

Identification and biotechnological characterization of Lactic Acid Bacteria Isolated from White Cheese Samples

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Research Article

Keywords: Lactic acid bacteria, Bacteriocin, Molecular characterization, biotechnological enzymes

Posted Date: June 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-630567/v1>

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Version of Record: A version of this preprint was published at Journal of Pure and Applied Microbiology on December 1st, 2022. See the published version at <https://doi.org/10.22207/JPAM.16.4.66>.

Abstract

In this study, the isolation of lactic acid bacteria were carried out from approximately one hundred white cheese samples collected from different regions of Turkey. Subsequently, phenotypic and genotypic characterization of the isolates were performed. Finally, biotechnological enzyme and bacteriocin production potentials of the isolates were determined. As a result of the analysis, a total of forty one bacteria were isolated and seventeen of them were found to be different species. The isolates generally grew at 4–6 pH values, 0–8% NaCl and 30–40 °C. Genomic fingerprint profiles of the isolates were determined by using BOX-PCR. According to 16S rRNA sequence results, test strains belong to *Lentilactobacillus kefir*, *Levilactobacillus brevis*, *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Pediococcus lolii*, *Staphylococcus haemolyticus*, *Lysinibacillus sinduriensis*, *P. parvulus*, *Lactiplantibacillus paraplantarum*, *Staphylococcus hominis*, *Lactiplantibacillus plantarum*, *Enterococcus faecium*, *Micrococcus yunnanensis*, *Microbacterium paraoxydans* and *Micrococcus aloeverae* species. Since the isolate coded MA56 is 96.41% similar to *Lentilactobacillus buchneri*, it is thought to be a new species. Also MA19, MA25, MA43 and MA47 were determined to have multi-enzyme production potential. MA43 was found to be the only isolate producing bacteriocin.

1. Introduction

Cheese is produced by coagulating the milk with the effect of a suitable proteolytic enzyme or organic acids. and processing, filtering, suppressing and maturing in various ways (Carafa et al. 2015; Domingos-Lopes et al. 2017). The unique taste and aroma of cheese is obtained by the addition of lactic acid bacteria to the culture. Lactic acid bacteria (LAB) are gram positive bacteria that produce lactic acid as the main product during the fermentation of carbohydrates (Alvarez-Sieiro et al. 2016; Varoquaux and Wiley 2017). They are non-spore-forming, anaerobic or microaerophilic and acid-tolerant organisms with rod or coccal cells (Agriopoulou et al. 2020; Ayeni et al. 2009; Shoukat 2020). LAB is a group of bacteria that consists of genus such as *Streptococcus*, *Lactococcus*, *Pediococcus*, *Enterococcus*, *Lactobacillus* is commonly found in dairy and fermented foods (Azam et al. 2017; Barbieri et al. 2019; Szutowaska and Gwiazdowska 2021). There are many studies reporting the health benefits of fermented dairy products. Fermented foods typically contain microorganisms considered to be generally regarded as safe (GRAS), which can produce a range of beneficial by-products / metabolites such as antimicrobial peptides (e.g. bacteriocins), ethanol, organic acids, fatty acids, carbon dioxide (Macori and Cotter 2018; Marco et al. 2017). It is known that products resulting from LAB-induced fermentations have anti-cancer (Bindu and Lakshmidivi 2021), immunomodulatory (Qian et al. 2011), anti-gastritis (Rodriguez et al. 2009), Antihypertensive (Mallappa et al. 2021) and, anti-allergenic effects (Drywien et al. 2015). In addition, Mozaffarian et al. were reported that consuming LAB fermented foods had positive effects on weight (Chen et al. 2014; Mozaffarian et al. 2011). Other studies have shown that the consumption of fermented yogurt and dairy products can reduce the risk of developing cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (Eussen et al. 2016; Soedamah-Muthu et al. 2013). In addition, researchers reported that fermented milk and dairy products associated with LAB have hypocholesteremic (Lye et al. 2010) and anti-cancer properties (Kapila et al. 2007).

Besides the health aspect, LABs can also be the source of new species with enzymatic activities for biotechnological process (Mathur et al. 2020). Microbial enzymes are more preferred than other enzymes because they have high catalytic activity and efficiency. Major enzymes used in biotechnological processes include amylases, proteases, and lipases. LAB with amylase, lipase xylanases and protease activities have been reported in previous studies. (Adiguzel et al. 2019; Konkrit and Kim 2016; Saez et al. 2018; Unban et al. 2017). Considering these properties, interest in lactic acid bacteria is increasing day by day and there are many LAB species with technological potential yet to be discovered. In the light of all this information, in this study, isolation, identification and molecular characterization of lactic acid bacteria from cheese samples collected from different regions (Erzurum, Van, Konya, Karaman and Kars) of Turkey were carried out. Later, biotechnologically important enzyme and bacteriocin production potentials of the isolates were determined.

