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Molecular Identification and Partial Characterization of *Pediococcus* sp. and *Leuconostoc* sp. Isolated from Traditionally Made Dairy Products

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ABSTRACT

Lactic acid bacteria (LAB) are group of commercially important organisms that play an important role in the fermentation process of hexose sugars into lactic acid and they are videly used in dairy industrial applications since they were designated as "generally recognised as safe" organisms. In order to isolate laktic acid bacteria (LAB), forty fermented food samples were collected and 160 bacterial strains were purified and identified from those samples. According to morphological characteristics, 62 isolates (38.75%) were classified as cocci and all these cocci were found to be as catalase negative and grown well on MRS agar plates. *Coccobacilli* and *bacilli* forms were observed in 66 (41.25%) and 19 (11.875%) isolates respectively. Total of 13 isolates (8.125%) were classified as "others" (according to morphological characteristics) and excluded from rest of experimental process. For molecular identification of *Leuconostoc* sp. and *Pediococcus* sp., 16S and 23S rDNAs (respectively) were amplified with the aid of PCR and 34 of them were classified as *Pediococcus* whilst 4 of them were designated as *Leuconostoc*. These newly isolated strains were tested for their antimicrobial activity and more than one strains exhibited antimicrobial activity against to S. paratyphi and E. *faecali*. All tested strains showed relatively lower (≤ 10mm in diameter) antimicrobial effect against P. *mirabilis*, E. *coli* and C. *jejuni* while P. aeruginosa showed resistancy against all bacteriocins produced tested isolates. Result of this study revealed that majority of the tested (n=20) isolates were resistant to erythromycin, chloramphenicol and rifampin. All isolates were investigated for the presence of plasmid vectors and 10 of them found having at least 1 plasmid vector.

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Geleneksel Yöntemlerle Yapılmış Peynirlerden Leuconostoc sp ve Pediococcus sp.'lerin İzolasyonu, Moleküler Tanımlanması ve Kısmi Karakterizasyonu

ÖZET

Laktik asit bakterileri heksoz şekerlerin laktik asite fermentasyon prosesinde önemli rol oynayan ticari bakterilerdir ve "güvenli mikroorganizmalar" olmaları nedeniyle süt endüstrisinde yaygın olarak kullanılırlar. Laktik asit bakterilerinin izolasyonu amacıyla 40 adet fermente gıda örneklenmiş ve bu örneklerden 160 bakteri izolatı saflaştırılarak tanımlanmıştır. Morfolojik karakterlerine göre 62 izolat (38.75%) cocci olarak sınıflandırılmış, bunların MRS agarda iyi yetişdiği ve tamamının katalaz-negatif olduğu belirlenmiştir. *Coccobacilli* ve *bacilli* formlar sırasıyla 66 (41.25%) ve 19 (11.875%) adet olarak gözlemlenmiş, 13 izolat ise morfolojik özelliklerine göre "diğerleri" şeklinde tanımlanmış ve deneme dışı bırakılmıştır. *Pediococcus* ve *Leuconostoc* suşlarının moleküler tanımlaması sırasıyla 23S ve 16S rDNA'larının PZR yardımıyla

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Anahtar Kelimeler
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Leuconostoc,
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Pediococcus

Araştırma Makalesi

amplifiye edilmesiyle yapılmış ve 34 izolat *Pediococcus* olarak tanımlanırken 4 izolatın *Leuconostoc* suşu olduğu tespit edilmiştir. Yeni izole edilen bu suşların antimikrobiyal aktiviteleri test edilmiş ve birden fazla izolatın S. *paratyphi* ve E. *faecali*'ye karşı antimikrobiyal aktivitelerinin olduğu belirlenmiştir. Tüm test edilen izolatların P. *mirabilis*, E. *coli* ve C. *jejuni*'a karşı göreceli olarak düşük antimikrobiyal etki gösterirken P. *aeruginosa*'nın izolatların sentezlediği tüm bakteriyosinlere karşı dirençlilik gösterdiği belirlenmiştir. Sonuçlar test edilmiş izolatların (n=20) erythromycin, chloramphenicol ve rifampine karşı dirençlilik gösterdiğini ortaya koymuştur. Tüm izolatların plasmid içerikleri araştırılmış ve 10 izolatın en az 1 adet plasmid vektör içerdiği bulunmuştur.

