GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2022) 47 (3) 387-398
doi: 10.15237/gida.GD22011

INVESTIGATION OF STARTER CULTURE PROPERTIES AND ANTIFUNGAL ACTIVITIES OF PICKLE-DERIVED LACTIC ACID BACTERIA

Haktan Aktaş*, Bülent Çetin
Department of Food Engineering, Ataturk University, Erzurum, Turkey

Received / Geliş: 04.01.2022; *Accepted* / Kabul: 23.03.2022; *Published online* / Online baskı: 01.04.2022

Aktaş, H., Çetin, B. (2022). Investigation of starter culture properties and antifungal activities of pickle-derived lactic acid bacteria. GIDA (2022) 47 (3) 387-398 doi: 10.15237/gida.GD22011

Aktaş, H., Çetin, B. (2022). Turşu kökenli laktik asit bakterilerinin starter kültür özellikleri ve antifungal aktivitelerinin incelenmesi. GIDA (2022) 47 (3) 387-398 doi: 10.15237/gida.GD22011

ABSTRACT
Fermented foods, which are widely consumed around the world, are of great importance for human health. Standard production should be carried out in order to extend the shelf life of fermented products and increase their positive effects on human health. Therefore, there is a need for starter cultures that can be used in the production of fermented products. In this study, antifungal activity on 9 yeast isolates, antibiotic resistance, growth at different temperatures, pH and salt concentrations, arginine hydrolysis and gas production from glucose of 5 lactic acid bacteria (*Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus paracasei, Pediococcus parvulus, Leuconostoc holzapfel*) were investigated. The lactic acid bacteria have antifungal effects on all yeasts except *Hanseniaspora opuntiae, Kazachstania exigua* and *Pichia fermentans*, and they grow at 10 and 25 °C, 3.9 and 5 pH and 4% salt concentration. In addition, all of the isolates showed resistance to vancomycin. The results indicated that *L. brevis, L. plantarum, L. paracasei* strains can be used as starter cultures in term of standart pickle production and antifungal effect.
Keywords: Lactic acid bacteria, antifungal activity, starter culture, pickles

TURŞU KÖKENLİ LAKTİK ASİT BAKTERİLERİNİN STARTER KÜLTÜR ÖZELLİKLERİ VE ANTİFUNGAL AKTİVİTELERİNİN İNCELENMESİ

ÖZ
Dünyada yaygın olarak tüketilen fermente gıdalar insan sağlığı için büyük öneme sahiptir. Fermente ürünlerin raf ömürlerinin uzatılması ve insan sağlığına olumlu etkilerinin artırılması için standart üretimlere ihtiyaç duyulmaktadır. Bu sebeple, fermente ürünlerin üretiminde kullanılabilecek starter kültürlere duyulan ihtiyaç giderek artmaktadır. Bu çalışmada, 5 laktik asit bakterisinin (*Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus paracasei, Pediococcus parvulus* ve *Leuconostoc holzapfel*) 9 maya izolatı üzerinde antifungal aktivitesi, antibiyotik dirençleri, farklı sıcaklık, pH ve tuz konsantrasyonlarında gelişim, arginin hidrolizi etme ve glukozdan gaz oluşturma yetenekleri incelenmiştir. Laktik asit bakterilerinin *Hanseniaspora opuntiae*, *Kazachstania exigua* ve *Pichia fermentans* hariç tüm mayalar üzerinde antifungal etkiye sahip olduğu, 10 ve 25 °C'de, 3.9 ve 5 pH'da ve %4 tuz konsatrasyonunda gelişim gösterdikleri tespit edilmiştir. Bunun yanında, izolatların tamamı vankomisine direnç göstermiştir. Çalışma sonuçları değerlendirildiğinde *L. brevis, L. plantarum, L. paracasei* suşlarının standart turşu üretimi ve antifungal etki açısından starter kültür olarak kullanılabileceği görülmektedir.
Anahtar kelimeler: Laktik asit bakterisi, antifungal aktivite, starter kültür, turşu

* *Corresponding author* / Yazışmalardan sorumlu yazar
: haktan.aktas@atauni.edu.tr : (+90) 442 231 2482 : (+90) 442 231 5878

Haktan Aktaş; ORCID no: 0000-0002-1067-061X
Bülent Çetin; ORCID no: 0000-0002-4679-2555

INTRODUCTION

Fermentation is one of the oldest food processing methods used to preserve foods and improve their flavor. With fermentation, new properties can be added to foods, such as increasing the nutritional value of foods, reducing toxic and spoilage factors, and improving shelf life. The production of traditional fermented foods is mostly uncontrolled and spontaneous. This event can cause quality losses in fermented foods from time to time (Misihairabgwi and Cheikhyoussef, 2017; Chang, 2018). Therefore, it is important to develop starter cultures that can be used in traditional production.

Fermented foods have important effects on human health, such as regulating and protecting the intestinal microbiota, preventing colon cancer, reducing or stabilizing the cholesterol level in the blood, increasing the absorption of minerals such as Fe, Mg and Ca, increasing the bioavailability of nutrients, and treating diarrhea cases (especially products containing probiotic microorganisms) (Demirgul and Sagdıc, 2018; Akdeniz Oktay and Özbaş, 2020).

The production of pickles, which is an important traditional fermented food, dates back to ancient times. Today, pickle production is carried out spontaneously both at home and industry. In particular, lactic acid bacteria (LAB) such as *L. plantarum*, *L. brevis*, *Leu. mesenteroides* and *P. pentocaceus* play a role in pickle fermentation by contributing to the taste, flavor and texture (Aktan et al., 2003). Pickles have properties such as anticarcinogenic (Lee et al., 2016), antiobesity (Kim et al., 2011), antioxidant (Lee et al., 2004), anti-aging (Kim et al., 2011) and antidiabetic (Islam and Choi, 2009; Kaur et al., 2017; Tang et al., 2019). Pickles have many bioactive functions, including preventing constipation, lowering serum cholesterol, enhancing immunity and fibrinolytic activity (Xia et al., 2017). In addition to the positive effects of pickles on health, LABs can inhibit some microorganisms by producing lactic acid, bacteriocin, ethanol, aroma compounds, exopolysaccharides, enzymes and other antagonistic substances (Cetin, 2011; Elmaci et al., 2015; Chen and Ying, 2017).