2. Material And Methods

Sampling and lactic acid bacteria isolation

A total of one hundred Cow cheese samples taken from markets in different regions of Turkey were brought to the laboratory under aseptic conditions and kept at +4°C until they were studied. 225 ml of sterile physiological water (0.9% NaCl) was added to 25g cheese sample and homogenization process was carried out and dilution series (10^0 - 10^{-7}) were prepared (Luiz et al. 2017). The dilution liquids were spread on MRS and M17 Agar media and incubated at 35°C for 48 hours. After the incubation, isolates were stored at -86 °C in a stock medium containing 15% glycerol (Kirmaci et al. 2016).

Phenotypic Characterization

To determine phenotypic characterization, test strains were grown in MRS and M17 medium at different temperatures 15°C to 50°C (with 5°C intervals) for up to 72 h. The pH ranges were analyzed in MRS and M17 at pH 3.0–11.0 (1 pH unit intervals), Tolerance of NaCl was determined using MRS and M17 supplemented with 0–12 % NaCl (at intervals of 1.0 %) for 72 h. All experiment were tested in triplicate and growth was measured at OD600 nm (Baltaci et al. 2020). Gram-staining of test strains was performed according to method of Gerhardt et al (Gerhardt et al. 1981). Catalase activity was performed by the production of bubbles of a drop of 3 % H₂O₂ (v/v). Oxidase reagent (Sigma) was used for testing oxidase activity (Adiguzel et al. 2020). Biochemical characterization of isolates were conducted using API 50CHL test (Anekella and Perez-Diaz 2020).

Genotypic Characterization

Genomic DNA isolation were performed according to the Promega WizardR Genomic DNA Purification Kit (A2360) protocol. 16S rRNA region was amplified using 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers (Baltaci and Adiguzel 2016). 30 µL volume of PCR mixture containing, 13.1 µl ddH₂O, 3 µl 10X PCR buffer, 1.8 µl MgCl₂, 1.2 µl DMSO, 0.6 µl dNTP, 3 µl (5 µM) forward primer (27F), 3 µl (5 µM) reverse primer (1492R), 0.3 µl Taq DNA polymerase and 4 µl template DNA. The amplified fragments were cloned into *Escherichia coli* JM101 strain with the pGEM-T Easy Cloning Vector (Promega, Southampton, UK) according to the instructions of the manufacturer. After the cloning, plasmid isolation was performed by selecting colonies that gave the positive result, and the sequence analysis was made by the Macrogen Company (Netherlands). The 16S rRNA obtained was compared with the other bacterial series in GenBank and EzTaxon (<http://blast.ncbi.nlm.nih> and <http://www.eztaxon.org>), similarity rate between them was determined and GenBank accession numbers were received (Baltaci et al. 2017).

rep-PCR

The rep-PCR reactions were carried out in a Sensequest Thermal Cycler (Göttingen, Germany) using BOXAIR primer (5'-CTACGGCAAGGCGACGCTGACG-3'), PCR mixtures contained: 5 µl Gitschier Buffer, 12.7 µl ddH₂O, 2.5 µl dimethyl sulfoxide, 1.25 µl bovine serum albumin, 1.25 µl dNTP, 4 µl primer, 0.3 µl Taq DNA polymerase and 3 µl template DNA. Pcr Cycles were; initial denaturation at 94°C for 7 min., 36 cycles of 1 min. at 94°C, 1 min. at 45°C, 8 min. at 65°C. Final extension at 65°C for 16 min. At the end of PCR, samples were run in 1% agarose gel for 90 minutes (Saez et al. 2017).

Determination of Industrial Enzyme Production Potentials of Isolates

Lipase

To test lipase enzyme production, isolates were inoculated into tributyrin agar medium containing 1 % tributyrin (glycerol tributyrate) and incubated at 35°C for 48 h. Isolates with clear zone were considered positive for lipase (Linares-Morales et al. 2020).

Amylase

Test strains were streaked on plates containing MRS agar with 1% starch instead glucose that were incubated at 35°C for 48 h. Then, petri dishes were treated with Lugol's solution. Isolates showing clear zones were evaluated as amylase positive (Padmavathi et al. 2018).

Protease

To test whether isolates were producers of protease enzymes inoculated into MRS agar and containing 1% Skim Milk Powder and incubated at 35°C for 48 hours. Isolates with clear zone were considered positive for protease (Linares-Morales et al. 2020).