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INTRODUCTION

Lactic acid bacteria (LAB), Gram-positive, non-sporulating microaerophilic bacteria, are group of commercially important organisms that are classified by their ability for the fermentation of hexose sugars into lactic acid mainly. Vast majority of the lactic acid bacteria, including species of the genera *Pediococcus* and *Leuconostoc*, are used as starter cultures in vegetable and dairy fermented products causing characteristic flavour change, improving hygienic quality and extending the shelf life (Mora et al., 1997; Akyol et al., 2009). Screening of these microorganisms isolated from naturally occurring processes is the most common method of obtaining strains useful for industrial applications for the production of various fermented foods worldwide (Pal et al., 2005).

Pediococcus sp. is homofermentative Gram-positive cocci that produce D- and L-lactate from carbohydrates. These bacteria are regularly found in the nonstarter population of raw milk cheeses at the end of the ripening process. Thus, *Pediococcus* spp. could be isolated from cheddar, ewes' milk cheeses, and some other raw milk cheeses traditionally produced in various geographic regions of the World (Noohi et al., 2016). Studies on the physiological and biochemical activities of *Pediococcus* spp. have mainly focused on carbohydrate metabolism and proteolytic and lipolytic activities (Irmler et al., 2013).

Although *Leuconostocs* are traditionally used for the fermentation of vegetables particularly cabbage to make sauerkraut (Holzapfel et al., 2008), dairy products such as kefir, wines and meats, they are specific to particular cheese varieties. Their proteolytic and lipolytic enzymes play a remarkable role in texture, flavour, chemical composition, aroma and some other quality criteria of the fermented products. *Leuconostoc* spp. contribute to the aroma formation in cheeses through its heterofermentative metabolism and ability to degrade citrate (Hemme and Foucaud-Scheunemann, 2004).

Cheese production is based on the use of added commercially developed starter cultures of lactic acid bacteria that initiate rapid milk acidification; however, many cheeses, produced locally, obtain their flavor intensity from the presence and activity of traditionally obtained starter cultures. For the identification of novel starter strains, working with fresh cheese is important because fermentation occurs at the beginning of production process.

Although several studies have been performed on the characterisation of microbial population of traditional dairy products, there is still little information available about their antibacterial activity, antibiotic resistance, proteinase activity and the presences of plasmids of these localy made fermented foods (Gezginc, 2010). The exploration of naturally occurring antimicrobials in food preservation receives increasing attention due to consumer awareness of natural food products. For this aim, isolation and partial characterization of the antimicrobial activity, antibiotic resistance, enzymatic properties and plasmid profiles of newly isolated *Leuconostoc* sp. and *Pediococcus* sp., which are responsible for aroma of some dairy products, were conducted in this study.

MATERIALS and METHODS:

Samples

A total of 40 samples (4 cottage cheeses, 3 cheeses with garlic, 1 sausage, 4 yogurts and 28 local cheeses, all home made) were collected from the Northern Iraq and they were kept in sterilised universal tubes. All samples kept at 4 ºC until delivery to laboratory and stored in fridge conditions until their usage for microbial isolation.

Culture conditions and isolation of bacterial strains

Samples (*ca* 1 g) were aseptically transferred into tubes containing either 5 mL acetate, MSE, MRS or GM17 broth media and inoculated tubes were incubated at 25°C for MSE medium, 30°C for MRS medium and 37°C for GM17 and acetate media for overnight. Aliquots (100 µl) of the samples were plated into appropriate medium and plates were incubated at the same temperatures for 48h.

After incubation, individual colonies were selected and transferred into sterilized broth media. Following preliminary characterization of 160 isolates, they were initially tested for colony morphology, Gram stain characters, cell morphology and H2O2 production for catalase test (Harrigan and McCance, 1976). The strains, which were morphologically identified as being Gram positive, coccus in tetrads, ovoid cocci shape and catalase negatives, were selected for detailed identification process. All isolates were coded accordingly and stored (-80°C) under the treatment of 30% glycerol as cryoprotectant.

