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Isolation, Identification, and Biochemical Characterization of Lactic Acid Bacteria from Okara

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Abstract
During the process of making soymilk, soybean pulp (okara) is produced as a solid waste product. However, this waste product is rich in nutrient contents such as essential fatty acids, proteins, carbohydrates, vitamins, and minerals. Hence okara is favorable for the growth of microorganisms such as lactic acid bacteria (LAB). The purpose of this study was to isolate, identify and characterize LAB from fermented okara. Isolation of LAB was carried out on MRS agar media supplemented with $CaCO_3$. LAB was selectively screened based on their morphological, biochemical, and physiological characterizations. A total of 133 different colonies were selected from fermented okara, of which 20 colonies were confirmed as LAB. The entire LAB isolates were catalase negative, indicating that all the identified isolates did not produce the enzyme catalase. Out of 20 LAB, 14 isolates were whitish and six isolates were found to be creamy in colony appearance. The entire LAB isolates were circular in colony shape and found to be rod in cell shape. Based on the cell shape of LAB; isolates were belongs to the genera Lactobacillus. Out of 20 isolates; SL-3, SL-7, SL-10, SL-14, and SL-19 were hetero-fermentative; and the remaining fifteen (15) isolates were found to be homo fermentative. Further studies on the properties of LAB isolate was conducted through a growth test at different temperature (15^0C, 37^0C, and 45^0C) and in different NaCl concentrations. The result of the present study will contribute to the future applications and utilization of LAB as starter cultures for the production of soybean yoghurt and its use in food preservation.
Keywords: Lactic acid bacteria, Soybean, Okara
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Introduction

Soymilk is made by grinding soybeans that have been soaked in water, filtered, cooked until boiling, and then supplemented with sugar essence to enhance the flavor. During the process of making soy milk, soybean pulp or okara is produced as solid waste products and it is dumped into the environment causing pollution (Suruga *et al.*, 2011). However, it is rich in nutrient content such as essential fatty acids, proteins, carbohydrates, vitamins, and minerals (Hsieh and Yang, 2003). Hence okara is favorable for the growth of microorganisms such as lactic acid bacteria (Kitawaki *et al*., 2009).

Lactic acid bacteria (LAB) are Gram positive, catalase negative, non-spore former, usually non motile, nonacid fast, non-respiring rods or coccobacilli with frequently in single, pairs, or chains. They grow well under anaerobic conditions but may grow in micro aerophilic as well as aerobic conditions. They exhibit optimum growth at slightly lower acidic conditions (pH 5.5 – 6.0) while growth is often restricted at neutral or somewhat alkaline conditions (pH above 7.0 to 7.5). They are strictly fermentative, with lactic acid as the major end product during sugar fermentation (Schleifer and Ludwig, 1995). LAB can be classified based on their morphology (cocci or rods), mode of glucose fermentation, and growth at different temperatures and salt concentrations (Axelsson, 2004). They have two different metabolic pathways for hexose fermentation. In the homo fermentative pathway, lactic acid (more than 85%) is the major end product whereas, in the hetero fermentative pathway lactic acid, ethanol/acetone, and CO_2 are the terminal products (Schleifer and Ludwig, 1995).

Lactic acid bacteria are among the most important groups of microorganisms and are used as a starter cultures for the processing of functional food, probiotic effects, and food preservation (Frick *et al*., 2007). Lactic acid bacteria are also used for the production of dairy, meat, and vegetables fermented products (De Vuyst and Leroy, 2007) and are generally recognized as safe (GRAS) (Avail-Jaaskelainen and Palva, 2005). In addition to their fermentation properties, a large number of LAB strains exhibit very good antifungal and antibacterial activities. Therefore, they are used as bio preservatives (Lavermicocca, 2000). A lactic acid bacterium (LAB) is not toxic to the host but can kill pathogenic bacteria (Akbar *et al.*, 2016). *Lactobacillus* is one of the largest genera in this category with almost 80 species that are widely used in different food products including beer, wine, juices, cheese, yoghurt, and sausage (Prescott *et al.*, 2002). *Lactobacillus casei* inhibits the growth of *A. parasiticus* and its aflatoxin production (El-Gendy, 1981). *Lactobacillus fermentum* has strong antifungal properties against candida albicans and candida glabrata (Masood *et al.*, 2011). Lactic acid bacteria (LAB) can inhibit the growth of spoilage and pathogenic microbes by producing growth inhibiting substances such as

primary (lactic acid) and secondary metabolites such as bacterocin (Mazzoli *et al.*, 2014). Bacteriocin act as an antimicrobial compound that is effective, safe, and able to inhibit the growth of pathogenic bacteria compared to other antimicrobial compounds (Marshall and Arenas, 2003) due to the presence of bacteriocin increased the microbial activity of LAB against pathogenic bacteria.