Yeasts are the oldest unicellular fungi used in food fermentation. While it is difficult to determine when they were first used, there is evidence that yeasts were used in the production of bread and fermented beverages in Neolithic Age in 7000 BC. Today, yeasts are used in the production of many foods, especially alcoholic beverages and bread (Hernández et al., 2018; Hittinger et al., 2018). Yeasts can also cause spoilage in foods with high acidity and/or sugar content. As a result of the spoilage, some properties and shelf life of the product are adversely affected. (Hernández et al., 2018). In addition to the fact that yeasts in fermented foods can cause spoilage, if there are opportunistic yeasts in the product and they are taken into the body through food, they can also cause negative effects on human health. Yeasts that may have negative effects on consumption quality or human health should be kept under control during production and storage (Karasu Yalçın et al., 2010). In order to ensure this control, it is necessary to pay attention to parameters such as raw material quality, hygiene during production, conditions of the storage environment. In addition, the most important method for controlling the yeast content of fermented foods is the use of starter cultures, which have the ability to inhibit yeasts that may occur in the product (Erdem Buyukkiraz et al., 2020). However, for microorganisms to be used as starter culture in fermented food production, it is not enough only to have an antifungal effect. Besides, they must have some properties. These features can be listed as ability to compete well with other microorganisms in the food matrix (antagonistic activity), lack of resistance to any antibiotic, rapid and good acid formation ability, development at different pH, salt and temperature degrees (may vary according to the product), not forming biogenic amines, being homofermentative etc. (Lee et al., 2015).

In this study, seven starter culture tests were performed on LABs isolated from pickles in previous studies. Thus, it is aimed to understand how effective the LABs isolated from the pickle can be on the fermentation and the quality of the product. For this purpose, the antifungal effect and its source on yeasts, antibiotic resistance,

growth at different temperatures, pH and salt values, ability to hydrolyze arginine and gas production from glucose of LABs were investigated.

MATERIAL AND METHOD

Material

Five LAB and 9 yeast strains which isolated from previous studies on pickle and stored in the Microbiology Laboratory of the Atatürk University Food Engineering Department were used in the study (Gözütok, 2013; Dündar, 2017; Çetin et al., 2017). The strains had been enumerated, isolated and identified according to Tournas et al. (1998), Speck (1984), Pichhardt (2004), Tamang et al. (2005), and Vasdinyei and Dea´k (2003). For this aim, MRS, M17 and PDA media were used for enumeration and isolation. Obtained isolates were genetically grouped by using $(GTG)_5$ and M13 RAPD PCR. Representive isolate of each group was identified with 16S and 26S rRNA sequencing analysis. The strains used in the study are shown in Table 1.

Method

Investigation of the source of the antifungal effect by LABs

Yeast isolates kept in 40% glycerol solution at –80 °C were revived on Sabouraud-2% Dextrose Agar and incubated at 25 °C for 3-5 days. At the end of the incubation, the yeast isolates were adjusted to a concentration of 10^4 CFU/mL in 0.85% physiological saline (FTS). For each yeast isolate, 0.1 mL of these suspensions was spread on Sabouraud-2% Dextrose Agar and 7 (5 LAB, 1 negative control and 1 positive control) wells were drilled into the solidified petri dishes (Magaldi et al., 2004).

LABs stored in 40% glycerol solution at –80 °C were revived on MRS (de man, Rogosa, Sharpe Agar) Agar and incubated at 30 °C for 2-3 days. Then, the colonies were inoculated into MRS broth and incubated at 30 °C for 24 h. At the end of this period, the growing LABs in MRS brohts were centrifuged at 10000 *g* for 5 min. Thus, cell-free supernatants (CFS) were obtained. Neutralization, enzyme application and heat treatment were performed on the CFS for determining the source of the antifungal effect. For the neutralization process, pH of the CFS was adjusted about 7±0.1 by 1 N NaOH in order to eliminate the probable inhibitory effect of acids produced by LABs on yeasts. Proteinase K, pepsin and trypsin enzymes were added into the CFS to investigate the effect of proteins by LABs. In addition, to determine the inhibitory properties of molecules (such as heat sensitive proteolytic enzymes and hydrogen peroxide), CFS were subjected to heat treatment at 80 °C for 10 min (Barbosa et al., 2016; Gutiérrez-Cortés et al., 2018; Khodaei and Soltani Nezhad, 2018).

100 µl of the CFS was added into the wells on Sabouraud-2% Dextrose Agar and incubated at 25 °C for 18 h. Sterile MRS broth was used as negative control and Amphotericin-B was used as positive control. At the end of the incubation, the antifungal effects of LABs on yeasts were determined by measuring the diameters of the clear zones formed around the wells (Balouiri et al., 2016). The Analysis was performed at two duplicates for two independent experiments.

Determination of antibiotic resistance of LABs

To determine the antibiotic resistance of the LABs, disc diffusion method was used. 9 different antibiotics (ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, and chloramphenicol) recommended by European Food Safety Authority (EFSA) were used. Fresh culture was suspended in FTS and adjusted at 0.5 McFarland ($1.5x10^8$ CFU/mL) by Biosan DEN-1 McFarland Densitometer. This suspension was spread homogeneously on MRS agar using a cotton swab. Then, the antibiotic discs were placed on the media. After the petri dishes were incubated at 30 °C for 24 h, the diameters of the clear zones around the discs were measured (EUCAST, 2021). This analysis was carried out duplicately.

Determination of growth abilities of LABs at different temperatures, pH and salt concentrations

In this study, the growth abilities of LABs at different temperatures (at 10, 25 and 45 °C for 7

days) were investigated. Readings were carried out by a plate reader (Epoch, BioTek) at 600 nm. The absorbance values obtained from LABs were determined by comparing with the data obtained from the negative control (sterile MRS broth). LABs with the same absorbance as the negative control were expressed as "–", and LABs with higher absorbance compared to the negative control were expressed as "+" (Şimşek et al., 2006).