Molecular identification of the bacterial strains

All isolated and purified bacterial strains were subjected to the molecular identification process. Total DNA was extracted from colonies grown on agar plates. Bacterial colonies were scraped using sterilized toothpick from the surface of agar plates and suspended in 10 µl sterilized distilled water. The suspension used as template for polymerase chain reaction (PCR) process. For molecular identification of the bacterial strains, 16S rDNA for *Leuconostoc* strains and 23S rDNA for *Pediococcus* strains were used. The primer sets for the amplification of 16S rDNA were designed according to the report of Jang et al. (2003). The sequence of forward primer was 5'-CGA AAG GTG CTT GCA CCT TTC AAG-3' whilst the reverse primer was 5'- TTT GTC TCC GAA GAG AAC A-3'. For PCR amplification of the 23S region of rDNA, forward and reverse primers were designed as described by Pfannebecker and Fröhlich (2008) as follow; F: 5'-GAA CTC GTG TAC GTT GAA AAG TGC TGA-3' and R: 5'-GCG TCC CTC CAT TGT TCA AAC AAG-3'.

PCR reactions were carried out using a Thermal Cycler (Favorgen) and each PCR reaction was consisted of 1 µl of template DNA, 1 µl of each dNTP, 1 µl of each primer, 1 µl of MgCl2, 1 µl of Taq (5U) DNA polymerase and 4 µl of 10X buffer and 30 µl distilled water in a total volume of 40 µl. The PCR conditions were conducted as; pre-denaturation at 95ºC for 3 min followed by 35 cycles of 94ºC for 1 min, 55ºC (58ºC for 23S region) for 50 sec, 72ºC for 1 min and a one cycle of final extension at 72ºC for 4 min. The PCR products (5 µl) were loaded into 1% agarose gel and run for the yield of amplicons of expected sizes of *ca* 700 bp for the identification of the typical *Pediococcus* sp. (23S rRNA) and *ca* 973 bp for the identification of *Leuconostoc* sp. (16S rRNA).

Antimicrobial activity detection

The antibacterial activity test was performed using the agar-well-diffusion method described by Tagg and McGiven (1971) with slight modifications detailed as follow. The pure cultures of food borne pathogens namely *Salmonella paratyphi, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Campylobacter jejuni, Klebsiella pneumoniae* and *Yersinia* sp. P1 were used. All microbiome was obtained from the culture collection of BİGEM Laboratory, Animal Science department of KSU and they were inoculated in Muller Hilton Broth (MHB). After 24 h incubation at 37 ºC, this suspension was used for inoculation of the pathogenic strains for the antimicrobial activity determination of the *Leuconostoc* and *Pediococcus*. The plates were incubated at 37ºC to allow for the growth of the target microorganisms and then controlled for the presence of inhibition zones around the wells. Tests were carried out in triplicate and inhibition zones were measured using an electronic-digital caliper.

Determination of antibiotic susceptibility

Antibiotic susceptibility of *Leuconostoc* and *Pediococcus* isolates were tested against to antibiotics discs of penicilline (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), erythromycin (15 µg), amoxicillin (25 µg), streptomycin (10 µg), rifampin (5 µg), kanamycin (30 µg), vancomycine (30 µg) and gentamicin (10 µg) using agar disc diffusion test (Barry and Thornsberry, 1980). Based on the inhibition zone size, the results were interpreted as resistant (R), intermediate resistant (IR), or sensitive (S) to the antimicrobial agents.

Plasmid isolation of bacterial strains

Bacterial stock cultures were reactivated in 5 ml of acetate broth at 37 ºC and plasmids isolations were carried out using Gene Jet plasmid extraction kit (Thermo Scientific) according to the manufacturer's instruction. Isolated plasmids were electrophoresed on agarose gel (1% w/v) and positive bands were visualized on UV transilluminator and photographed using Olympus C-5060 camera.