Xylanase

Isolates were inoculated into medium containing xylan (10 gL⁻¹), NaNO₃ (1.2 gL⁻¹), KH₂PO₄ (13 gL⁻¹), K₂HPO₄ (6 gL⁻¹), CaCl₂ (0.05 gL⁻¹), MgSO₄ (0.01 gL⁻¹), ZnSO₄ (0.001 gL⁻¹) and agar (15 gL⁻¹) incubated at 35°C for 48 hours. After incubation, the plates were stained for 20 min with 0.1% congo red and washed with 1M NaCl. Isolates with orange colored zones were evaluated xylanase as positive (Adiguzel et al. 2019).

Determination of Bacteriocin Production Potentials

After determination of isolates species, The presence of related bacteriocin genes were investigated. For this purpose, PCR analyzes were performed using primers specific to each bacteriocin gene (Chiorean et al. 2018). The 16S PCR cycle given above was performed by changing annealing temperatures of bacteriocin primers.

Detection of Antibacterial Activity

For detection of antimicrobial activity, disc diffusion assay was used. Pathogenic bacteria were spread on the surfaces of Mueller Hinton agar media. Overnight cultures, on MRS medium, of the strains to be tested were centrifuged and cell free supernatant was loaded on discs. The plates were incubated at 37°C for 24h. The antagonistic effects of the test strains were determined by measuring the zone diameters (Mezaini et al. 2009). The target test strains used in this study are *Escherichia coli* O157:H7 (ATCC 43888), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 810), *Pseudomonas aeruginosa* (ATCC 9027), *Streptococcus pyogenes* (ATCC 12344), *Klebsiella pneumoniae* (ATCC 13883), *Listeria monocytogenes* (ATCC 7644), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (ATCC 13313) and *Staphylococcus aureus* (ATCC 6538).

3. Results And Discussion

Isolation of LAB

In the study, a total of 41 bacteria isolated from cheese samples obtained from Erzurum Kars, Karaman, Konya and Van provinces for the isolation of lactic acid bacteria and stock cultures were prepared. Since seventeen of the isolated bacteria belonged to different species, the study was continued with these isolates.

Phenotypic Characterization

According to conventional analysis; all isoates were gram positive, catalase and oxidase negative and showed cocci or bacil cell morphology. Generally isolates grow at 4–6 pH values, 0–8% NaCl and 30–40 °C. Similarly, Kuikui et al., was determined isolated lactic acid bacteria can usually grow at 35–45 °C at pH 3 and 6.5% NaCl (Ni et al. 2015). Interestingly, it has been found that MA7 strain can thrive in wide range of pH and salt concentrations such as pH:2–11 and 0–10% NaCl. So, MA7 can be suitable for many biotechnological processes. Detailed phenotypic characteristics and API test results are given in the Table 1.

Table 1

Phenotypic properties of isolates 1: MA4, 2: MA7, 3: MA10, 4: MA12, 5: MA19, 6: MA25, 7: MA 27, 8: MA28, 9: MA31, 10: MA33, 11: MA34, 12: MA35, 13: MA39, 14: MA43, 15: MA47, 16: MA55, 17: MA56

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
pH	4-6	2-11	4-6	2-8	3-6	4-6	4-7	4-5	4-6	4-6	4-6	4-6	5-7	4-6	3-6	4-6	2-8
NaCl	0-9	0-10	0-9	0-8	4-6	0-6	0-8	0-11	0-8	0-6	0-8	0-8	0-8	0-5	0-8	0-10	0-8
Temperature	30-40	30-40	15-45	30-40	30-40	30-40	15-45	15-45	15-45	30-40	30-40	15-45	30-40	30-40	30-40	30-40	30-40
Morphology	Bacil	Bacil	Bacil	Bacil	Coc	Coc	Coc	Bacil									
Oksidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Arabinose	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+
Ribose	-	+	+	-	-	-	+	+	d	-	+	+	-	+	-	-	+
Xylose	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+
Adonitol	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	-	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+
D-Glucose	-	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+
D-Fructose	-	+	+	-	-	-	+	+	-	+	+	+	+	+	-	+	+
D-Mannose	-	+	+	-	-	-	+	-	-	-	+	+	d	+	-	+	-
Rhamnose	-	-	+	-	-	-	-	-	-	-	-	d	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Mannitol	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
Sorbitol	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Met-D-Gluc.	-	-	+	-	-	-	-	d	-	-	-	-	-	-	-	-	+
NAG	-	+	+	-	-	-	+	d	d	+	+	+	-	+	-	+	+
Amygdaline	-	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-
Arbutine	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-
Esculine	d	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	-
Salicine	-	+	+	-	-	-	+	-	-	-	+	d	d	+	-	d	-
Cellobiose	-	+	+	-	-	-	+	-	-	-	+	+	-	+	-	+	-
Maltose	-	d	+	-	-	-	+	+	+	+	+	-	+	+	-	-	+
Lactose	-	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+
Melibiose	-	-	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+
Saccharose	-	d	+	-	-	-	-	-	+	d	-	-	+	+	-	-	-
Trehalose	-	+	+	-	-	-	+	-	+	+	+	+	+	+	-	d	+
Melezitose	-	+	+	-	-	-	-	-	-	-	+	-	d	+	-	-	-
D-Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Gentiobiose	-	-	+	-	-	-	+	-	-	-	+	+	-	+	-	+	-
D-Turanose	-	+	-	-	-	-	-	-	d	d	+	-	d	-	-	-	-
D-Tagatose	-	+	-	-	-	-	-	-	-	-	+	d	-	-	-	+	-
D-Arabitol	-	-	+	-	-	-	-	-	-	-	-	-	-	d	-	-	-