In order to determine the growth abilities of LABs at different pH levels, the pH values of MRS broth were adjusted to 2.5, 3.9, 5.0, 8.5 and 9.6 with HCl or NaOH. At end of the incubation (at 30 °C for 48 h) in 96-well plates, readings were performed at 600 nm in a plate reader (Epoch, BioTek). The growth of LABs was investigated by comparing the absorbances obtained from the negative (pH-adjusted sterile MRS broth) and positive control (LAB isolate inoculated into not pH-adjusted MRS broth). As a result of this comparison, growing isolates were expressed as "+" and those not growing were expressed as "–" (Kask et al., 2003; Papamanoli et al., 2003; Terzic-Vidojevic et al., 2009).

To determine the growth abilities of LABs at different salt concentrations, MRS broth containing 4, 6.5 and 10% NaCl was used. The absorbance of the LABs incubated at 30 °C for 48 h in the media in 96-well plate was determined at 600 nm with a plate reader (Epoch, BioTek). The absorbance values were compared with negative (MRS broth containing 4, 6.5 or 10% NaCl) and positive control (LAB isolate inoculated into MRS broth without NaCl), and the LABs that showed growth were expressed as "+" and those not growing as "–" (Papamanoli et al., 2003).

Investigation of arginine hydrolysis of LABs

In order to determine the arginine hydrolysis of the LABs, modified MRS broth recommended by Drosinos et al. (2007) was used. LABs were inoculated into the modified MRS broth and incubated at 30 °C for 3 days. Then, Nessler reagent (Merck, Darmstadt, Germany) was used to determine the hydrolysis of arginine. The transformation of color to orange-brown has been accepted as positive.

Gas formation from glucose by LABs

LABs were inoculated into modified MRS broth recommended by Yildiz (2019) and incubated at 30 °C for 24 h. At the end of the incubation, the gas formation ability of the LABs from glucose was determined in the durham tubes. The gas-forming LABs were heterofermentative (creating CO_2 from glucose), and the non-gas-forming bacteria were homofermentative (not creating CO_2 from glucose) (Yildiz, 2019).

Statistical analysis

Statistical analyzes were performed by using SPSS version 17.0 package program (SPSS Inc., Chicago, IL, USA). The results were evaluated by using the Duncan multiple comparison test. Analyzes in the study were carried out as 2 duplications, and $P < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Antifungal effect of LABs on yeasts and determination of its source

The antifungal effects of the LABs on yeasts are given in Table 1. The LABs used in the study had antifungal effect on all yeasts, except *Hanseniaspora opuntiae*, *Kazachstania exigua*, *Pichia fermentans*. Some studies shown that *L. paracasei* strains isolated from fermented dairy products have an inhibitory effect on yeasts (Shehata et al., 2018). In this study, the isolate was effective on 6 of 9 yeast isolates. In addition, *L. paracasei* strains isolated from dairy products had antifungal effect on *Candida* spp., the same effect was found in the previous studies (Crowley et al., 2013; Ribes et al., 2018). Abouloifaa et al. (2021) investigated the antifungal activity of *L. plantarum, L. pentosus* and *L. brevis* species isolated from green olive brine, and reported the inhibitory effect of LABs on *Candida* sp. Similar result was found in this study, i.e. the inhibitory effect of *L. plantarum* and *L. brevis* on *Candida sorboxylosa*. Bulgasem et al. (2016) investigated the inhibitory effect of LABs (*L. plantarum*, *L. curvatus*, *P. acidilactici* and *P. pentosaceus*) isolated from honey on *Candida* spp. According to the results, as in the study, the inhibitory effect of *L. plantarum* isolate on *Candida* spp. was reported.

Table 1. The inhibition effect of LABs on the yeast isolates (inhibition zone, mm)

		Hanseniaspora opuntiae	*Candida sorboxylosa*	*Pichia kudriavzevii*	*Pichia kluyveri*	*Pichia kluyveri var. kluyveri*	*Kazachstania exigua*	*Pichia fermentans*	*Pichia barkeri*	*Pichia membranifaciens*
L. brevis	P	ND	14.0±2.8^{aAB}	13.0±2.8^{aABC}	9.5±0.7^{aC}	10.5±0.7^{aBC}	ND	ND	15.5±0.7^{aA}	11.0±2.8^{aBC}
	E	ND	12.5±3.5^{aAB}	12.0±0.0^{aAB}	15.0±2.8^{aA}	10.0±0.0^{aB}	ND	ND	14.5±2.1^{aA}	8.5±2.1^{aB}
	S	ND	14.0±1.4^{aA}	14.5±2.1^{aA}	13.0±1.4^{aA}	12.0±2.8^{aA}	ND	ND	15.0±0.0^{aA}	14.0±2.8^{aA}
L. plantarum	P	ND	10.5±3.5^{aAB}	12.5±3.5^{aAB}	12.5±3.5^{aAB}	12.5±0.7^{aAB}	ND	ND	15.5±0.7^{aA}	9.5±2.1^{aB}
	E	ND	11.0±2.8^{aAB}	11.0±0.0^{aAB}	13.5±0.7^{aA}	10.5±2.1^{aAB}	ND	ND	13.0±1.4^{aA}	9.0±1.4^{aB}
	S	ND	12.0±1.4^{aA}	10.0±0.0^{aA}	11.5±3.5^{aA}	10.5±3.5^{aA}	ND	ND	14.5±2.1^{aA}	10.0±0.0^{aA}
L. paracasei	P	ND	12.0±0.0^{aA}	13.5±0.7^{aA}	10.5±3.5^{aA}	12.5±2.1^{aA}	ND	ND	14.5±0.7^{aA}	10.0±4.2^{aA}
	E	ND	13.5±2.1^{aAB}	11.0±1.4^{aABC}	12.5±2.1^{aAB}	10.0±2.8^{aBC}	ND	ND	14.0±1.4^{aA}	8.5±0.7^{aC}
	S	ND	12.5±0.7^{aAB}	10.5±0.7^{aB}	12.0±4.2^{aAB}	10.5±0.7^{aB}	ND	ND	15.0±0.0^{aA}	10.5±0.7^{aB}
P. parvulus	P	ND	14.0±0.0^{aAB}	17.0±1.4^{aA}	8.0±0.0^{aC}	12.0±1.4^{aB}	ND	ND	16.5±0.7^{aA}	14.0±4.2^{aAB}
	E	ND	12.5±0.7^{bABC}	17.0±2.8^{aA}	8.0±0.0^{aC}	9.5±2.1^{aBC}	ND	ND	16.0±1.4^{aA}	14.5±6.4^{aAB}
	S	ND	13.0±0.0^{abBC}	13.0±0.0^{aBC}	10.0±2.8^{aC}	10.0±2.8^{aC}	ND	ND	17.0±0.0^{aA}	14.5±2.1^{aAB}
Leu. holzapfel	P	ND	10.0±0.0^{abB}	13.0±2.8^{aAB}	11.0±1.4^{aB}	10.5±2.1^{aB}	ND	ND	16.0±1.4^{aA}	11.5±2.1^{aB}
	E	ND	9.5±0.7^{bB}	12.0±1.4^{aAB}	9.5±2.1^{aB}	12.0±2.8^{aAB}	ND	ND	14.5±0.7^{aA}	9.5±2.1^{aB}
	S	ND	11.5±0.7^{aB}	12.0±1.4^{aAB}	11.5±0.7^{aB}	10.0±0.0^{aB}	ND	ND	14.0±1.4^{aA}	14.0±1.4^{aA}