RESULTS and DISCUSSION

Identification and morphology of isolates

Initially 160 strains were isolated from various types of home-made fermented foods collected from the Northern part of Iraq. All isolates were tested to observe their Gram positive/negative and catalase negative characteristics. Gram positive and catalase negative strains (which are basic characteristics of the genera *Pediococcus* and *Leuconostoc*) were preliminarily characterised and grouped by biochemical tests and morphological features (Haakensen et al., 2009; Kulwichit et al., 2007). Globus shaped cells were observed in 38.75% and they were designated as *coccus*. All *cocci* isolates were found to be catalase negative and grown well on MRS agar plates. *Coccobacilli* and *bacillus* forms were observed at the ratio of 41.25% and 11.87%, respectively. The remaining cells (8.13%) were not classified accordingly and therefore, they were discarded from the experimental process. The isolates were cultured in the media containing various energy sources, namely acetate, MSE, GM17 and MSE, which are selective in some extend for lactic acid bacteria. Morphologically grouped isolates were further subjected to colony PCR for molecular identification and 38 straisns were identified as either *Pediococcus* (Figure 1) sp. or *Leuconostoc* sp. (Figure 2).

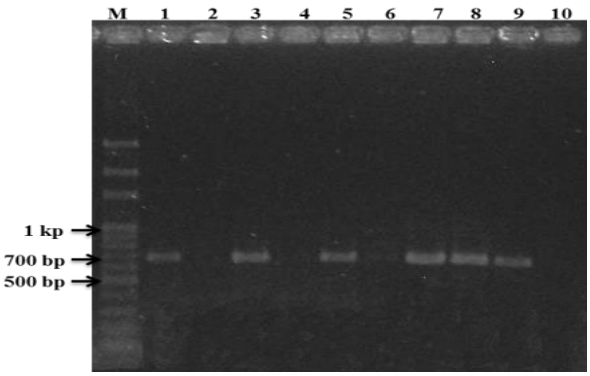

Figure 1. Exemplified gel electrophoresis of the PCR products obtained using *Pediococous* –spesific primers (PedioF and PedioR). M: 100 bp markler ladder (Favorgen-Taiwan).

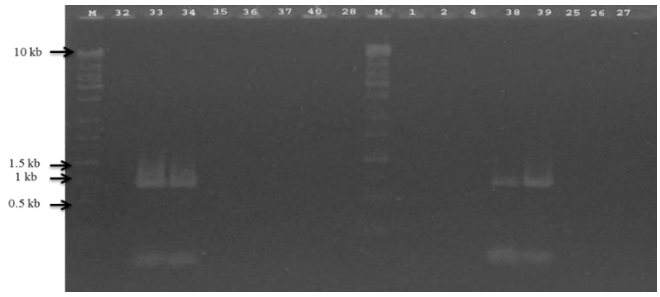

Figure 2. Exemplified gel electrophoresis of the PCR products obtained using *Leuconostoc* – spesific primers (LeuF and LeuR) for 16S rDNA (*ca* 973 bp). M: 1 kb marker ladder (Favorgen-Taiwan)

All the isolates were subjected to molecular identification at the species level by using *Leuconostoc* sp. and *Pediococcus* sp. specific primers. Molecular identification studies (by colony PCR) using 16S rDNA and 23S rDNA sequencing showed that these isolates were belong to the *Leuconostoc* sp. (n=4) and *Pediococcus* sp. (n=34). *Leuconostoc* isolates were differentiated from the other genera of LAB by their coccobacillus cell morphology (Haakensen et al., 2009). Only 4 isolates were found belonging to the genus *Leuconostocs* and this lower number of *Leuconostocs*, isolated and identified in current study, is probably due to their inability to compete with other LAB in mixed cultures under *in vitro* conditions (Teuber and Geis, 1981; Togo et al., 2002). Although, 122 isolates displayed LAB profile by phenotypic tests (Gram positive and catalase negative), no amplification band, either for 16S rDNA of *Pediococcus* or 23S rDNA of *Leuconostoc* primers, was observed (data not shown) and therefore they are not included for the remaining parts of the study.

Antimicrobial effect of Pediococcus sp. and Leuconostoc sp.

