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Identification of Lactic Acid Bacteria in Fermented Cornichon Pickles Produced with Acid Whey and Vinegar

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Abstract

The purpose of this study is to isolate, identify and compare lactic acid bacteria responsible for fermentation from pickles produced with vinegar and acid whey (AW). For this reason, fermented cornichon pickles were produced by using AW and vinegar as brine media. Colonies with different morphologies were selected and isolated at the 0, 7, 14, 21, 35, 49, 77 and 105th days of fermentation. A total of 139 isolates were obtained. Salt, acidity and pH values of brine and cornichon were followed on each analysis day. Growth and gas formation from glucose of isolates at different temperatures (10, 45, and 50 ^{o}C), salt concentrations (2, 4.5, and 10% (w/w)), different pH values (3, 4.5, and 9.6), were analyzed. Eight isolates were determined as heterofermentative according to their ability to produce CO_2 from glucose. The isolates were observed to show the highest growth at 10 ^{o}C, and a very few isolates developed at 45 ^{o}C and 50 ^{o}C. No isolates were able to grow at pH 9.6, 25 of the isolates were able to grow at pH 4, and only 4 isolates were able to grow at pH 3. Only one isolate was able to show resistance to 10% salt concentration. Almost all isolates grew at 2% and 6.5% salt concentrations. 48 selected isolates were identified with API 50 CHL. 19 isolates were determined as *Lactobacillus pentosus* (8 vinegar, 11 AW) and 29 isolates as *Lactobacillus plantarum* (16 vinegar, 13 AW). As a result, *L. plantarum* and *L. pentosus* species were the dominant bacteria for cornichon pickles produced with AW and vinegar. Microbial flora in the pickles produced with two different brines were similar. The results show that AW can be successfully used for pickle production.

Keywords: Lactic acid bacteria, identification, acid whey, cornichon pickle

1. INTRODUCTION

By pickling, foods can be preserved for longer and consumed even when little or no available [1]. Lactic acid fermentation and salt are two important factors in pickle production. In pickle fermentation, the lactic acid bacteria in the natural flora come from the raw material. Lactic acid fermentation inhibits the development of pathogenic microorganisms, provides protection against the growth of microorganisms that cause degradation and toxin formation, and also increases the nutritional value of the product [2].

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The success of the lactic acid fermentation is significant for pickle quality [3].

Greek yogurt is a fermented milk product in which the serum part of yogurt is filtered and removed. The demand for Greek yogurt has increased rapidly in recent years. Thus, acid whey (AW), a by-product of Greek yogurt manufacture, also increased. Approximately 2-3 kg of AW emerges from the production of 1 kg of Greek yogurt [4]. In the USA, approximately 4 million tons of acid whey was produced as by product of Greek yogurt manufacture in 2015 [5]. Acid whey is difficult to powder as it contains high levels of lactic acid and calcium [6]. The presence of high amounts of lactic acid reduces the commercial value of AW, limits potential food applications, making it very difficult to process [7]. However, AW can cause serious environmental problems if not properly disposed of. Various evaluation methods have been investigated in order to increase the value of AW and to eliminate its harm to the environment [5, 8].

Lactic acid bacteria (LAB) is very important for fermented foods with its ability to inhibit pathogens and spoilage microorganisms. Organic acids, hydrogen peroxide, exopolysaccharides, antimicrobial and aromatic compounds synthesized by LAB during fermentation contribute to the texture, shelf life, flavor and aroma development of foods [9]. In addition, it has a protective effect by lowering the pH of the environment with the organic acids it produces [10]. Lactic acid bacteria are resistant to gastric acid and pancreatic secretions in the stomach. Lactic acid bacteria that are resistant to conditions in the digestive system and survive are probiotic [11, 12].

The amount of LAB in cornichons is very low. But when cornichons meet with brine, the existing LAB adapt quickly to the environment and begin to develop rapidly. Coliform group bacteria coming from the raw material are inhibited with increasing acidity during fermentation. Another undesirable microorganism is yeasts, competing with LAB for fermentable sugars [2]. The dominant LAB in the pickled environment are listed as *Leu. mesenteroides*, *Stp. faecalis*, *P. pentosaceus*, *L. brevis*, *L. plantarum*. *P. pentosaceus*, *L. brevis*, *L. plantarum* are generally used in commercial fermentation [13].