The diameter of the wells used in the analysis was 6 mm; ND: not determined, P: pH neutralization, E: Enzyme application, S: Heat treatment

Different letters in the same column (series "a and b") and in the same row (series "A-D") indicate significant differences ($P < 0.05$).

The source of the antifungal effect of LABs was investigated. Neutralization, enzyme application and heat treatment were applied on the CFSs. It was determined that the antifungal effect of any isolate did not change statistically with these applications ($P > 0.05$), except for the antifungal effect of *P. parvulus* and *Leu. holzapfel* on *Candida sorboxylosa* ($P < 0.05$). For *P. parvulus*, the neutralized CFSs showed the highest antifungal activity, while the enzyme-applicated CFSs showed the lowest effect ($P < 0.05$). Besides, for CFS of *Leu. holzapfel,* heat treatment had the highest antifungal activity, while enzyme application had lowest ($P < 0.05$). According to these results, the proteins produced by LABs have a significant effect on the growth of yeasts. In this study, proteinase K, pepsin and trypsin enzymes were used to reduce or eliminate the effect of these proteins. It was observed that antifungal effect of the LABs on yeasts decreased significantly with the addition of these enzymes ($P < 0.05$). In addition, these proteins, which have antifungal effect, may differ in their resistance to temperature and pH changes due to their configurational differences. Therefore, neutralized and heat-treated CFS of *P. parvulus* and *Leu. holzapfel* may have exhibited different antifungal effects among themselves. In the study by Barbosa et al. (2016), the sensitivity of compounds produced by LABs to enzyme and pH applications were investigated. According to the results, the antimicrobial effect of these compounds decreased when exposed to enzymes such as proteinase K, trypsin and pepsin, but they remained stable at pH 6. Gutiérrez-Cortés et al. (2018) tested whether the activities of antimicrobial substances from *Pediococcus* sp. decreased with neutralization, enzyme application and heat treatment. The results showed that the application of proteinase K and pepsin enzymes reduced the antifungal effect by 53-95%, pH 6 by 0-15%, and heat treatment at 80 °C by 21-32%. The results obtained from this study are in accordance with the above study.

The zone was not observed in the negative controls. The sensitivity of *Hanseniaspora opuntiae, Candida sorboxylosa*, *Pichia kudriavzevii, Pichia kluyveri, Pichia kluyveri var. kluyveri*, *Kazachstania exigua, Pichia fermentans, Pichia barkeri* and *Pichia membranifaciens* to amphotericin-B was determined as 21.0, 23.0, 17.5, 21.5, 16.5, 15.0, 18.0, 16.0 and 20.5 mm, respectively.

Determination of antibiotic resistance of LABs

The resistance of the LABs to antibiotics (ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline and chloramphenicol) was investigated (Table 2). All of the LABs were found to be sensitive to all antibiotics, except vancomycin for all LABs, gentamicin for *L. paracasei*, kanamycin and streptomycin for *P. parvulus*. It is known that LABs are naturally resistant to some antibiotics (Meral and Korukluoglu, 2014). The resistance of the above-mentioned LABs to antibiotics vancomycin, gentamicin, kanamycin and streptomycin may be due to natural resistance.

Table 2. Resistance of LABs against antibiotics (inhibition zone, mm)

	Antibiotics								
	AMP	VA	CN	K	S	E	DA	TE	C
L. brevis	28.0±0.0	ND	18.5±0.5	16.5±0.5	19.0±0.0	38.0±0.0	16.5±0.5	26.5±0.5	27.0±1.0
L. plantarum	41.5±0.5	ND	24.0±0.0	21.0±1.0	21.0±1.0	41.0±1.0	37.0±1.0	39.0±1.0	51.0±1.0
L. paracasei	32.0±0.0	ND	ND	15.0±1.0	23.0±1.0	45.0±1.0	47.0±1.0	33.0±1.0	34.5±0.5
P. parvulus	40.0±1.0	ND	21.0±1.0	ND	ND	37.0±1.0	42.0±0.0	33.0±1.0	35.0±1.0
Leu.holzapfel	27.5±0.5	ND	13.5±0.5	10.0±0.0	11.0±0.0	27.5±0.5	36.0±0.0	33.5±0.5	27.5±0.5

Abbreviations: AMP: ampicillin (2 μg), VA: vancomycin (5 μg), CN: gentamicin (10 μg), K: kanamycin (30 μg), S: streptomycin (25 μg), E: erythromycin (15 μg), DA: clindamycin (2 μg), TE: tetracycline (10 μg), C: chloramphenicol (10 μg), ND: not determined

In the study by Zeng et al. (2020), the antibiotic resistance of *L. plantarum* isolated from homemade Chinese pickles was investigated. As a result of the study, *L. plantarum* isolates were sensitive to all antibiotics, except vancomycin. Similar results were reported by Guo et al. (2017). The obtained results in this study are in accordance with the above studies.