+:Positive -: Negative d:Delayed

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
L-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+:Positive -: Negative d:Delayed																	

Genotypic Characterization

16S rRNA sequence analysis is used as a powerful tool in determining the prokaryotic diversity in almost every environment (Edgar 2018a; Martinez-Porchas et al. 2017). So, we performed molecular identification of lactic acid bacteria isolated from cheese samples based on 16 S rRNA sequence. Except for MA56, all isolates are 99% similar to related standard type strains. MA56 has 96.41% similarity with *Lentilactobacillus buchneri* DSM 20057. According to general acceptance, the 16 S rRNA gene sequence similarity ratio below 97% is a new species indicator of the isolate. In recent years, it has been reported that this rate is 98.2% – 99% and that the whole genome sequence and DNA:DNA hybridization is required in addition to the 16S rRNA gene sequence (Edgar 2018b; Meier-Kolthoff et al. 2013). According to this information, MA56 might be a novel species belonging to the genus *Lentilactobacillus*. It is thought that MA56 will be added to the literature as a novel species as a result of the whole genome sequence analysis in future studies. Detailed sequence results of the isolates and related species are given in Table 2. Also, 16S rRNA gene based phylogenetic tree is shown in Fig. 1.

Table 2
Related species and similarity rates of isolates according to 16S rRNA sequence analysis

Isolate Code	Product size (bp)	Related species	Similarity rate (%)	Genbank No.
MA 4	1535	<i>Lentilactobacillus kefir</i>	99	KY425772
MA 7	813	<i>Lacticaseibacillus casei</i>	99	KY425775
MA 10	1528	<i>Lactiplantibacillus paraplantarum</i>	99	KY425790
MA 12	1515	<i>Lysinibacillus sinduriensis</i>	99	KY425788
MA 19	1484	<i>Micrococcus yunnanensis</i>	99	KY425784
MA 25	1486	<i>Microbacterium paraoxydans</i>	99	KY425786
MA 27	1387	<i>Enterococcus faecium</i>	99	KY425810
MA 28	1527	<i>Levilactobacillus brevis</i>	99	KY425773
MA 31	1512	<i>Staphylococcus haemolyticus</i>	99	KY425785
MA 33	885	<i>Staphylococcus hominis</i>	99	KY425791
MA 34	1531	<i>Lacticaseibacillus paracasei</i> subsp. <i>paracasei</i>	99	KY425778
MA 35	1538	<i>Pediococcus lolii</i>	99	KY425782
MA 39	1488	<i>Rothia dentocariosa</i>	99	KY425811
MA 43	1528	<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	99	KY425796
MA 47	1462	<i>Micrococcus aloeverae</i>	99	KY425780
MA 55	1539	<i>Pediococcus parvulus</i>	99	KY425789
MA 56	1491	<i>Lentilactobacillus buchneri</i>	96	KY425792

Previous studies have reported that rep-PCR is an easy method that can be used to classify bacteria. Mohammed et al. used BOX-PCR analysis to characterize lactic acid bacteria isolated from traditional milk samples in their study (Mohammed et al. 2009). We also performed genomic fingerprint analysis of isolates using BOX-PCR in this study. While 12 polymorphic bands were observed in some of the test strains, it was observed that there was 1 band in some isolates Fig. 2. It was observed that the BOX-PCR was not sufficient to classify the all LAB.

Determination of Biotechnological Enzyme Production Potentials of Isolates

The potential of isolates to produce amylase, lipase, protease and xylanase enzymes, which are biotechnologically important, were determined. As a result of analysis one strains have xylanase and lipase activity, five strains with amylase activity, and ten strains with protease activity were observed (Table 3). Also petri images of some isolates are given in Fig. 3. Matthews et al. investigated the enzyme production potential of lactic acid bacteria and determined that especially *Lactobacillus* and *Pediococcus* species are important producers of lipase, cellulase and xylanase enzymes. In another study, Konkrit and Kim examined that *Lactococcus chungangensis* produces amylase, proteinase, and lipase enzymes (Konkrit and Kim 2016).