Some phenotypic and genotypic tests are used to identify LAB. Phenotypic tests determine the characteristics of bacteria such as growth at different temperatures, resistance to high acidity and salt concentrations, gas formation from glucose, cell morphology, and thus identification from family to genus can be provided [14, 15]. API 50 CHL can identify the species and subspecies by examining the carbohydrate fermentation characteristics of bacteria. Genotypic tests are aimed at determining the DNA characters of bacteria [16, 17].

In this study, our objective was to isolate and identify LAB responsible for fermentation of cornichon pickles produced by using vinegar and AW as brine.

2. MATERIALS AND METHODS

2.1. Pickle samples

Three independent batches of cornichon pickles were manufactured with brines produced from AW and vinegar. Cornichons were washed with water, 290-300 g cornichons were placed in 600 cc glass jars and plastic pressure apparatus were placed on top to keep cucumbers in brine. Vinegar used in the production was obtained from a local market and diluted with distilled water until the titration acidity was 1% (w / w). The AW was obtained from a local dairy plant and was kept at room temperature until the titration acidity reached 1% (w / w). The final salt concentration of the brines were adjusted to 8% by using coarse salt. Acid whey was boiled at 100^{o}C for 10 minutes, and coagulated proteins (if any) were removed by filtering with a cloth. Pickles were placed for fermentation in an incubator set at 25 ° C. Chemical and microbiological analysis were conducted on the 0, 7, 14, 21, 35, 49, 77, 105th days of fermentation. At each analysis point, two jars of pickles (one jar of pickle manufactured with AW and one jar pickle manufactured with vinegar) were opened and used for analysis.

2.2. Chemical and microbiological analysis of pickle samples

The pH of brine samples was measured by a pH meter (Inlab® solid pro; Mettler Toledo, Columbus, OH, ABD). Titration acidity (947.05; AOAC 2007) and salt analysis (975.20; AOAC, 2007) of all samples taken from the brine were measured. Colonies of lactic acid bacteria were isolated from pickled samples at the 0, 7, 14, 21, 35, 49, 77, 105th days of fermentation.

10 ml of pickle brine was taken under aseptic conditions, and necessary dilutions were made with sterile peptone water. 0.1 µL of brine sample from each dilution was inoculated into petri plates containing MRS (DeMan, Rogosa and Sharpe Agar) and M17 agar. Petri plates were incubated for 48 hours at 30°C. Petri plates containing MRS agar were incubated under anaerobic conditions.

2.3. Isolation of lactic acid bacteria

Colonies with different morphologies were taken from MRS and M17, then inoculated on MRS agar. Petri plates were incubated for 48 hours at 30°C. After incubation, a single colony was taken with a loop and dipped into tubes containing MRS Broth, incubated at 30°C for 48 hours. When the incubation was over, the liquid medium was homogenized with vortex and inoculated again on MRS agar and MRS Broth. The pure cultures with sterile glycerol (30%) were kept as frozen culture at -80°C until the day of identification analysis.

2.4. Identification of lactic acid bacteria

The isolates kept at -80°C were transferred to 10 mL MRS Broth for revival. Gram stain and cell morphologies of 18-24 hour fresh cultures grown at 30°C were examined under the microscope. The shapes of the bacteria were determined as cocci or bacilli. The catalase reaction was investigated by dripping 3-4 drops of %30 H_2O_2 solution on the colonies developed for 24 hours at 30 °C in MRS agar and observing the gas evolution. Gram-positive and catalase-negative bacteria were assumed as LAB.

To test CO_2 production; samples from the activated culture were inoculated into MRS Broth containing a Durham tube and incubated at 30 ^{O}C for 7 days. It was evaluated as heterofermentative if CO_2 formation was detected and homofermentative if CO_2 formation was not detected [18, 19].

To observe the activities of bacterial cells at different temperatures, pH and salt concentration; they were incubated in MRS broth at three different temperatures (10ºC, 15ºC and 45ºC for 5 days), in MRS broth adjusted different pH conditions using lactic acid (3, 4.5 and 9.6 for 3 days, 30°C) and at different salt concentrations (2%, 6.5% and 10% NaCl (w/v) for 3 days, 30°C) [18, 19].

Carbohydrate fermentation patterns were determined using API 50 CHL test strips according to manufacturer's instructions (BioMérieux, Marcy l'Etolie, France). API 50 CH kit (BioMérieux, Marcy l'Etolie, France) was used to determine the isolates at the species level.

