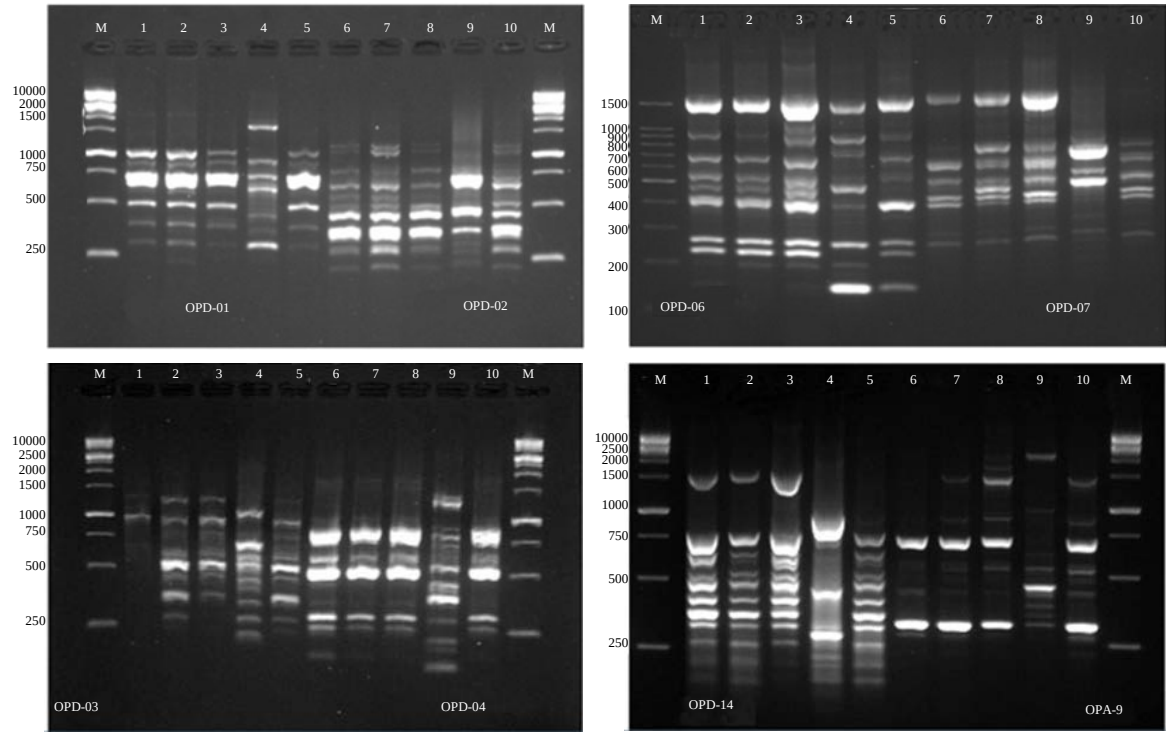

Fig. 6: RAPD-PCR image of the donor, recipient and resulted transconjugants. Lane M: 1 kb ladder, Lane 1, 2, 3, 6, 7, 8: Transconjugants, Lane 4-9: *Lactococcus lactis* donor, Lane 5-10: *Enterococcus* AH2 recipient

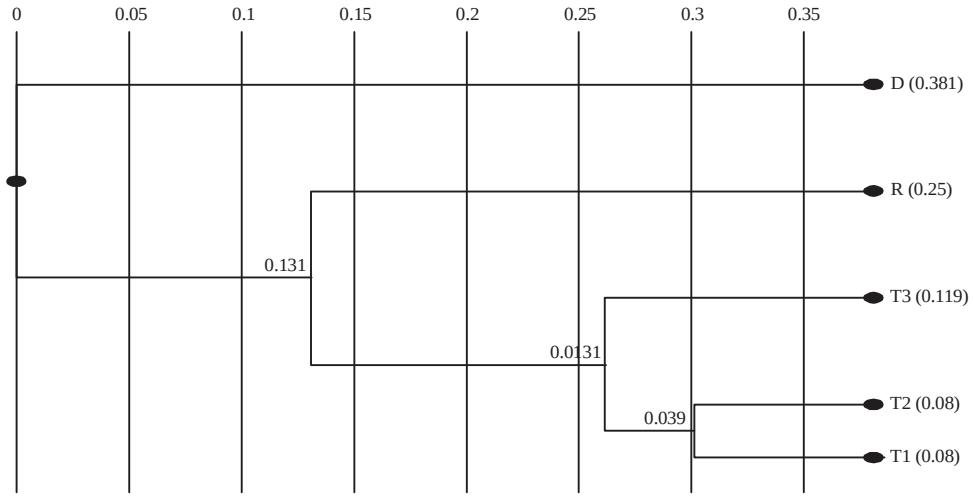

Fig. 7: Dendrogram demonstrating the relationships among the donor, recipient and transconjugants strains. Based on RAPD-PCR analysis using Jaccard coefficient

grow at temperatures of 45°C due to an increased growth rate, while donor strain decreased in growth rate. The growth rate was determined as OD at 600 nm. The data collected were examined with the ANOVA technique; differences between strains were statistically analysis. Transconjugants strains gave significant variance with donor and high similarity with recipient at the significance level of LSD $p<0.05 = 0.0859$ (Table 10). On the other hand, all transconjugants strains and recipient tolerate to high osmotic concentrations of NaCl (2-10%), while donor could be tolerate to (2-6%).