Table 3
Screening Industrial Enzyme Profiles of Isolates

Isolate Code	Related species	Amylase	Lipase	Protease	Xylanase
MA4	<i>Lentilactobacillus kefir</i>	-	-	++	-
MA7	<i>Lactocaseibacillus casei</i>	-	-	++	-
MA10	<i>Lactiplantibacillus paraplantarum</i>	-	-	-	-
MA12	<i>Lysinibacillus sinduriensis</i>	-	-	++	-
MA19	<i>Micrococcus yunnanensis</i>	++	-	++	-
MA25	<i>Microbacterium paraoxydans</i>	+	+	+	-
MA28	<i>Levilactobacillus brevis</i>	-	-	++	-
MA31	<i>Staphylococcus haemolyticus</i>	-	-	-	-
MA33	<i>Staphylococcus hominis</i>	-	-	++	-
MA34	<i>Lactiplantibacillus paracasei subsp. paracesei</i>	-	-	-	-
MA35	<i>Pediococcus lolii</i>	+	-	-	-
MA39	<i>Rothia dentocariosa</i>	-	-	-	-
MA43	<i>Lactiplantibacillus plantarum subsp. plantarum</i>	+	+	++	-
MA47	<i>Micrococcus aloeverae</i>	-	-	++	+
MA55	<i>Pediococcus parvulus</i>	+	-	-	-
MA56	<i>Lentilactobacillus buchneri</i>	-	-	++	-

-: Negative, +: Positive, ++: Strong positive

Table 4
The result of disc diffusion test. The diameter of inhibition zone of MA43.

Pathogens	MA43
<i>Serratia marcescens</i> ATCC 810	5 ± 0.4
<i>Shigella dysenteriae</i> ATCC 13313	12 ± 1.3
<i>Klebsiella pneumoniae</i> ATCC 13883	12 ± 0.8
<i>Streptococcus pyogenes</i> ATCC 12344	10 ± 0.4
<i>Staphylococcus epidermidis</i> ATCC 12228	9 ± 0.3
<i>Staphylococcus aureus</i> ATCC 6538	12 ± 0.8
<i>Pseudomonas aeruginosa</i> ATCC 9027	16 ± 1.1
<i>Salmonella typhimurium</i> ATCC 14028	7 ± 0.6
<i>Listeria monocytogenes</i> ATCC 7644	9 ± 0.7
<i>Escherichia coli</i> O157:H7 ATCC 43888	19 ± 0.5

The measures of the inhibition zone are expressed in mm.

There are many studies in the literature in which enzymes obtained from lactic acid bacteria are used in biotechnological processes. For example, The xylanase enzyme purified from *Pediococcus acidilactici* was applied in clarification of fruit juices (Adiguzel et al. 2019). In another study, it was reported that the protease enzyme obtained from *Lactobacillus plantarum* had an antimicrobial effect on pathogenic microorganisms (Lin and Pan 2019). So, in this study, isolates coded MA19, MA25, MA43 and MA47 were determined to have multi-enzyme production potential (Table 3) and these isolates are attractive for biotechnological processes, because they have more than one enzyme activity.

Determination of Bacteriocin Production Potential

Whether the isolates produced bacteriocin was determined using specific bacteriocin primers with PCR. As a result of the PCR analysis, it was determined that only the MA43 coded isolate produced bacteriocin. The gel image of plantaricin belonging to MA43 and *Lactobacillus plantarum* ATCC 8014 are shown in Fig. 4.

After it was determined that MA43 has bacteriosin gene, its effect against pathogenic bacteria was investigated. It has been determined that MA43 has a highest antagonistic effect against *Escherichia coli* O157:H7 ATCC 43888 and *Pseudomonas aeruginosa* ATCC 9027. Previous studies have also reported that *Lactobacillus plantarum* has an antimicrobial effect against various pathogens (Layus et al. 2020; Wang et al. 2017). In this respect, MA43 has an antagonistic effect against foodborne pathogens shows that it can use food preservatives.

4. Conclusions

With this study, it was determined that the white cheese samples have a very wide microflora. 16S rRNA sequence similarity to the closest species of the MA56 was determined as 96.41%. This isolate is highly likely to be a novel species of lactic acid bacteria. MA43 not only has amylase, lipase and protease activity but also produces bacteriosin makes it unique for biotechnological processes. In addition, this study leads to new studies for MA56 and MA43.