3. RESULTS AND DISCUSSION

3.1. Chemical and microbiological characteristics of pickle samples

Salt, acidity and pH analyzes of the brines were made to have information about the general condition of the pickles. The titration acidity values increased from 0.61 to 1.06% and 0.71 to 1.85% and at the end of fermentation for the pickles with vinegar and AW, respectively.

The pH values were between 3.59 and 3.34 in brines produced with vinegar, and between 4.21 and 3.48 in brines produced with AW at the manufacture and at the end of fermentation, respectively. The initial and final pH of the vinegar brines was lower than the brine produced with AW.

Table 1 pH, titration acidity and salt values of pickle brines

Fermantation	pH		Titration Acidity (%)		Salt (%)	
Day	AW	Vinegar	AW	Vinegar	AW	Vinegar
0	4.21±0.04	3.59±0.11	0.71±0.07	0.61±0.01	4.93±0.12	4.53±0.48
7	3.71±0.03	3.53±0.08	1.32±0.03	0.79±0.05	3.58±1.46	4.68±0.44
14	3.64±0.02	3.58±0.29	1.49±0.11	0.80±0.17	5.07±0.81	4.69±1.05
21	3.62±0.03	3.36±0.30	1.71±0.16	1.06±0.22	5.47±0.45	4.79±0.20
35	3.61±0.06	3.52±0.28	1.78±0.16	0.93±0.25	4.97±0.53	4.78±0.31
49	3.57±0.01	3.55±0.14	1.85±0.16	0.95±0.12	5.30±0.54	4.41±0.21
77	3.48±0.02	3.52±0.33	1.84±0.19	0.94±0.24	5.18±0.52	4.51±0.19
105	3.53±0.04	3.49±0.31	1.75±0.13	0.97±0.22	5.21±0.51	4.57±0.26

The titration acidity of pickles produced with AW increased at a higher rate compared to pickles produced with vinegar. The higher numbers of lactic acid bacteria and also lactose content in pickles produced with AW may have caused the higher titration acidity. Although the titration acidity of pickles produced with AW was about twice of the pickles produced with vinegar at the end of fermentation, the pH values of both pickles were found to be similar. Calcium salts and/or protein in the AW may have caused buffering effect, preventing pH decrease and causing observing similar pH values with pickles produced with vinegar.

Salt concentrations are between 4.41 and 4.79 in brine produced with vinegar, and between 3.58 and 5.30 in brine produced with AW. It has been stated that the salt ratio in vegetable fermentations should be in the range of 1-8%. Excessive salt content inhibits the growth of lactic acid bacteria [20].

3.2. Identification results of lactic acid bacteria

It is known that all LAB are Gram-positive bacteria. According to gram stain results of 139 isolates, 109 isolates were defined as Gram-positive. Gram-negative bacteria (30) were eliminated because they did not show the characteristic of LAB. Lactic acid bacteria are catalase negative. All 109 of Gram-positive bacteria also gave catalase negative results.

53 isolates were obtained from pickles produced with vinegar: 16 cocci and 37 bacilli. 56 isolates were obtained from pickles produced with AW: 14 cocci and 42 bacilli.

Many studies report cultures grown on MRS agar as lactobacilli and on M17 agar as lactococci [21, 22]. This information is incomplete and can cause confusion. Polysorbate, acetate and magnesium in the composition of MRS agar are supportive factors for the development of Lactobacilli. MRS agar does not show a feature such as inhibiting the growth of other microorganisms. Other bacteria and yeasts can also grow on MRS agar. M17 agar promotes the development of lactic streptococci by increasing the buffering capacity of the medium, due to β-glycerophosphate it contains. Similar to MRS agar, there is no selective additive to inhibit the growth of other microorganisms in M17 agar, only the growth of Lactococci is supported. As a result, MRS is the most suitable medium for the growth of Lactobacillus. However, defining all the bacteria that develop in MRS as Lactobacillus is incorrect, the fact is we do not know for sure without further analyses. This is also the case for M17 agar [1, 23].