The antimicrobial activities of newly isolated strains were determined by the agar well diffusion method (Tagg et al., 1976) using cell-free culture supernatants and presented as mm in diameter (Table 1). Their antimicrobial properties were tested against eight food borne pathogenic bacteria namely *Salmonella paratyphi, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Campylobacter jejuni, Klepsiella pneumoniae* and *Yersinia* sp. P1 and an exemplified photograph showing these activities is given in Figure 3.

None of the 38 strains showed antimicrobial effect with the activity zones of ≥10 mm against *P. mirabilis, E. coli* and *C. jejuni*, while *P. aeruginosa* was found to be resistant to the all antimicrobial agents synthesized by these 38 strains (Table 1). Similar results were reported by Herreros *et al.,* (2005) who screened thirty-one lactic acid bacteria isolated from dairy products, that none of the strains showed antimicrobial activity against several pathogenic and spoilage bacteria.

The most notable results for the antimicrobial activity of newly isolated strains were recorded against to *S. paratyphi* and *E. faecalis* that they were remarkably inhibited by the majority of isolates. The highest antimicrobial effects were recorded for the isolate GMLAK8 (*Pediococcus*) and GMLAK38 (*Leuconostoc*) against *E. faecalis*, and GMLAK23, 27 (both *Pediococcus*) against *S. paratyphi*. No isolate which has not antimicrobial activity against tested pathogenic bacteria (apart from *P. aeruginosa*), was recorded suggesting that all isolates are forming at least one type of bacteriocin. The bacteriocins produced by *Pediococcus* appear to have a relatively broad spectrum of activity, including non-lactobacillaceae gram positive bacterial species (Daeschel and Klaenhammer 1985; Hoover, 1988). The findings of current study, about antimicrobial effects of the genus *Pediococcus*, are similar to those of Nghe and Nguyen (2014), who reported remarkable inhibitory effect of the bacteriocin(s) produced by *Pediococus pentosaceus* against *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococus aureus* and *Micrococcus luteus*.

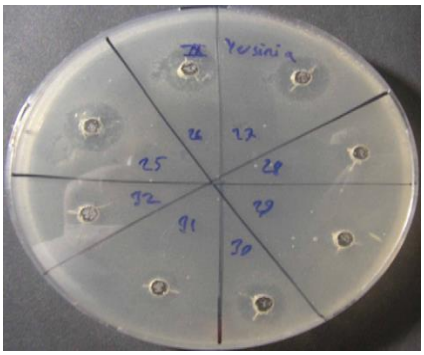

Figure 3. An exemplified view showing antimicrobial activity of newly isolated *Pediococcus* and *Leuconostoc* strains against to *Yersinia* P1 is given in petri dish containing Muller Hilton agar

Antibiotic susceptibility of the strains

Total of 20 out of 38 isolates were checked for antibiotic sensitivity against 11 different antibiotics and results were presented in tabulated form (Table 2). Majority of the 20 isolates were resistant to erythromycin, chloramphenicol and rifampin. None of the isolates had resistant gene

against vancomycine as all the isolates were highly sensitive to this drug. All the tested isolates were intermediate to highly sensitive to streptomycin, however, GMLAK1, 5, 11 and 16 were found to be resistant to this antibiotic.

Penicillin G, gentamicin, netilmicin, erythromycin, clindamycin, rifampin, chloramphenicol, were found to be active against *Pediococcus* species (Zarazaga et al, 1999). However, susceptibility of LAB against these antibiotics is thought to be species dependent (Danielsen et al., 2007). The presence of the antibiotic resistance genes in LAB, on the other hand, is a growing concern since they have a remarkable potential for transfer to other spoilage or pathogenic microorganisms inhabiting in the same environment (Herrores et al., 2005; Korhonen et al., 2010) and therefore it is recommended that LAB used as starters should not carry antibiotic resistance genes. These microorganisms showed a multiresistance to chloramphenicol, erythromycin, rifampin, penicillin and amoxicillin. Our knowledge about the antibiotic resistivity of LAB is still limited, due to the fact that notably large numbers of genera and species encountered in this group, as well as variances in their resistance spectra (Hummel et al., 2007). Thus, susceptibility tests against different antibiotics should be conducted perior to usage of LAB in fermentation process of food products (Jansson, 2005).