Declarations

Declaration of competing interest

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

Acknowledgements

Not applicable.

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Figures

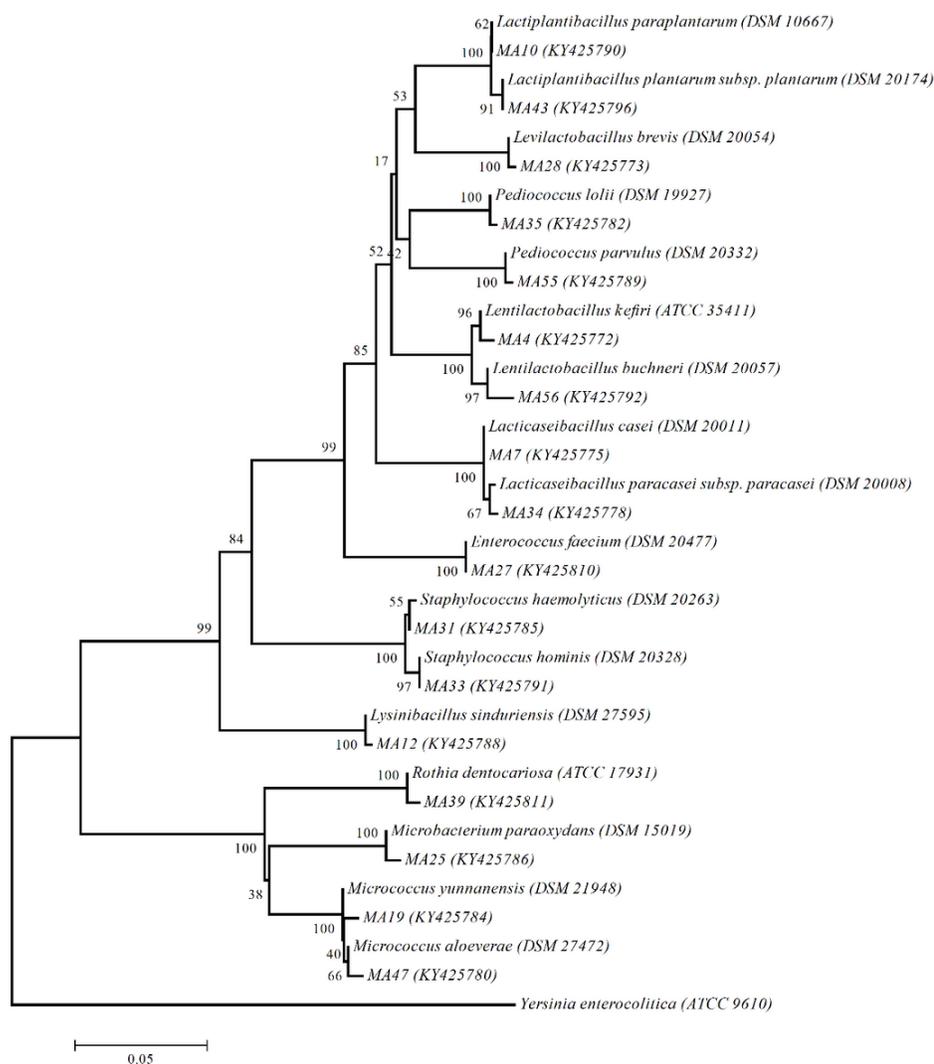


Figure 1

Neighbour joining phylogenetic tree based on 16S rRNA gene sequences of test strains and related type species. *Yersinia enterocolitica* ATCC 9610 was used as out-group. Bootstrap values based on 1000 replications are listed as percentages at branching points. The accession numbers are given in parentheses. The scale bar represented 0.5% divergence.