Gas formation of lactic acid bacteria by utilization of glucose usage was monitored with Durham tubes. In total, 8 heterofermentative bacteria were detected: 3 in pickles produced with AW, and 5 in pickles produced with vinegar. In cucumber pickles, it is desirable that CO_2 formation is as low as possible during fermentation as it causes swelling problems. Therefore, heterofermentative lactic acid bacteria are less desirable than homofermentative lactic acid bacteria [24].

The isolates, activated twice, were incubated for 5 days at three different temperatures, 10 °C, 45 °C and 50 °C. Bacteria obtained from the pickles produced with vinegar, 14 isolates grew at 10 °C, 11 isolates at 45°C and 4 isolates at 50 °C. From

the bacteria isolated from pickles produced with AW, 13 isolates grew at 10 ^{o}C, 5 isolates at 45 ^{o}C, and 3 isolates at 50 ^{o}C. The optimum growth temperature of lactic acid bacteria in cucumber fermentations was determined as 16-32 °C [24].

After the isolates were activated, they were incubated for 3 days at three different pH values; 3, 4.5 and 9.6. At pH 3, 4 isolates, and at pH 4.5, 20 isolates were able to grow. No growth was observed at pH 9.6 for pickles manufactured with vinegar. A similar situation exists in the isolates of pickles produced with AW. Four isolates obtained from pickles produced with AW showed growth at pH 3.0, and 26 isolates at pH 4.5. No isolate showed growth at pH 9.6.

The ability of bacterial isolates to grow at three different salt concentrations were evaluated at 2%, 6.5% and 10% salt concentrations. There are a total of 53 isolates from the pickles produced with vinegar. 50 isolates showed growth at 2% concentration, 44 isolates at 6.5% concentration. Two isolates were able to grow at 10% salt concentration. Most of the isolates obtained from pickles produced with AW showed growth at 2% and 6.5% concentrations. There are a total of 56 isolates obtained from AW brine. 55 isolates were able to show growth at 2% salt concentration and 50 isolates at 6.5% salt concentration. No isolates obtained from AW brine could grow at 10% salt concentration.

Table 2 Phenotypic characteristics of LAB isolated from pickles and identification result with API 50 CHL test kit

Brine Type	Isolate No.	GFG	Morf.	pH			T (ºC)			NaCl (%)			LAB identification	%
				3	4,5	9,6	10	45	50	2	6,5	10		
Vinegar	112	-	Bacil	-	-	-	-	-	-	++	++	-	*L.plantarum*	99.8
	131	-	Bacil	-	+	-	-	-	++	+	+	-	*L.pentosus*	97.4
	141	-	Bacil	-	+	-	-	-	-	+	+	-	*L.plantarum*	99.9
	152	-	Bacil	-	-	-	-	-	-	++	+	-	*L.pentosus*	98.6
	162	-	Bacil	-	+	-	+	+	-	-	++	-	*L.plantarum*	99.9
	172	+	Bacil	-	+	-	+	++	-	+	++	+	*L.plantarum*	99.1
	183	-	Bacil	-	+	-	+	-	-	++	++	-	*L.plantarum*	99.9
	184	-	Bacil	+	+	-	-	-	-	++	++	-	*L.plantarum*	99.8
	313	-	Bacil	-	-	-	-	++	-	++	+	-	*L.plantarum*	99.6
	331	-	Bacil	-	-	-	-	-	-	+	+	-	*L.plantarum*	99.9
	363	-	Cocci	-	-	-	-	-	-	+	+	-	*L.pentosus*	60.7
	381	-	Bacil	-	+	-	-	++	-	-	-	-	*L.plantarum*	99.9
	721	-	Bacil	-	-	-	-	-	-	+	+	-	*L.plantarum*	999
	761	-	Cocci	-	-	-	-	-	-	+	+	-	*L.pentosus*	98.6
	X152	-	Bacil	-	-	-	-	-	-	+	++	-	*L.plantarum*	99.9
	X183	-	Bacil	-	+	-	-	-	-	+	+	-	*L.plantarum*	99.9
	X323	-	Cocci	-	+	-	-	-	-	+	++	-	*L.plantarum*	99.9
	X354	-	Bacil	-	-	-	-	-	-	++	+	-	*L.pentosus*	97.2
	X361	+	Bacil	-	+	-	-	-	-	++	+	-	*L.pentosus*	98.6
	X362	+	Bacil	-	+	-	-	-	-	++	-	-	*L.pentosus*	97.4
	X711	-	Cocci	+	-	-	++	++	+	+	+	-	*L.pentosus*	88.7
	X751	-	Bacil	-	-	-	+	-	-	++	++	-	*L.plantarum*	99.8
	X753	-	Cocci	-	-	-	-	-	-	++	++	-	*L.plantarum*	66.6
	X771	-	Bacil	-	-	-	++	-	-	+	++	-	*L.plantarum*	99.9
AW	241	-	Bacil	-	-	-	-	-	-	+	+	-	*L.pentosus*	97.4
	263	-	Cocci	-	+	-	-	+	-	+	+	-	*L.plantarum*	99.9
	272	-	Cocci	+	-	-	-	-	-	++	+	-	*L.plantarum*	99.9
	411	-	Cocci	-	-	-	-	-	-	+	+	-	*L.plantarum*	99.9
	432	-	Bacil	-	-	-	-	-	-	+	+	-	*L.pentosus*	99.8
	444	-	Bacil	-	+	-	-	-	-	+	+	-	*L.pentosus*	98.6
	463	-	Cocci	-	-	-	-	-	-	+	++	-	*L.pentosus*	91.6
	474	-	Bacil	-	-	-	+	-	+	+	++	-	*L.plantarum*	99.2