The antimicrobial activity of LAB against spoilage pathogenic bacteria isolated from the small – scale meat facility, dairy products, and cassava processing of waste water; increase the shelf life of products without affecting the organoleptic qualities (Arques *et al.*, 2015; Siroli *et al*., 2015). LAB produces antimicrobial compounds that can increase the shelf of the food and can inhibit the growth of undesirable microorganisms and are safe for humans to consumption (Fontana *et al.*, 2013). Bacterial food poisoning is commonly caused by bacterial pathogenic species. In general, bacteria can contaminate different foods depending on the physical-chemical preservation profile (Gram *et al*., 2002). So, LAB can control the quality of different foods by killing or inhibiting the growth of pathogenic microorganisms. The main objective of this research work is to isolate, identify and characterize LAB from okara.

Materials and methods

Description of the study site and Experimental designs

The study was conducted at Melkassa Agricultural Research and Holeta Agricultural Research centers under laboratory conditions in complete randomized design (CRD).

Sample collection and processing

Soybean samples used in this study were collected from the local market using an aseptic plastic bag. The collected samples were cleaned manually to remove stones, damaged grains, and extraneous matters. The sample was packed properly and stored in closed sterile polyethylene bags and used for the entire study for the preparation of soymilk from the soybean after the required flour was prepared.

Preparation of soymilk

Soymilk was prepared according to the local practice. About 1 kg of soybean seed sample was weighted. The weighted sample was sorted out in to closed packed beaker; washed and soaked in 3000 mL of water for 14hr at room temperature. The soaked soybean seeds were blanched for 5 minutes. The liquid was drained of and the soybean seed was de-hulled and milled using a miller. Water was added to soybean flour and mixed with a 1:3 soybean water ratio and finally filtered in a filter press (Muslin cloth) to separate the soluble and insoluble parts of the soybean.

Isolation of Lactic Acid Bacteria from okara

Isolation of lactic acid bacteria was done according to (Chen *et al*., 2005). Isolation and purification of LAB were carried out on MRS (de Man, Rogosa, and Sharpe) agar media supplemented with calcium carbonate ($CaCO_3$). The insoluble part of soybean (okara) was fermented spontaneously for 48hr. About 1 mL of fermented okara was taken and homogenized with 9 mL of 0.85% sterile sodium chloride solution. After this process, a serially diluted mixture of 0.1 mL was taken from each serially diluted mixture and was spread directly on the surface of MRS agar and 1% $CaCO_3$ was added on (MRS) agar to differentiate acid – producing bacteria from other bacteria and incubated at 37°C for 48h. Randomly colonies were collected from colonies surrounding the clear zone because they are considered as acid – produce bacteria. The collected colonies were purified using the streak plate technique. Finally, the purified lactic acid bacteria (LAB) were stored in 15% glycerol and 85% MRS broth.

Characterization of Lactic Acid Bacteria

Lactic acid bacteria were identified using morphological, biochemical, and Physiological Characteristics. The identification of LAB was determined by using the method of (Felten *et al.*, 1999). First Gram positive and gram negative bacteria were differentiated using the KOH test of Gregersen (1978) and then microscopic observation was done to determine the shape of the bacteria.

Morphological Characterization

Gram Staining

Cell morphology of LAB was determined by using gram staining according to Ismail *et al*. (2018). Gram staining morphological identifications included the color and shape of a bacterial cell. Gram-positive bacteria were marked by purple or red color which indicates that the bacteria cell wall is capable of binding to crystal violet dye, whereas gram-negative were characterized by the formation of pink color which indicates that the bacteria cell wall is not able to bind to the crystal violet dye.