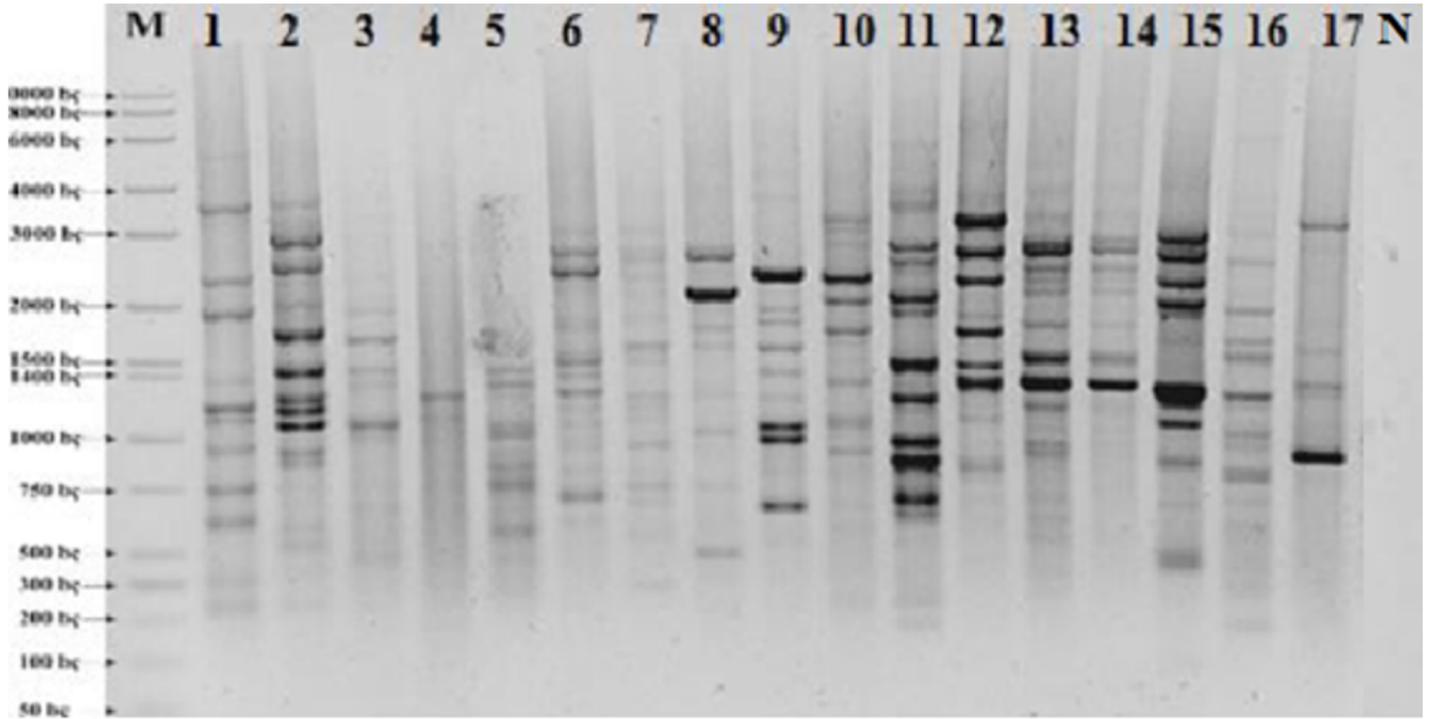


Figure 2

BOX-PCR profiles of isolates (M:Marker, 1: MA4, 2: MA7, 3: MA10, 4: MA12, 5: MA19, 6: MA25, 7: MA27, 8: MA28, 9: MA31, 10: MA33, 11: MA34, 12: MA35, 13: MA39, 14: MA43, 15: MA47, 16: MA55, 17:MA56, N: Negative Control).