482	-	Bacil	-	+	-	-	-	-	+	+	-	*L.plantarum*	99.9
483	-	Bacil	-	+	-	++	++	+	++	++	-	*L.plantarum*	99.6
823	-	Bacil	-	+	-	-	-	-	++	++	-	*L.plantarum*	99.9
831	+	Bacil	-	-	-	+	-	-	++	-	-	*L.pentosus*	98.6
833	-	Bacil	-	-	-	-	-	-	++	-	-	*L.plantarum*	99.9
881	-	Bacil	-	+	-	+	+	-	++	+	-	*L.plantarum*	99.9
X221	-	Bacil	-	+	-	-	-	-	++	+	-	*L.plantarum*	99.9
X253	-	Bacil	-	-	-	-	-	-	++	+	-	*L.pentosus*	99.9
X273	-	Bacil	+	+	-	++	-	-	+	+	-	*L.pentosus*	51.5
X281	-	Bacil	-	+	-	-	-	-	-	-	-	*L.pentosus*	99.9
X283	-	Bacil	-	+	-	+	-	++	++	+	-	*L.pentosus*	99.8
X452	-	Bacil	-	-	-	-	-	-	+	-	-	*L.plantarum*	99.9
X472	-	Cocci	-	+	-	-	-	-	+	+	-	*L.pentosus*	97.4
X481	-	Bacil	-	-	-	-	-	-	+	+	-	*L.plantarum*	99.9
X862	+	Cocci	-	-	-	++	-	-	+	++	-	*L.plantarum*	99.9
X874	+	Bacil	-	+	-	+	-	-	++	++	-	*L.pentosus*	98.6

-: negatif reaction, +: positive reaction, ++: strong positive reaction
GFG: Gas from glucose, T: Temperature, NaCl: Salt concentration

3.3. Carbohydrate Fermentations

Isolates were grouped according to the results of phenotypic analysis. 48 bacteria with different phenotypic characteristics were selected and identified with API 50 CH test kit. Selection of bacteria was carried out based on their resistance to high acidity, high salt concentrations, and their behavior at different temperatures. *L. plantarum* appeared to be the dominant microorganism for both brine types.

L. plantarum and *L. pentosus* species isolated from the pickles produced with vinegar were identified with high percentage (~99%). Only two species were identified with 60%. We observed that *L. planturum* species was dominant in pickles produced with vinegar. Bacteria isolated from M17 agar turned out to be *L. pentosus* species and bacteria isolated from MRS agar, *L. plantarum* species were dominant.

L. plantarum and *L. pentosus* species were isolated from the pickles produced with AW. Unlike the pickles produced with vinegar, *L.pentosus* species seems to be more dominant in pickles manufactured with AW. Similarly, *L.pentosus* species were dominant in the isolated samples from M17 agar.

According to API test results, genus characteristics of some species are inconsistent with previously determined cell morphologies. Although samples 323, 761, X323, X711, X753, X771 isolated from the pickles produced with vinegar and samples 263, 272, 411, 463, X472, and X862 were isolated from pickles produced with AW were determined as cocci, they were identified as Lactobacilli according to API test results.