In the study conducted by Wu et al. (2021), antibiotic resistance analysis was applied to 17 LABs isolated from local pickled leaf mustard from Wuwei. The LABs were sensitive to chloramphenicol, tetracycline and erythromycin, while they were resistant to streptomycin, kanamycin, gentamicin, and vancomycin. The obtained results in this study are consistent with the study.

Determination of growth abilities of LABs at different temperatures, pH and salt concentrations

The growth abilities of 5 pickle-derived LABs at different temperatures, pH and salt concentrations were investigated. The results obtained from these analyzes are given in Table 3. The LABs grew at 10 °C, except for *L. plantarum* and *Leu. holzapfel* (weak growth). On the other hand, all isolates grew at 25 °C. The growth abilities of the bacteria at 45 °C were weak, except for *L. plantarum* (no growth). In the studies of Yildiz (2011) and Bayrak (2019), LABs isolated from pickles can grow at moderate temperatures (10 and 15 °C). In addition, their development was weak at 45 °C. In this respect, the results obtained from the study are in agreement with these literatures. In addition, since pickle fermentation is carried out at room temperatures, it is an expected result that all strains will develop at 25 ˚C (Diker et al., 2021; Gezginc and Inanc, 2021). In the study of Basdogan (2020), all of 114 LABs isolated from pickles showed growth at 25 ˚C.

The growth abilities of the LABs at different pH values (2.5, 3.9, 5.0, 8.5 and 9.6) were investigated. None of the isolates could grow at pH values of 2.5 and 9.6. All of the LABs grew at pH 3.9 and 5.0. On the contrary, *L. plantarum*, *L. paracasei* and *Leu. holzapfel* showed better growth at pH 8.5,

while *L. brevis* and *P. parvulus* failed to grow. In the study conducted by Saez et al. (2018), the growth abilities of *L. plantarum* isolated from pickle samples at different pH were investigated. This isolate showed growth at pH 4 and 5. Tokatli (2013) determined that pickle-derived *L. plantarum* isolates showed good resistance to high pH, while *P. parvulus* and *L. brevis* strains were found to be more sensitive to that. The results obtained from this study are in accordance with the above studies.

Table 3. Growth abilities of LABs at different temperatures, pH and salt concentrations

	Temperature (°C)			pH				Salt concentration (%)		
	10	25	45	2.5	3.9	5.0	8.5	9.6	4	6.5	10
L. brevis	++	+++	+	–	++	++	–	–	++	+	–
L. plantarum	+	+++	–	–	++	+++	+++	–	+++	+++	–
L. paracasei	++	+++	+	–	++	++	++	–	+++	++	–
P. parvulus	++	+++	+	–	+	++	–	–	++	–	–
Leu. holzapfel	+	+++	+	–	+	+++	+++	–	++	–	–

–: No growth; +: absorbance close to negative control, weak growth; ++: good growth; +++: absorbance close to positive control, very good growth

The growth abilities of the LABs at 4, 6.5 and 10% salt concentrations were investigated. None of the isolates could grow at 10% salt concentration. On the other hand, *L. plantarum* and *L. paracasei* showed better growth at 4% salt concentration than *L. brevis, P. parvulus* and *Leu. holzapfel*. In addition, *L. brevis*, *L. plantarum* and *L. paracasei* showed growth at 6.5% salt concentration while *P. parvulus* and *Leu. holzapfel* did not (Table 3). It is known that LABs isolated from pickles show a decrease in their growth abilities with the increase of salt content in the medium. It is also available in the literature that these isolates can tolerate 4% salt concentration (Tokatli, 2013; Saez et al., 2018). As above-mentioned, in this study, the growth decreased with the increasing salt content in the medium and all of the isolates tolerated 4% salt concentration. Considering the ability of the LABs to grow at different salt concentrations, growth performance of *L. plantarum* in medium containing 6.5% salt draws attention. In the study conducted by Karasu et al. (2010), 11 of 12 pickle-derived *L. plantarum* isolates could tolerate 8% salt content.

Investigation of arginine hydrolysis of LABs

The ability of the LABs used in the study to form ammonia from arginine was investigated (Table 4). According to the results obtained, none of the LABs hydrolyzed to arginine. Arginine hydrolysis is an important criteria for the selection of starter cultures. Because, ammonia from arginine has toxic effect and plays an important role in the formation of biogenic amines (Özogul and Özogul 2007). In the study by Tokatli (2013), 87 of 142 LABs isolated from pickle samples did not hydrolyze arginine. Sanchez et al. (2000) reported that 96 of 149 LABs isolated from Almagro eggplants, a fermented vegetable, did not hydrolyze arginine. According to the studies above-mentioned, the majority of LABs do not hydrolyze arginine. Therefore, the data obtained from our study are similar to these studies, since none of the 5 LABs obtained from pickles, which constitute the dominant flora, did not hydrolyze arginine.

Table 4. Ability of forming gas from glucose and arginine hydrolysis of LABs

	L. brevis	*L. plantarum*	*L. paracasei*	*P. parvulus*	*Leu. holzapfel*
Arginine hydrolysis	–	–	–	–	–
Gas from glucose	+	–	–	–	+

Gas formation from glucose by LABs

The results of the gas formation test from glucose of the LABs are given in Table 4. According to the results, *L. brevis* and *Leu. holzapfel* formed gas from glucose. These isolates were evaluated as heterofermentative. It is known that *Leuconostoc* spp. originating from pickles is heterofermentative (Dallal et al., 2017). In addition, in the study conducted by Tokatli (2013), the technological properties of LABs isolated from pickle samples were investigated. He reported that most of *L. brevis* strains producted gas from glucose, that is, they were heterofermentative. The results obtained from this study are in accordance with the above study.