Gas production from Glucose/Glucose fermentation

The test aimed to identify homo-fermentative and hetero-fermentative LAB isolates. It was assessed based on gas production from glucose using MRS broth with an inverted Durham tube according to Fguiri *et al*. (2017). A loop full of overnight cultures was transferred in to 8ml of autoclaved MRS broth media supplemented with 1% glucose with an inverted Durham tube and incubated anaerobically at 32°C for 5 days. Gas accumulation in Durham tubes was taken as evidence for CO_2 production from glucose. If CO_2 is produced from glucose, the isolate is hetro-fermentative and if not the isolate is homo-fermentative also un-inoculated broth media was used for the control experiment.

Biochemical and Physiological Characterizations

KOH string test

A loop full of the bacterial colonies from the culture plate was emulsified over a glass slide in the suspension of 3% KOH. The suspension was stirred continuously for one minute and then the loop was gently pulled up from it. The test was considered positive if the string was seen within the first 30 seconds after mixing in KOH solution, KOH positive bacteria are Gram negative whereas KOH negative is Gram positive bacteria (Chinmaya Dash *et al*., 2016).

Catalase reaction

The test was done according to Abdulkadir *et al*. (2011). A drop of 3% hydrogen peroxide was placed on a clean microscope slide. A visible amount of bacterial growth (colony) was streaked on the glass slide with the inoculating loop and observed for gas (bubble) production. Non-catalase producers do not release any gas (oxygen) from hydrogen peroxide and are considered positive LAB tests.

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Citrate test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. About 2.4 g of Simmons citrate agar was dissolved in 100 mL of distilled water. About ten milliliters of citrate medium was dispensed into each tube and the tubes were capped, and sterilized by autoclaving. The tubes were inoculated by streaking the organisms once across the surface and incubating at 37±0.2ºC for 24 hrs. A change from green to blue indicates utilization of the citrate (Melanie *et al*., 2009).

Starch hydrolysis test

The ability to degrade starch is used as a criterion for the determination of amylase production by a microbe. The ability of the isolates to hydrolyze starch was determined on starch agar (1 gram starch + 1 gram nutrient agar + 100 mL distilled water). After activation LAB isolates in MRS broth for 24hr, 0.1 mL were spread/streaked on the starch agar and incubated aerobically/anaerobically at 37°C for 2 days. The plates were then flooded with gram's iodine for 15 to 30 min as an indicator. Starch in the presence of iodine produces a dark blue coloration of the medium and a yellow zone (clear zone) around a colony in a blue medium indicates amylolytic activity and was considered a positive test (Estifanos *et al*., 2016).

Carbohydrate Fermentation Test

A sugar fermentation test of glucose, sucrose, lactose, mannitol, and starch, was carried out to determine the ability of organisms to produce acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1% fermentable sugar and 0.01% Phenol red. About ten milliliters of sugar broth was dispensed into each of the test tubes and the Durham tube which would trap the gas if produced was inverted and placed in a tube carefully. The test tubes were autoclaved and inoculated with a loop full of 24hrs old culture of the isolate and then they were incubated for 24-48hrs at 37±2°C and observed for acid and gas production. Yellow coloration indicates acid production, while gas production was indicated by the formation of gas bubble in the Durham tube (Fawole *et al*., 2004).

Tolerance to Different Temperatures

Gram positive and catalase negative isolates were tested for their growth at different temperature values (15ºC, 37ºC, and 45ºC) and tolerance to different concentrations of NaCl (3%, 4%, and 6.5%). A loop full of young culture (18-24hrs) of each isolate was inoculated in to 10 mL MRS broth. From the inoculate 1ml was poured plated on MRS agar and incubated at 37ºC for initial cell count. The left broth was incubated at different temperatures of 15ºC, 37ºC, and 45ºC separately for 48hrs. After 48hrs 1 mL of aliquots was pour plated on to MRS agar for final cell count. The plates were incubated at 37ºC for 48hrs. The colony was counted and a viability of greater than >50% was considered to be tolerant (Tambekar and Bhutada, 2010). The growths of the isolates were determined as follows:

$$Growth\ at\ n\ temp\ in\ \% \ = \frac{Final\ cell\ count\ at\ 48hr}{Initial\ cell\ count\ at\ 0hr} X\ 100$$

Tolerance to Different Salt Concentration

Salt tolerance of each isolate was assessed by inoculating young cultures of (18-24hrs) of LAB from MRS agar transferred to a 10 mL tube containing MRS broth with 3%, 4%, and 6.5% NaCl. The inoculated broth was incubated at 37ºC for 48 hrs. One milliliter of the broth pour plated on MRS agar and incubated at 37ºC for 48 hrs. The colony was counted and viability greater than >50% was considered to be tolerant (Hoque *et al*., 2010). The growths of the isolates were determined as follows:

$$NaCl\ tolerance\ in\ \% \ = \frac{Final\ cell\ count\ at\ 48hr}{Initial\ cell\ count\ at\ 0hr} X\ 100$$