Lactic acid bacteria that classified as heterofermentative according to the gas formation test were identified as *L. plantarum* and *L. pentosus*. These two bacteria were reported as "facultative heterofermentative", and they may exhibit gas-forming abilities [25, 26].

L. plantarum and *L. pentosus* are in the Streptobacterium group from Lactobacilli. The optimum growth temperature these species are 30-35 ^{o}C, and they are able to grow at 15 ^{o}C but mostly not at 50 ^{o}C. Growth test at different temperatures showed that *L. plantarum* and *L. pentosus* showed characteristics growth at temperatures studied, and several isolates were able to grow at 45 and 50 ^{o}C. The ability to grow at 10 ^{o}C was found to be higher for both bacteria. Şimşek (2003) reported that *L. plantarum* species isolated from pickles were able to grow at 15 ^{o}C, but only 1 isolate could grow at 45 ^{o}C [27].

L. plantarum and *L. pentosus* species seem to show the best growth at pH 4.5. The growth characteristics of both species at different pHs are quite similar. At pH 3, bacteria showed poor growth and at pH 9.6 no bacteria could grow. Even though *L. plantarum* and *L. pentosus* species were weakly able to grow at pH 3, and their acid resistance were quite high. Elmacı et al. [28] stated that *L. plantarum* bacteria isolated from cucumber pickle samples showed low growth at pH 3 and showed no growth at pH 9. However, they reported that *L. plantarum* showed the best growth at pH 4. In another study, *Lactobacillus* strains isolated from pickles showed growth at the level of 6.2-7.7 log cfu/mL at pH 3 [29].

Growth tests of different salt concentrations showed that most of the isolated LAB were able to grow at 2 and 6.5% salt concentrations (only 3 and 6 LAB were unable to grow at 2% and 6.5% salt concentrations, respectively). 10% salt concentration inhibited the growth of all isolated species except one bacterium (sample 172). Çetin [3] examined the growth conditions of the LAB isolated from pickles at 6.5%, 10% and 12% salt concentrations. They reported majority of the species isolated were *L. plantarum* showing growth at 6.5% concentration. They also reported that the growth ability of bacteria decreased considerably with the increase in salt concentration. In another study, *L. plantarum* bacteria isolated from cucumber pickles showed very good growth at 3% and 6.5% salt concentrations, while the growth level was very low level at 10% salt concentration [28].

The utilization of carbohydrates by the isolates exhibited similar results in the API test. All of the isolates were able to ferment D-ribose, D-fructose, D-glucose and D-mannose but not glycogen, xylitol. Especially the ability to use D-xylose and L-arabinose was definitive for identification.

L. plantarum and *L. pentosus* species show great similarity to each other in terms of phenotype. The biggest feature distinguishing *L.pentosus* from *L. plantarum* is that *L.pentosus* can ferment both xylose and arabinose [30]. We have observed similar results at the phenotypic tests for *L. plantarum* and *L. pentosus*. When the API test kit results were examined, samples showed a positive reaction by fermenting the D-xylose in microtube 6 were defined as *L. pentosus*.

Dursun [31] performed identification analyzes of LAB isolated from 15 different pickled cucumber samples, and stated that *L. plantarum*, *L. brevis*, and *L. acidophilus* species were dominant. Alan and Dığrak [32] isolated LAB from the pickled cucumber samples they produced and performed their identification analysis. According to the results of the analysis, they stated that the majority of the isolates were *L. plantarum* species. Tamminen et al. [33] stated that *L. plantarum* and *L. pentosus* constituted most of the bacteria they isolated from pickled cucumbers.

4. CONCLUSION

Isolates obtained from the pickles produced with two different brines prepared with vinegar and AW showed that *L.plantarum* and *L.pentosus* bacteria were the dominant flora. It was observed that the microbial flora of the pickles produced with two different brines were similar. Isolated bacteria from both brines exhibited similar resistance to acidity and salt concentration during fermentation, and their phenotypic characteristics were also quite similar. Some LAB isolated from both brines such as X273, 483, X884 and 184 exhibited growth at high acid and salt concentrations, which may indicate possible probiotic potential. The fact that the pickles produced with AW have a similar flora to the pickles produced with vinegar supports the conclusion that AW can be used successfully in pickle production.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The authors contributed equally to the study.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCES

[1] N. Aktan, U. Yücel, and H. Kalkan, Turşu Teknolojisi, Ege Üniversitesi Ege Meslek Yüksekokulu Yayınları, no. 23, 1998.