CONCLUSION

Fermentation is a widely used method for food preservation. In addition to increasing the shelf life of foods with fermentation, it is possible to produce more desired products by the consumer and to take compounds that have positive effects on human and public health. However, the spontaneous production of pickles, which is an important fermented product, may cause the standard product not to be obtained in every production. In order to obtain standard fermented products, the use of starter cultures should be encouraged and the use of microorganisms, which are safety and healthy, should be increased in traditional productions. In order for a microorganism to be used as a starter culture, it should show good growth in fermentation conditions and product matrix, should not have negative effects on health, should not cause undesirable changes in the product, and should compete with spoiling microorganisms. All LABs used in the study were found to have antifungal effects on all yeasts, except for *Hanseniaspora opuntiae*, *Kazachstania exigua*, *Pichia fermentans*, and these isolates developed at 25 °C, which is the pickle fermentation temperature. On the other hand, *L. brevis*, *L. plantarum* and *L. paracasei* showed growth at pH 3.9 and 6.5% salt concentration. Considering the pickle environment, it is thought that these isolates can be used as starter cultures in pickle production.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed significantly to different processes in the article.

REFERENCES

Abouloifaa, H., Gaamouchea, S., Roknia, Y., Hasnaouia, I., Bellaouchia, R., Ghabboura, N., Karbouneb, S., Brascac, M., D'Hallewind, G., Ben Salahe, R., Saalaouia, E., Asehraoua, A. (2021). Antifungal activity of probiotic *Lactobacillus strains* isolated from natural fermented green olives and their application as food bio-preservative. *Biological Control*, 152, 104450. https://doi.org/10.1016/j.biocontrol.2020.104450.

Akdeniz Oktay, B., Özbaş, Z.Y. (2020). The effects of fermented foods on human health. *The Journal of Food*, 45(6), 1215-1226. https://doi.org/10.15237/gida.GD20105.

Aktan, N., Yücel, U., Kalkan, H. (2003). *Pickle Technology*. Ege University Printing Office, İzmir, Turkey, 138p.

Balouiri, M., Sadiki, M., iIbnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005.

Barbosa, M.S., Todorov, S.D., Ivanova, I.V., Belguesmia, Y., Choiset, Y., Rabesona, H., Chobert, J.M., Haertlé, T., Franco, B.D.G.M. (2016). Characterization of a two-peptide plantaricin produced by *Lactobacillus plantarum* MBSa4 isolated from Brazilian salami. *Food Control*, 60, 103-112. https://doi.org/10.1016/j.foodcont.2015.07.029

Basdogan, M.G. (2020). Determination of probiotic potential of lactic acid bacteria isolated from pickles produced traditionally. MSc Thesis, Ege University, Graduate School of Natural and Applied Sciences, İzmir, Turkey, 98p.

Bayrak, N. (2019). Investigation of some probiotic properties of lactic acid bacteria obtained from pickle. MSc Thesis, Atatürk University, Graduate School of Natural and Applied Sciences, Erzurum, Turkey, 73p.

Bulgasem, Y.B. Mohd, N.L., Zaiton, H., Wan Mohtar, W.Y., Sumaya, G.F. (2016). Antifungal

activity of lactic acid bacteria strains isolated from natural honey against pathogenic candida species. *Mycobiology*, 44(4), 302-309. https://doi.org/10.5941/MYCO.2016.44.4.302.

Cetin, B. (2011). Production of probiotic mixed pickles (tursu) and microbiological properties. *African Journal of Biotechnology*, 10(66), 14926-14931. https://doi.org/10.5897/AJB11.2621.

Chang, H.C. (2018). Healthy and safe Korean traditional fermented foods: Kimchi and chongkukjang. *Journal of Ethnic Foods*, 5(3), 161-166. https://doi.org/10.1016/j.jef.2018.08.003.

Chen, Y., Ying, T.J. (2017). Isolation and identification of lactic acid bacteria from xiaoshan pickle radish, a traditional fermented vegetable. *Food Science and Technology Research*, 23(1), 129-136. https://doi.org/10.3136/fstr.23.129.

Crowley, S., Mahony, J., Sinderen, D. (2013). Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. *Trends in Food Science & Technology*, 33(2), 93-109. https://doi.org/10.1016/j.tifs.2013.07.004.

Çetin, B., Çakmakçı, S., Yıldız, H. (2017). Determination of lactic acid bacteria to be used in Turşu production and selection of suitable strains for the production of probiotic pickle (*in Turkish*). TUBITAK Project Number: 214O719, The Scientific and Technological Research Council of Turkey (TUBITAK), Tunus Street No:80 Ankara, TURKEY.

Dallal, M.M.S., Zamaniahari, S., Davoodabadi, A., Hosseini, M., Rajabi, Z. (2017). Identification and characterization of probiotic lactic acid bacteria isolated from traditional persian pickled vegetables. *GMS Hygiene and Infection Control*, 12, Article Number: Doc15. https://doi.org//10.3205/dgkh000300.

Demirgul, F., Sagdıc, O. (2018). The effect of fermented milk products on human health. *European Journal of Science and Technology*, 13, 45-53. https://doi.org/10.31590/ejosat.377798.

Diker, A., Akar, E., Akgun, R., Tarhan, Ö. (2021). Investigation of some quality parameters in cucumber pickles prepared by different types of vinegars. *Journal of Food and Feed Scence – Technology*, 26, 30-39.

Drosinos, E.H., Paramithiotis, S., Kolovos, G., Tsikouras, I., Metaxopoulos, I. (2007). Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greeece. *Food Microbiology*, 24, 260-270. https://doi.org/10.1016/j.fm.2006.05.001.

Dündar, R. (2017). Isolation and identification of lactic acid bacteria from traditionally produced home-type pickle. MSc Thesis, Atatürk University, Graduate School of Natural and Applied Sciences, Erzurum, Turkey, 65 p.

Elmaci, S.B., Tokatli, M., Dursun, D., Ozcelik, F., Sanlibaba, P. (2015). Phenotypic and genotypic identification of lactic acid bacteria isolated from traditional pickles of the Cubuk region in Turkey. *Folia Microbiologica*, 60(3), 241-251. https://doi.org/10.1007/s12223-014-0363-x.

Erdem Buyukkiraz, M., Avcı, E., Kahraman, N., Kesmen, Z. (2020). Identification of spoilage yeasts isolated from some foods. *Çukurova Journal of Agricultural and Food Science,* 35(1), 15-28. https://doi.org/10.36846/CJAFS.2020.14.