Table 1. Inhibition of some food-borne pathogens and spoliage bacteria by newly isolated *Pediococcus* (GMLAK1-34) and *Leuconostoc* (GMLAK35-38) strains, tested using agar well diffusion method (inhibition zone presented as mm in diameter).

Strains GMLAK	*S. paratyphi*	*E. faecalis*	*P. mirabilis*	*P. aeruginosa*	*E. coli*	*C. jejuni*	*K. pneumoniae*	*Yersinia P1*
1	10.33	13.00	5.05	0	7.15	8.00	5.00	0
2	5.00	8.00	0	0	0	0	0	9.10
3	13.00	13.33	0	0	0	7.25	10.00	7.85
4	5.00	8.05	0	0	0	7.32	8.25	11.20
5	7.00	9.33	0	0	0	0	0	7.25
6	11.50	5.52	0	0	5.50	0	8.15	5.00
7	6.50	12.00	8.00	0	0	0	0	6.00
8	0	14.00	0	0	9.00	8.55	0	5.20
9	0	9.25	0	0	0	0	5.05	0
10	7.45	6.66	0	0	6.55	0	0	0
11	11.03	10.33	7.25	0	8.20	0	6.00	7.33
12	6.05	8.00	0	0	0	6.02	6.25	0
13	7.33	10.55	5.15	0	0	0	0	6.12
14	10.66	11.22	0	0	0	6.03	0	8.10
15	6.00	9.25	0	0	0	0	0	0
16	11.20	13.55	0	0	0	5.00	0	9.05
17	11.00	8.00	0	0	0	0	0	0
18	8.33	8.55	8.22	0	6.15	0	8.05	0
19	11.33	12.32	0	0	7.00	5.15	5.15	7.22
20	13.32	9.00	5.25	0	0	0	0	10.35
21	9.05	0	5.33	0	8.95	6.00	0	0
22	9.33	13.00	7.00	0	6.03	0	6.73	8.55
23	14.00	10.00	6.10	0	0	0,75	10.45	11.11
24	10.02	10.04	0	0	5.02	9.00	0	11.00
25	10.05	9.00	7.55	0	0	6.15	0	10.34
26	10.32	8.67	0	0	0	8.20	10.00	9.66
27	14.02	0	0	0	0	8.35	0	11.65
28	12.00	11.22	0	0	8.40	0	0	6.25
29	13.05	11.15	7.00	0	0	0	6.65	0
30	13.00	6.00	0	0	0	0	8.10	10.45
31	9.01	13.65	0	0	6.00	0	0	0
32	9.66	13.00	5.00	0	7.00	0	0	0
33	0	10.33	0	0	0	0	0	5.00
34	13.00	10.52	0	0	8.10	6.04	11.22	7.15
35	5.00	11.00	5.45	0	0	0	0	0
36	5.02	12.65	6.15	0	6.00	0	6.15	6.32
37	0	9.00	0	0	0	0	6.00	9.22
38	12.55	14.00	0	0	0	7.02	12.33	0

0: No antimicrobial activity was observed; Activity zones with ≥10 mm are emphsized in grey colour

Table 2. Antibiotic susceptibility of newly isolated strains were tested against 11 antimicrobial agents using disc diffusion technique in Muller Hilton agar plates (inhibition zone presented as mm in diameter).