Table 10: Growth rate of donor, recipient and transconjugated strains OD at 600 nm at 45°C

Strain	Mean	SD
Lactococcus lactis sub sp. *lactis*	0.411[d]	0.0121
Enterococcus faecium AH2	1.04133[c]	0.0735
T1	1.404[a]	0.0137
T2	1.01766[c]	0.0715
T3	1.18533[b]	0.0172
p<000***		
LSD 0.05 = 0.0859		

p<000***, LSD 0.05 = 0.0859, ***ANOVA analysis, Means have the same letter are not significant according to LSD, p<0.05

These examinations gave an indication of the osmotolerance level of the LAB strains resulted from this experiment. These results accepted with Ibourahema *et al*.[37] bacterial cells cultured with a high osmotolerance would be a requirement of LAB strains to be used as commercial strains, because when lactic acid is produced by the strain, alkali would be pumped into the broth to prevent an excessive reduction in pH and the free acid would be converted to its salt form, increasing the osmotic pressure on the bacterial cells.

CONCLUSION

The results of the present study clearly suggest that three bacteriocin producing LAB isolates *Enterococcus faecium* (I_1-I_2) and *Pediococcus pentosaceus* (I_3) with probiotic properties. These LAB isolates could provide significant health benefits and enhance safety of the product and shelf life. However, they need further isolation, identification of new isolates of LAB producing bacteriocin and genetic improvement to use these isolates in different industrial application. Consumers are very concerned of chemical preservatives and processed foods, but they accept easily LAB as a natural way to preserve food and promote their health bacteriocinogenic LAB inhibit the growth of spoilage and pathogen bacteria in foods.

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REFERENCES

1. Hayek, S.A. and S.A. Ibrahim, 2013. Current limitations and challenges with lactic acid bacteria: A review. Food Nutr. Sci., 4: 73-87.
2. Leroy, F. and L. De Vuyst, 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci. Technol., 15: 67-78.
3. Klaenhammer, T., E. Altermann, F. Arigoni, A. Bolotin and F. Breidt *et al*., 2002. Discovering lactic acid bacteria by genomics. Antonie van Leeuwenhoek, 82: 29-58.
4. Randazzo, C.I., C. Caggia and E. Neviani, 2009. Application of molecular approaches to study lactic acid bacteria in artisanal cheeses. J. Microbiol. Methods, 78: 1-9.
5. Adimpong, D.B., D.S. Nielsen, K.I. Sorensen, P.M.F. Derkx and L. Jespersen, 2012. Genotypic characterization and safety assessment of lactic acid bacteria from indigenous African fermented food products. BMC Microbiol., Vol. 12. 10.1186/1471-2180-12-75.
6. Amor, K.B., E.E. Vaughan and W.M. de Vos, 2007. Advanced molecular tools for the identification of lactic acid bacteria. J. Nutr., 137: 741S-747S.
7. Knoll, C., B. Divol and M. du Toit, 2008. Genetic screening of lactic acid bacteria of oenological origin for bacteriocin-encoding genes. Food Microbiol., 25: 983-991.
8. Moraes, P.M., L.M. Perin, M.B.T. Ortolani, A.K. Yamazi, G.N. Vicosa and L.A. Nero, 2010. Protocols for the isolation and detection of lactic acid bacteria with bacteriocinogenic potential. LWT-Food Sci. Technol., 43: 1320-1324.
9. Leite, A.M.O., M.A.L. Miguel, R.S. Peixoto, P. Ruas-Madiedo, V.M.F. Paschoalin, B. Mayo and S. Delgado, 2015. Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. J. Dairy Sci., 98: 3622-3632.
10. Davison, J., 1999. Genetic exchange between bacteria in the environment. Plasmid, 42: 73-91.
11. De la Cruz, F. and J. Davies, 2000. Horizontal gene transfer and the origin of species: Lessons from bacteria. Trends Microbiol., 8: 128-133.
12. Huys, G., K. D'Haene, J.M. Collard and J. Swings, 2004. Prevalence and molecular characterization of tetracycline resistance in *Enterococcus* isolates from food. Applied Environ. Microl., 70: 1555-1562.
13. Grohmann, E., G. Muth and M. Espinosa, 2003. Conjugative plasmid transfer in gram-positive bacteria. Microbiol. Mol. Biol. Rev., 67: 277-301.
14. Lavanya, B., S. Sowmiya, S. Balaji and B. Muthuvelan, 2011. Screening and characterization of lactic acid bacteria from fermented milk. Br. J. Dairy Sci., 2: 5-10.
15. Wang, J., X. Chen, W. Liu, M. Yang, Airidengcaicike and H. Zhang, 2008. Identification of *Lactobacillus* from koumiss by conventional and molecular methods. Eur. Food Res. Technol., 227: 1555-1561.
16. Tejero-Sarinena, S., J. Barlow, A. Costabile, G.R. Gibson and I. Rowland, 2012. *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids. Anaerobe, 18: 530-538.