Results and Discussion

Soybean seeds were purchased from local markets and processed into soymilk and okara. The okara was let to ferment and aseptically analyzed for the presence of lactic acid bacteria. Isolation of LAB from okara in MRS agar medium supplemented with $CaCO_3$ yielded a total of 20 isolates confirmed as lactic acid bacteria (Table 1). A total of 133 different colonies were selected and purified from fermented okara, of which 20 colonies were confirmed as lactic acid bacteria. Lu *et al.* (2013) reported that okara still retained nutritional components such as protein, fat, dietary fiber, minerals, monosaccharaides, and oligosaccharides. This nutrient allows the growth of microorganisms such as LAB. In this study, the LAB isolated from fermented okara was identified by tests such as Gram staining, cell morphology, gas production, and catalase production.

Table 1 indicates that the colony size of LAB isolates ranges from large to pin point. Out of 20 LAB, seven isolates were found to be large in colony size, four isolates were medium, six isolates were found to be small and only three isolate namely; SL-1, SL-9, and SL-13 were found to be pin point in colony size. Out of 20 lactic acid bacteria, fourteen isolates were whitish and six isolates were found to be creamy in colony appearance. All isolates were found to be circular in colony shape and the isolates were found to be convex in colony consistency. The entire LAB isolates were circular in colony shape and found to be rod in cell shape. Based on the cell shape of LAB isolated from okara the isolates were belongs to the genera *Lactobacillus*. Holzapfel dan Wood *et al.* (2012) stated that the shape of LAB cells, consists of two families: (i) Lactobacillaceae, which are rod shaped and consist of the genera *Lactobacillus* and *Bifidobacterium,* and (ii) Streptococococaceae, which are round and consists of the genera *Streptococcos* and *Leuconostoc* and *Pediococcus.*

The ability of the LAB isolates to produce gas from glucose was then assayed to determine the types of fermentation that the LAB isolates could perform. The result in table 1 showed that isolates SL-3, SL-7, SL-10, SL-14, and SL-19 were hetero-fermentative; they produced CO_2 from carbohydrates fermentations and the remaining fifteen isolates were homo- fermentative and found that they did not produce CO_2 while fermenting carbohydrates. The LAB demonstrating homo-fermentative include *Streptococcus*, *Pediococcus,* and *Lactobacillus*, while some LAB is known to be hetero-fermentative, namely *Leuconosto* and some species of *Lactobacillus*.

The entire LAB isolates were catalase negative, indicating that all the identified isolates did not produce the enzyme catalase. Roberts and Greenwood (2003) stated positive reactions in the catalase test are indicated by the bubble (gas) formation due to the activity of the enzyme catalase, which breaks down H_2O_2 in to H_2O and CO_2 and these results indicate that the isolates were LAB. All the isolates were gram positive, citrate negative; rod shaped, and appeared in single, pairs, or chains in the cellular arrangement. All of the isolates were able to ferment glucose and mannitol and fourteen isolates were found to ferment sucrose whereas six isolates; SL-3, SL-6, SL-8, SL-12, SL-14, and SL-16 do not ferment sucrose. Ten isolates were found to ferment starch and the remaining ten isolates do not able to ferment starch (Table 2). Similarly, Salam *et al.* (2017) also reported that twenty four lactic acid bacteria were isolated from fermented okara in an MRS agar medium supplemented with $CaCO_3$. The $CaCO_3$ served as a buffer and was used for the initial selection of lactic acid producing bacteria. The detailed result of this study is also in agreement with Badarinath *et al.* (2017) in which LAB was selectively screened from okara based on their morphological, biochemical characterization, antimicrobial and probiotic properties.