EUCAST, 2021. European committee on antimicrobial susceptibility testing, Disk diffusion method for antimicrobial susceptibility testing, Version 9.0. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2022_manuals/Manual_v_10.0_EUCAST_Disk_Test_2022.pdf (Accessed: 1 January, 2022).

Gezginc, Y., Inanc, O. (2021). Chemical, microbiological and sensory properties of acur (cucumis melo var. flexuosus) pickles produced using salt and vinegar at different concentrations. *International Journal of Innovative Approaches in Agricultural Research*, 5(3), 290-302. https://doi.org/10.29329/ijiaar.2021.378.4.

Gözütok, E. (2013). Determination of yeast microflora in traditional and industrial produced turşu samples. MSc Thesis, Atatürk University, Graduate School of Natural and Applied Sciences, Erzurum, Turkey, 64 p.

Guo, H., Pan, L., Li, L., Lu, J., Kwok, L., Menghe, B., Zhang, H., Zhang, W. (2017). Characterization of antibiotic resistance genes from *lactobacillus* isolated from traditional dairy products. *Journal of Food Science*, 82(3), 724-730. https://doi.org/10.1111/1750-3841.13645.

Gutiérrez-Cortés, C., Suarez, H., Buitrago, G., Nero, L.A., Todorov, S.D. (2018). Characterization of bacteriocins produced by strains of *Pediococcus pentosaceus* isolated from Minas cheese. *Annals of Microbiology*, 68(6), 383-398. https://doi.org/10.1007/s13213-018-1345-z.

Hernández, A., Pérez-Nevado, F., Ruiz-Moyano, S., Serradilla, M.J., Villalobos, M.C., Martín, A., Córdoba, M.G. (2018). Spoilage yeasts: What are the sources of contamination of foods and beverages? *International Journal of Food Microbiology*, 286, 98-110. https://doi.org/10.1016/j.ijfoodmicro.2018.07.031.

Hittinger, C.T., Steele, J.L., Ryder, D.S. (2018). Diverse yeasts for diverse fermented beverages and foods. *Current Opinion in Biotechnology*, 49, 199-206. https://doi.org/10.1016/j.copbio.2017.10.004.

Islam, M.S., Choi, H. (2009). Antidiabetic effect of korean traditional Baechu (Chinese cabbage) kimchi in a type 2 diabetes model of rats. *Journal of Medicinal Food*, 12, 292–297. https://doi.org/10.1089/jmf.2008.0181.

Karasu, N., Şimşek, Ö., Çon, AH. (2010). Technological and probiotic characteristics of Lactobacillus plantarum strains isolated from traditionally produced fermented vegetables. *Annals of Microbiology*, 60, 227–234. https://doi.org/10.1007/s13213-010-0031-6.

Karasu Yalçın, S., Şenses Ergül, Ş., Özbaş, Y. (2010). Use of molecular based methods for identification of foodborne yeasts. *The Journal of Food,* 35(5), 379-386.

Kask, S., Adamberk, K., Orlowski, A., Vogensen, F.K., Møller, P.L., Ardo, Y., Paalme, T. (2003). Physiological properties of *Lactobacillus paracasei*, *L. danicus* and *L. curvatus* strains isolated from Estonian semi-hard cheese. *Food Research International,* 36, 1037-1046. https://doi.org/10.1016/j.foodres.2003.08.002.

Kaur, M., Singh, H., Jangra, M., Kaur, L., Jaswal, P., Dureja, C., Pinnaka, A.K. (2017). Lactic acid bacteria isolated from yak milk show probiotic potential. *Applied Microbiology and Biotechnology*, 101(20), 7635-7652. https://doi.org/10.1007/s00253-017-8473-4.

Khodaei, M., Soltani Nezhad, S. (2018). Isolation and molecular identification of bacteriocin producing enterococci with broad antibacterial activity from traditional dairy products in Kerman province of Iran. *Korean Journal for Food Science of Animal Resources*, 38(1), 172-179. https://doi.org/10.5851/kosfa.2018.38.1.172.

Kim, B., Park, K.Y., Kim, H.Y., Ahn, S.C., Cho, E.J. (2011). Antiaging effects and mechanisms of kimchi during fermentation under stress-induced premature senescence cellular system. *Food Science and Biotechnology*, 20, 643–649. https://doi.org/10.1007/s10068-011-0091-9.

Kim, E.K., An, S.Y., Lee, M.S., Kim, T.H., Lee, H.K., Hwang, W.S., Lee, K.W. (2011). Fermented kimchi reduces body weight and improves metabolic parameters in overweight and obese patients. *Nutrition Research*, 31, 436–443. https://doi.org/ 10.1016/j.nutres.2011.05.011.

Lee, H.A., Kim, H., Lee, K.W., Park, K.Y. (2016). Dietary nanosized *Lactobacillus plantarum* enhances the anticancer effect of kimchi on azoxymethane and dextran sulfate sodium-induced colon cancer in C57BL/6J mice. *Journal of Environmental Pathology, Toxicology and Oncology*, 35(2), 147–159. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2016015633.

Lee, M.E., Jang, J.Y., Lee, J.H., Park, H.W., Choi, H.J., Kim, T.W. (2015). Starter cultures for kimchi fermentation. *Journal of Microbiology and Biotechnology*, 25(5), 559-568. https://doi.org/10.4014/jmb.1501.01019.

Lee, Y.M., Kwan, M.J., Kim, J.K., Suh, H.S., Choi, J.S., Song, Y.O. (2004). Isolation and identification of active principle in Chinese cabbage kimchi responsible for antioxidant effect. *Korean Journal of Food Science and Technology*, 36, 129–133.

Magaldi, S., Mata-Essayag, S., de Caprilesa, C.H., Perez, C., Colella, M.T., Olaizola, C., Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8(1), 39-45. https://doi.org/10.1016/j.ijid.2003.03.002.

Meral, H., Korukluoglu, M. (2014). Antibiotic resistance mechanisms of lactic acid bacteria. *Journal of Agricultural Faculty of Uludag University*, 28(2), 71-82.