[2] M. Tokatlı, D. Dursun, N. Arslankoz, P. Şanlıbaba, and F. Özçelik, "Turşu üretiminde laktik asit bakterilerinin önemi," Akad. Gıda Derg., vol. 10, no. 1, pp. 70–76, 2012.

[3] B. Çetin, "Turşu fermantasyonunda kullanılabilecek laktik asit bakterilerinin belirlenmesi ve probiyotik turşu üretimi için uygun suşların seçimi," 2017.

[4] P. Menchik and C. I. Moraru, "Nonthermal concentration of liquid foods by a combination of reverse osmosis and forward osmosis. Acid whey: A case study," J. Food Eng., vol. 253, no. February, pp. 40–48, 2019.

[5] M. J. Lindsay, T. W. Walker, J. A. Dumesic, S. A. Rankin, and G. W. Huber, "Production of monosaccharides and whey protein from acid whey waste streams in the dairy industry," Green Chem., vol. 20, no. 8, pp. 1824–1834, 2018.

[6] S. Talebi, F. Suarez, G. Q. Chen, X. Chen, K. Bathurst, and S. E. Kentish, "Pilot study on the removal of lactic acid and minerals from acid whey using membrane technology," ACS Sustain. Chem. Eng., vol. 8, no. 7, pp. 2742–2752, 2020.

[7] J. Chandrapala et al., "Properties of acid whey as a function of pH and temperature," J. Dairy Sci., vol. 98, no. 7, pp. 4352–4363, 2015.

[8] E. M. Silva and S. T. Yang, "Kinetics and stability of a fibrous-bed bioreactor for continuous production of lactic acid from unsupplemented acid whey," J. Biotechnol., vol. 41, no. 1, pp. 59–70, 1995.

[9] J. P. Salminen, T. Roslin, M. Karonen, J. Sinkkonen, K. Pihlaja, and P. Pulkkinen, "Seasonal variation in the content of hydrolyzable tannins, flavonoid glycosides and proanthocyanidins in oak leaves," J. Chem. Ecol., vol. 30, no. 9, pp. 1693-1711, 2004.

[10] E. Yang, L. Fan, Y. Jiang, C. Doucette, and S. Fillmore, "Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts," AMB Express, vol. 2, no. 1, pp. 1–12, 2012.

[11] H. Rodríguez et al., "Food phenolics and lactic acid bacteria," Int. J. Food Microbiol., vol. 132, no. 2–3, pp. 79–90, 2009.

[12] G. Klein, A. Pack, and G. Reuter, "Antibiotic resistance patterns of enterococci and occurrence of vancomycin-resistant enterococci in raw minced beef and pork in Germany," Appl. Environ. Microbiol, vol. 64, pp. 1825–1830, 1998.

[13] C. S. Pederson and M. N. Albury, "Effect of temperature upon bacteriological and chemical changes in fermenting cucumbers," N. Y. Agr. Expt. Sta. Bull, p. 744, 1950.

[14] E. Çetinkaya and K. Ayhan, "Mikrobiyolojide Kullanılan Bazı Moleküler Teknikler Molecular Technics Used in Microbiology," Karaelmas Fen ve Mühendislik Derg., vol. 2, no. 1, pp. 53–62, 2012.

[15] F. Kıran and Ö. Osmanağaoğlu, "Laktik asit bakterilerinin (LAB) identifikasyonunda/tiplendirmesinde kullanılan moleküler yöntemler," Erciyes Üniversitesi Fen Bilim. Enstitüsü Derg., vol. 27, no. 1, pp. 62–74, 2011.

[16] E. Papamanoli, N. Tzanetakis, E. Litopoulou-Tzanetaki, and P. Kotzekidou, "Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties," Meat Sci., vol. 65, no. 2, pp. 859–867, 2003.

[17] O. Yerlikaya, "Laktik asit bakterilerinin tanılanmasında kullanılan başlıca fenotipik ve moleküler yöntemler," Gıda ve Yem Bilim. Teknol. Derg., vol. 14, pp. 8–22, 2014.