Further identification of LAB isolate was conducted through a growth test at different temperatures (15°C, 37°C, and 45°C) and in different NaCl salt concentrations. The entire isolates were able to grow at different temperatures (15°C, 37°C, and 45°C) but six (6) isolates namely SL-2, SL-4, SL-5, SL-14, SL-18 and SL-20 do not able to grow at 45^0C. All the LAB isolates were also able to grow in 3% and 4%NaCl except four isolates (SL-3, SL-13, SL-16, and SL-19) which are not able to grow in 4% NaCl. Ten isolates (SL-1, SL-4, SL-6, SL-7, SL-8, SL-9, SL-12, SL-13 SL-17, and SL-19 were able to grow in 6.5% NaCl. The remaining ten isolates are not able to grow in 6.5% NaCl (Table 3). Badarinath *et al.* (2017) demonstrated that LAB was isolated from fermented soybean okara by tests such as various physiological tests including growth at various temperatures,

different NaCl salt concentrations, and biochemical tests like growth in 0.04% sodium azide focusing on probiotic lactic acid bacteria. Similarly, the result of this study is in agreement with Salam *et al.* (2017) in which identification of LAB was performed through the tests at 15°C, 60°C, and 70°C for 48hr, and all the isolates were demonstrated growth at various temperatures.

Table 1: Morphological characteristics of cultured lactic acid bacteria

Sample Code	Isolate Code	Morphological and Cultural characteristics					
		Colony size	Colony color	Colony shape	Consistency	Glucose fermentation	Cell shape
S1	SL-1	Pin point	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-2	Small	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-3	Large	Whitish	Circular	Convex	Hetro Fer	Rod in chains
	SL-4	Large	Creamy	Circular	Convex	Homo Fer	Rod in chains
S2	SL-5	Medium	Creamy	Circular	Convex	Homo Fer	Rod in chains
	SL-6	Small	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-7	Medium	Whitish	Circular	Convex	Hetro Fer	Rod in chains
	SL-8	Large	Whitish	Circular	Convex	Homo Fer	Rod in chains
S3	SL-9	Pin point	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-10	Small	Creamy	Circular	Convex	Hetro Fer	Rod in chains
	SL-11	Medium	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-12	Large	Whitish	Circular	Convex	Homo Fer	Rod in chains
S4	SL-13	Pin point	Creamy	Circular	Convex	Homo Fer	Rod in chains
	SL-14	Small	Whitish	Circular	Convex	Hetro Fer	Rod in chains
	SL-15	Medium	Whitish	Circular	Convex	Homo Fer	Rod in chains
	SL-16	Large	Whitish	Circular	Convex	Homo Fer	Rod in chains
S5	SL-17	Small	Creamy	Circular	Convex	Homo Fer.	Rod in chains
	SL-18	Small	Creamy	Circular	Convex	Homo Fer	Rod in chains
	SL-19	Large	Whitish	Circular	Convex	Hetro Fer	Rod in chains
	SL-20	Large	Whitish	Circular	Convex	Homo Fer	Rod in chains

SL-1, SL-2, SL-3, SL-4, SL-5, SL-6, SL-7, SL-8, SL-9, SL-10, SL-11, SL-12, SL-13, SL-14, SL-15, SL-16, SL-17, SL-18, SL-19, and SL-20 were lactic acid bacterial isolates

Table 2: Biochemical characterizations of LAB

Sample code	Isolate code	Biochemical characteristics							
		Gram stain	Catalase	Citrate	Glucose	Sucrose	Lactose	Mannitol	Starch
S1	SL-1	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	SL-2	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	SL-3	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
	SL-4	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
S2	SL-5	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
	SL-6	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
	SL-7	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	SL-8	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
S3	SL-9	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve
	SL-10	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
	SL-11	+ve	-ve	-ve	+ve	+ve	- ve	+ve	+ve
	SL-12	+ve	-ve	-ve	+ve	-ve	- ve	+ve	-ve
S4	SL-13	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
	SL-14	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
	SL-15	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
	SL-16	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
S5	SL-17	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	SL-18	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve
	SL-19	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
	SL-20	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve

SL-1, SL-2, SL-3, SL-4, SL-5, SL-6, SL-7, SL-8, SL-9, SL-10, SL-11, SL-12, SL-13, SL-14, SL-15, SL-16, SL-17, SL- 18, SL-19 and SL-20 were lactic Acid bacterial isolates