17. Yamamoto, Y., Y. Togawa, M. Shimosaka and M. Okazaki, 2003. Purification and characterization of a novel bacteriocin produced by *Enterococcus faecalis* strain RJ-11. Applied Environ. Microbiol., 69: 5746-5753.
18. Adebayo-Tayo, B.C. and A.A. Onilude, 2008. Screening of lactic acid bacteria strains isolated from some Nigerian fermented foods for EPS production. World Applied Sci. J., 4: 471-747.
19. Maqsood, S., F. Hasan and T. Masud, 2013. Characterization of lactic acid bacteria isolated from indigenous dahi samples for potential source of starter culture. Afr. J. Biotechnol., 12: 5226-5231.
20. Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., 173: 697-703.
21. Gevers, D., G. Huys and J. Swings, 2003. *In vitro* conjugal transfer of tetracycline resistance from Lactobacillus isolates to other gram-positive bacteria. FEMS Microbiol. Lett., 225: 125-130.
22. Plengvidhya, V., F.Jr. Breidt and H.P. Fleming, 2004. Use of RAPD-PCR as a method to follow the progress of starter cultures in sauerkraut fermentation. Int. J. Food Microbiol., 93: 287-296.
23. De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of *Lactobacilli*. J. Applied Bacteriol., 23: 130-135.
24. Mossel, D.A., I. Eelderink, H. de Vor and E.D. Keizer, 1976. Use of Agar Immersion, Plating and Contact (AIPC) slides for the bacteriological monitoring of foods, meals and the food environment. Lab. Pract., 25: 393-395.
25. Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic Streptococci and their bacteriophages. Applied Environ. Microbiol., 29: 807-813.
26. Khalid, K., 2011. An overview of lactic acid bacteria. Int. J. Biosci., 1: 1-13.
27. Holzapfel, W.H., P. Haberer, R. Geisen, J. Bjorkroth and U. Schillinger, 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin. Nutr., 73: 365S-373S.
28. Buntin, N., S. Chanthachum and T. Hongpattarakere, 2008. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin J. Sci. Technol., 30: 141-148.
29. Bassyouni, R.H., W.S. Abdel-All, M.G. Fadl, S. Abdel-All and Z. Kamel, 2012. Characterization of lactic acid bacteria isolated from dairy products in Egypt as a probiotic. Life Sci. J., 9: 2924-2930.
30. Du Toit, M., C.M.A.P. Franz, L.M.T. Dicks and W.H. Holzapfel, 2000. Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. J. Applied Microbiol., 88: 482-494.
31. Vitali, B., G. Minervini, C.G. Rizzello, E. Spisni and S. Maccaferri *et al*., 2012. Novel probiotic candidates for humans isolated from raw fruits and vegetables. Food Microbiol., 31: 116-125.
32. Axelsson, L., 2004. Lactic Acid Bacteria: Classification and Physiology. In: Lactic Acid Bacteria: Microbiological and Functional Aspect, Salminen, S., A. Von Wright and A. Ouwehand (Eds.). 3rd Edn., Marcel Dekker Inc., New York, ISBN-13: 9780824752033, pp: 1-66.
33. Wagner, R.D., D.D. Paine and C.E. Cerniglia, 2003. Phenotypic and genotypic characterization of competitive exclusion products for use in poultry. J. Applied Microbiol., 94: 1098-1107.
34. Lampkowska, J., L. Feld, A. Monaghan, N. Toomey and S. Schjorring *et al*., 2008. A standardized conjugation protocol to asses antibiotic resistance transfer between lactococcal species. Int. J. Food Microbiol., 127: 172-175.
35. Llosa, M. and F. de la Cruz, 2005. Bacterial conjugation: A potential tool for genomic engineering. Res. Microbiol., 156: 1-6.
36. Toomey, N., A. Monaghan, S. Fanning and D.J. Bolton, 2009. Assessment of horizontal gene transfer in Lactic acid bacteria-A comparison of mating techniques with a view to optimising conjugation conditions. J. Microbiol. Methods, 77: 23-28.
37. Ibourahema, C., R.D. Dauphin, D. Jacqueline and P. Thonart, 2008. Characterization of lactic acid bacteria isolated from poultry farms in Senegal. Afr. J. Biotechnol., 7: 2006-2012.