[18] J. Yu, W. Goa, M. Qing, Z. Sun, W. Wang, W. Liu, L. Pan, T. Sun, H. Wang, N. Bai, and H. Zhang, "Identification and characterization of lactic acid bacteria isolated from traditional pickles in Sichuan," China. J. Gen. Appl. Microbiol., vol. 58, no. 3, pp. 163–172, 2012.

[19] S. Bağder Elmacı, M. Tokatlı, D. Dursun, F. Özçelik, ve P. Şanlıbaba, "Phenotypic and genotypic identification of lactic acid bacteria isolated from traditional pickles of the Çubuk region in Turkey," Folia Microbiologica, vol. 60, no. 3, pp. 241–251, 2015.

[20] M.G. Başdoğan, "Geleneksel olarak üretilen turşulardan izole edilen laktik asit bakterilerinin probiyotik potansiyelinin belirlenmesi," Ege Üniversitesi, 2020.

[21] B. Şimşek and O. Sağdıç, "Isparta ve yöresinde üretilen dolaz (tort) peynirinin bazı kimyasal ve mikrobiyolojik özellikleri," Süleyman Demirel Üniversitesi Fen Bilim. Enstitüsü Derg. vol. 10, no. 3, pp. 346-351, 2006.

[22] P. Ertürkmen, Z. Öner, "Beyaz peynir örneklerinden izole edilen laktik asit bakterilerinin başlatıcı (starter) kültür özelliklerinin biyokimyasal yöntemlerle belirlenmesi," Süleyman Demirel Üniversitesi Fen Bilim. Enstitüsü Derg. vol. 19, no. 3, pp. 9-16, 2015.

[23] K. Halkman and Ö. Sağdaş, Mikrobiyoloji El Kitabı. 2014.

[24] M. A. Daeschel and H. P. Fleming, "Selection of lactic acid bacteria for use in vegetable fermentations," Food Microbiol., vol. 1, no. 4, pp. 303–313, 1984.

[25] G. Bustos, A. B. Moldes, J. M. Cruz, and J. M. Domínguez, "Influence of the metabolism pathway on lactic acid production from hemicellulosic trimming vine shoots hydrolyzates using Lactobacillus pentosus," Biotechnol. Prog., vol. 21, no. 3, pp. 793–798, 2005.

[26] L. Axelsson, "Lactic Acid Bacteria: Classification and Physiology. in: Salminen, S.von Wright, A. and Ouwehand A. (Eds.), Lactic Acid Bacteria: Microbiological and Functional Aspects". 3rd rev. and exp. ed. Marcel Dekker, Inc., New York, pp.1-66, 2004.

[27] B. Şimşek, "Koyun Sütünden İşlenen Beyaz Peynirlerde İnek Sütü Varlığını Saptama Düzeyinin Belirlenmesi," 2003.

[28] S. Bağder Elmacı, M. Tokatlı, D. Dursun, F. Özçelik, and P. Şanlıbaba, "Phenotypic and genotypic identification of lactic acid bacteria isolated from traditional pickles of the Çubuk region in Turkey," Folia Microbiol. (Praha)., vol. 60, no. 3, pp. 241–251, 2015.

[29] A. A. Boricha, S. L. Shekh, S. P. Pithva, P.

S. Ambalam, and B. R. Manuel Vyas, “In vitro evaluation of probiotic properties of Lactobacillus species of food and human origin,” LWT, vol. 106, pp. 201–208, 2019.

[30] M. C. Curk, J. C. Hubert, and F. Bringel, “Lactobacillus paraplantarum sp. nov., a new species related to Lactobacillus plantarum,” Int. J. Syst. Bacteriol., vol. 46, no. 2, pp. 595–598, 1996.

[31] D. Dursun, “Ankara çubuk yöresi turşularından izole edilen laktik asit bakterilerinin tanımlanması ve toplam hücre protein profillerinin belirlenmesi,” Ankara Üniversitesi, 2010.

[32] Y. Alan and M. Dığrak, “Doğal turşulardan izole edilen lactobacillus plantarum suşlarının İzolasyonu ve Tanımlanması,” KSÜ Doğa Bilim. Derg., vol. 15, no. 2, pp. 46–49, 2012.

[33] M. Tamminen et al., “Screening of lactic acid bacteria from fermented vegetables by carbohydrate profiling and PCR-ELISA,” Lett. Appl. Microbiol., vol. 39, no. 5, pp. 439–444, 2004.