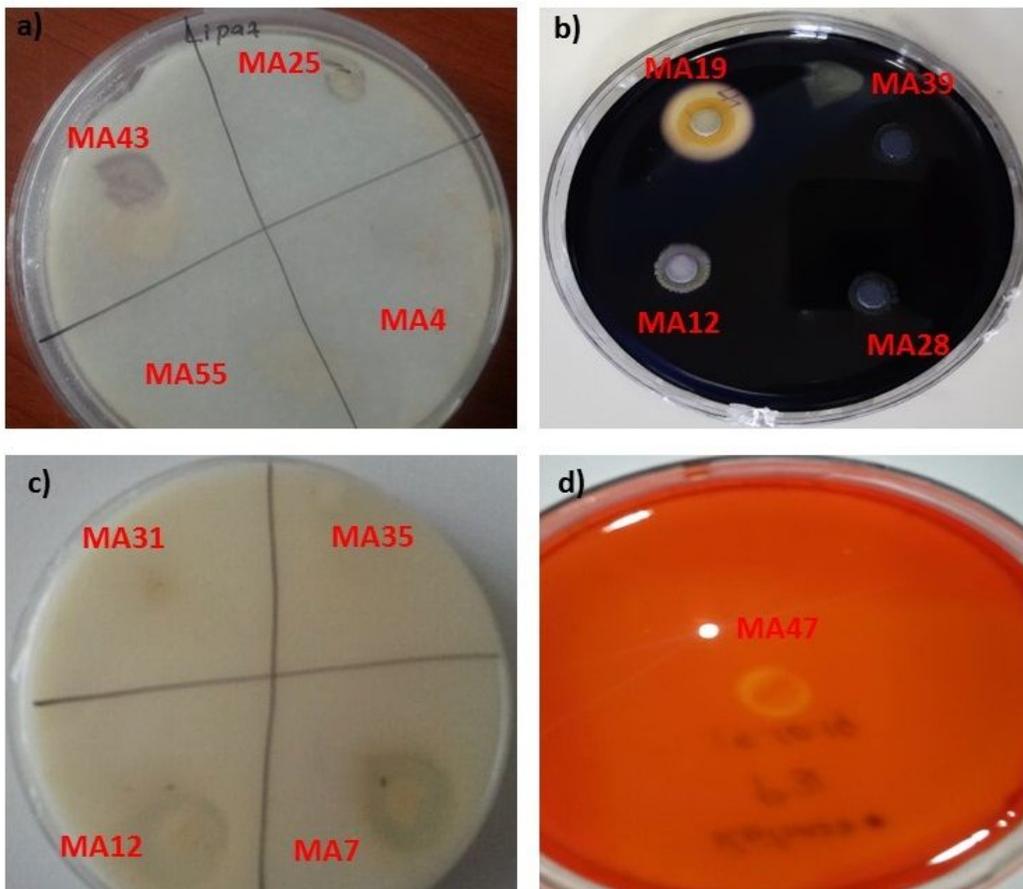


Figure 3

Screening biotechnological enzymes profiles of some isolates a) Petri image of lipase b) Petri image of amylase c) Petri image of protease d) Petri image of xylanase

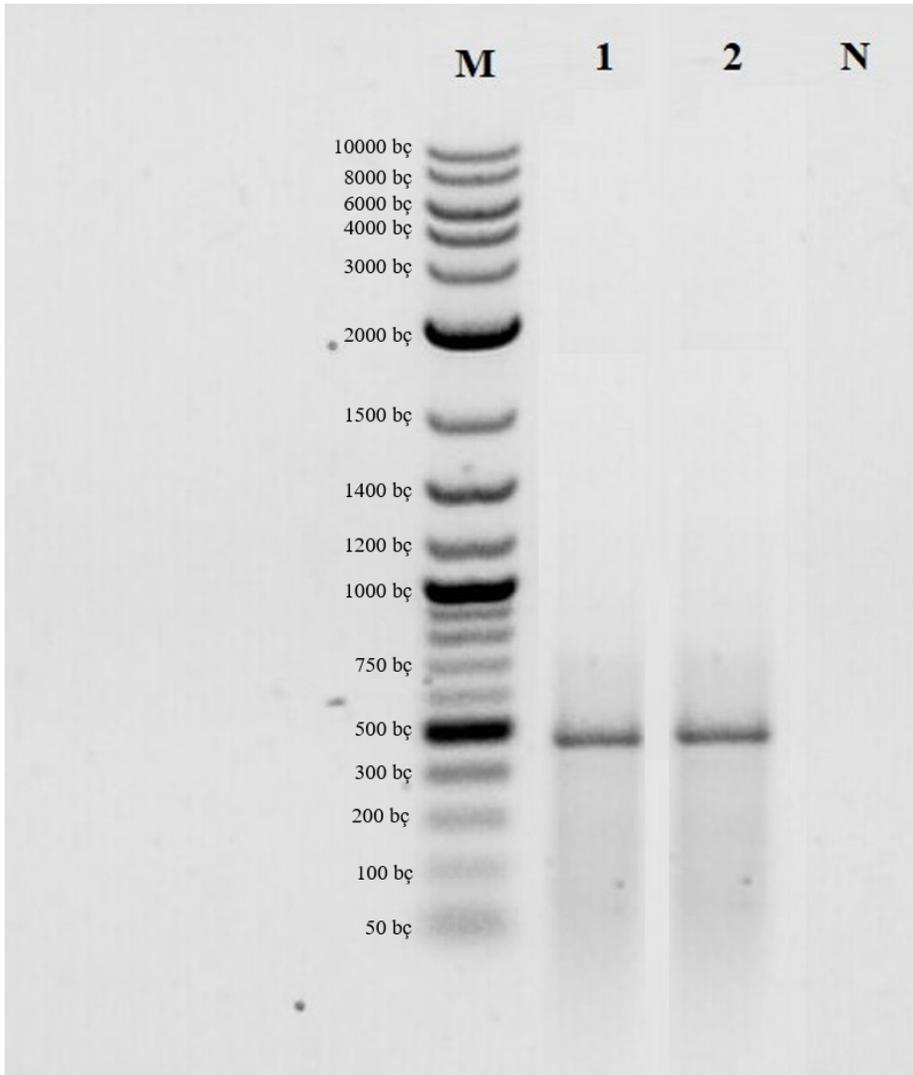


Figure 4

PCR analysis result of plantaricin genes. M: Marker, 1: *Lactobacillus plantarum* ATCC 8014, 2: MA43, 3: Negative Kontrol.