Misihairabgwi, J., Cheikhyoussef, A. (2017). Traditional fermented foods and beverages of Namibia. *Journal of Ethnic Foods*, 4(3), 145-153. https://doi.org/10.1016/j.jef.2017.08.001.

Özogul, F., Özogul, Y. (2007). The ability of biogenic amines and ammonia production by single bacterial cultures. *European Food Research and Technology*, 225(3-4), 385-394. https://doi.org/10.1007/s00217-006-0429-3.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meet Science*, 65, 859-867. https://doi.org/10.1016/S0309-1740(02)00292-9.

Pichhardt, K. (2004). *Food Microbiology: Basic Principles and Applications for the Food Industry)* (Gıda Mikrobiyolojisi: Gıda Endüstrisi için Temel Esaslar ve Uygulamalar, Translated by Sekin, Y., Karagözlü, N.), Literatür Publishing, İstanbul, Turkey, 358p.

Ribes, S., Fuentes, A., Talens, P., Barat, J.M. (2018). Prevention of fungal spoilage in food products using natural compounds: A review. *Critical Reviews in Food Science and Nutrition*, 58(12), 2002-2016. https://doi.org/10.1080/10408398.2017.1295017.

Saez, G.D., Flomenbaum, L., Zarate, G. (2018). Lactic acid bacteria from Argentinean fermented foods: isolation and characterization for their potential use as starters for fermentation of vegetables. *Food Technology and Biotechnology*, 56(3), 398-410.
https://doi.org/10.17113/ftb.56.03.18.5631.

Sanchez, I., Palop, L., Ballesteros, C. (2000). Biochemical characterization of lactic acid bacteria isolated fromspontaneous fermentation of 'Almagro' eggplants. *International Journal of Food Microbiology*, 59, 9-17. https://doi.org/10.1016/S0168-1605(00)00256-7.

Shehata, M.G., Badr, A.N., El Sohaimy, S.A. (2018). Novel antifungal bacteriocin from *Lactobacillus paracasei* KC39 with anti-mycotoxigenic properties. *Bioscience Research,* 15(4), 4171-4183.

Speck, M.L. (1984). *Compendium of Methods for The Microbiological Examination of Foods*. American Puplic Healt Association, Washington, DC, USA, 914p.

Şimşek, Ö., Çon, A.H., Tulumoğlu, Ş. (2006). Isolating lactic starter cultures with antimicrobial activity for sourdough processes. *Food Control*, 17, 263-270. https://doi.org/10.1016/j.foodcont.2004.10.011.

Tamang, J.P., Tamang, B., Schillinger, U., Franz, C.M.A.P., Gores, M., Holzapfel, W.H. (2005). Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. *International Journal of Food Microbiology*, 105, 347-356. https://doi.org/10.1016/j.ijfoodmicro.2005.04.024.

Tang, Y.Y., Zhou, X.R., Huang, S.L., Li, Y.Z., Long, M., Zhao, X., Du, M.Y. (2019). Microbial community analysis of different qualities of pickled radishes by Illumina MiSeq sequencing. *Journal of Food Safety*, 39(2), 1-8. https://doi.org/10.1111/jfs.12596.

Terzic-Vidojevic, A., Tolinacki, M., Nikolic, M., Lozo, J., Begovic, J., Gurban oglu Gulahmadov, S., Kuliev, A.A., Dalgalarrondo, M., Chobert, J.M., Haertlé, T., Topisirovic, L. (2009). Phenotypic and genotypic characterization of lactic acid bacteria isolated from Azerbaijani traditional dairy products. *African Journal of Biotechnology*, 8, 2576-2588.

Tokatli, M. (2013). Identification of lactic acid bacteria isolated from pickles in Ankara Çubuk region, determination of their technological and functional properties and their potential for using

as a starter culture. Ph.D. Dissertation, Ankara University, Graduate School of Natural and Applied Sciences, Ankara, Turkey, 183p.

Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., Bandler, L. (1998). Yeasts, molds and mycotoxins. In: *Bacteriological Analytical Manual*, Food and Drug Administration, Gaithersburg, MD, USA, pp.227-234.

Vasdinyei, R., Dea´k, T. (2003). Characterization of yeast isolates originating from Hungarian dairy products using traditional and molecular identification techniques. *International Journal of Food Microbiology*, 86(1-2), 123-130. https://doi.org/10.1016/S0168-1605(03)00251-4.

Wu, C.J., Lin, X.P., Tong, L., Dai, C.W., Lv, H., Zhou, X.H., Zhang, J. (2021). *In vitro* evaluation of lactic acid bacteria with probiotic activity isolated from local pickled leaf mustard from Wuwei in Anhui as substitutes for chemical synthetic additives. *Open Chemistry*, 19(1), 755-771. https://doi.org/10.1515/chem-2021-0054.

Xia, Y., Liu, X., Wang, G., Zhang, H., Xiong, Z., Sun, Y., Ai, L. (2017). Characterization and selection of *Lactobacillus brevis* starter for nitrite degradation of Chinese pickle. *Food Control*, 78, 126-131. https://doi.org/10.1016/j.foodcont.2017.02.046.

Yildiz, H. (2011). Isolation-identification of lactic acid bacteria and yeasts from pickle and olive and determination of some properties of isolates. Ph.D. Dissertation, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Turkey, 134p.

Yildiz, S. (2019). Investigation of starter culture and exopolysaccharide production characteristics of yoghurt derived lactic acid bacteria. MSc Thesis, Atatürk University, Graduate School of Naturel and Applied Sciences, Erzurum, Turkey, 70p.

Zeng, Y., Li, Y., Wu, Q.P., Zhang, J.M., Xie, X.Q., Ding, Y., Cai, S.Z., Ye, Q.H., Chen, M.T., Xue, L., Wu, S., Zeng, H.Y., Yang, X.J., Wang, J. (2020). Evaluation of the antibacterial activity and probiotic potential of lactobacillus plantarum isolated from chinese homemade pickles. *Canadian Journal of Infectious Diseases & Medical Microbiology*, Article ID 8818989. https://doi.org/10.1155/2020/8818989.