Strains	Antibiotics*										
GMLAK	C30	TE30	E15	GN10	S10	RA5	AM10	P10	K30	VA30	AX25
1	12.42	10.65	11.75	0	11.22	12.60	10.28	12.07	10.55	0	14.88
2	20.12	10.87	17.75	10.10	0	19.80	18.39	16.81	0	0	17.49
3	15.31	0	14.80	0	0	14.65	9.75	13.52	0	0	18.10
5	18.60	8.52	20.20	9.77	12.10	16.00	13.82	20.02	0	0	17.32
8	25.10	10.55	14.36	13.43	0	21.75	16.25	17.95	8.02	0	19.35
11	23.64	14.32	19.93	0	14.46	19.72	19.05	20.77	13.20	0	0
15	14.02	16.28	18.60	9.95	0	19.60	15.20	15.91	0	0	23.10
16	21.02	9.42	23.25	0	12.30	14.45	15.02	13.64	0	0	13.33
18	23.17	14.00	11.03	15.05	0	18.60	22.87	18.75	11.62	0	19.31
20	25.60	0	21.75	0	0	16.74	16.48	21.60	10.69	0	17.69
21	17.95	12.06	16.85	21.10	0	18.57	16.72	15.93	9.37	0	18.12
22	16.50	12.90	22.75	0	0	17.95	16.46	0	0	0	15.85
23	14.82	0	26.33	0	0	16.39	11.40	9.63	0	0	0
24	12.95	0	19.06	0	0	18.70	17.39	17.80	14.60	0	18.75
25	13.81	0	0	0	0	19.64	0	0	0	0	0
27	21.34	0	20.32	8.35	0	17.63	18.42	13.20	0	0	16.09
28	27.65	0	21.00	9.82	0	18.02	18.98	15.85	12.10	0	9.95
31	18.41	14.07	17.10	0	0	18.70	0	14.45	11.03	0	0
37	23.02	11.90	15.61	11.48	0	17.50	19.37	27.60	8.30	0	19.28
38	21.84	12.82	19.22	0	0	19.20	15.17	24.46	0	0	26.66

C30: Chloromphenicol; TE30: Tetracycline; E15: Erythromycin; GN10: Gentamicin; S10: Streptomycin; RA5: Rifampin; AM10: Ampicilin; P10: Penicillin; K30: Kanamycin; VA30: Vancomycin; AX25: Amoxicillin. 0: Resistant against tested antibiotic. The activity zones with ≥ 20 mm are emphsized in grey colour.

Investigation of bacterial isolates for plasmid contents

Results revealed that among 38 isolates, only ten of them GMLAK1, 2, 3, 5, 15, 21, 22, 31, 37 and 38 showed the presence of plasmids. Agarose gel electrophoresis results revealed that only one isolate (GMLAK3) contained a single plasmid which is *ca* 2.7 kb. The isolates, designated as *Pediococcus*, GMLAK2, GMLAK5, GMLAK15 and GMLAK31 had same plasmid profiles, and similarly, plasmid profiles of the *Leuconostoc* isolates (GMLAK37, 38) were found to be identical, having 4 plasmids each (**Figure 4**). The smallest size of the plasmids was recorded as *ca* 2.1 kb (For isolates GMLAK 22, 37, 38) although the majority of them were found to be more than 10 kb in size. There was no linear correlation between the plasmid contents of the newly isolated strains and their antimicrobial resistancy against tested antibiotic agents and these results were similar to the findings of Gezginc (2010).

Although the starter cultures, commercially improved and used in dairy industry, do not contain extra-choromosomal structures, observation of the plasmid vectors in the LAB isolated from traditionally home made dairy products is common (Turgeon and Moineau, 2001). Usage of such fermented dairy products as inoculant, therefore, should be restricted due to their potential risks for the transferring of antibiotic-resistant encoding genes to the harmful pathogenic microbiome.

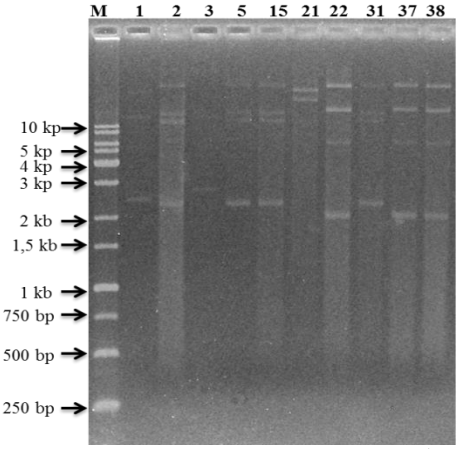

Figure 4. A total of 10 isolates (out of 38 isolates investigated) had various numbers (1-4) of plasmids. M: 1 kb DNA ladder Plus (Favorgen, Taiwan).

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