Table: 3 LAB growths at different temperatures and different salt concentrations

Sample code	Isolate code	Growth Characteristics					
		Temperature			Salt concentration		
		15^0C	37^0C	45^0C	3%NaCl	4%NaCl	6.5%NaCl
S1	SL-1	+	+	+	+	+	+
	SL-2	+	+	-	+	+	-
	SL-3	+	+	+	+	-	-
	SL-4	+	+	-	+	+	+
S2	SL-5	+	+	-	+	+	-
	SL-6	+	+	+	+	+	+
	SL-7	+	+	+	+	+	+
	SL-8	+	+	+	+	+	+
S3	SL-9	+	+	+	+	+	+
	SL-10	+	+	+	+	+	-
	SL-11	+	+	+	+	+	-
	SL-12	+	+	+	+	+	+
S4	SL-13	+	+	+	+	-	+
	SL-14	+	+	-	+	+	-
	SL-15	+	+	+	+	+	-
	SL-16	+	+	+	+	-	-
S5	SL-17	+	+	+	+	+	+
	SL-18	+	+	-	+	+	-
	SL-19	+	+	+	+	-	+
	SL-20	+	+	-	+	+	-

SL-1, SL-2, SL-3, SL-4, SL-5, SL-6, SL-7, SL-8, SL-9, SL-10, SL-11, SL-12, SL-13, SL-14, SL-15, SL-16, SL-17, SL- 18, SL-19, and SL-20 were lactic acid bacterial isolates

Conclusion

A total of twenty LAB were isolated and characterized by fermented okara and they were found to have better tolerance to low temperatures and different salt concentrations. The morphological, biochemical, and physiological characterization of LAB isolated from okara is different among isolates. Based on the morphological characterization of LAB isolated from okara the isolates belong to the genera *Lactobacillus.* The result of the present study will contribute to the future applications and utilization of LAB as starter cultures for the production of soybean yoghurt. The findings will also contribute to future applications and utilization of LAB in food preservation. However, there is a need for further studies on the antimicrobial activity of the isolate against food borne pathogens, probiotic characterization of the isolate, and acidifying capability of the isolate to use in food preservations and to use as a starter culture. Furthermore, they need to be further characterized at the molecular level (such as 16S rRNA sequencing) to use for all these types of practical applications.

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References

Akbar, A., Ali, I., & Anal, A. K. (2016). Industrial perspectives of lactic acid bacteria for biopreservation and food safety. *Journal of Animal & Plant Sciences*, 26(4): 938-948.

Arques, J. L., E. Rodriguez & S. Langa. (2015). Antimicrobial activity of lactic acid bacteria in dairy products and gut: Effect on pathogens. *BioMed Res*., 10.1155/2015/584183

Avall-Jaaskelainen S. & Palva A., (2005). *Lactobacillus* surface layers and their applications. FEMS Microbiol Rev., 29: 511–529. Pmid: 15935509

Axelsson, L. (2004). Lactic acid bacteria: classification and physiology. Food Science and Technology-New York-Marcel Dekker. 139:1-66.

Bromberg, R., I. Moreno, C. L. Zaganini, R. R. Delboni and J. de Oliveira. (2004). Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. *Braz. J. Microbiol*., 35: 137-144.

Chahrour, W., Merzouk, Y., Henni, J. E., Haddaji, M., & Kihal, M. (2013). Screening and identification of lactic acid bacteria isolated from sorghum silage processes in west Algeria. *African Journal of Biotechnology*, 12(14).

Chen, Y. S., F. Yangida & T. Shinahara. (2005). Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. Lett. *Applied Microbiol.*, 40: 195-200.

Dash, C., & Payyappilli, R. J. (2016). KOH string and Vancomycin susceptibility test as an alternative method to Gram staining. *Journal of International Medicine and Dentistry*, 3(2): 88-90.

De Vuyst L & Leroy F. (2007). Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. *J Mol Microbiol Biotechnol*. 13: 194-199.

El-Gendy, S. M. & Marth, E. H. (1981). Growth and aflatoxin production by *Aspergillus parasticus* in the presence of *Lactobacillus casei*. *J. Food Prot.,* 44: 211-212.

Felten, A., C. Barreau, C. Bizet, P.H. Lagrange & A. Philippon, (1999). Lactobacillus species identification, H2O2 production, and antibiotic resistance and correlation with human clinical status. *J. Clin*., 37: 729-733.

Fontana, L., M. Bermudez-Brito, J. Plaza-Diaz, S. Munoz-Quezada & A. Gil, (2013). Sources, isolation, characterisation, and evaluation of probiotics. *Br. J. Nutr*., 109: S35-S50

Frick, Julia-Stefanie, Katrin Schenk, Matteo Quitadamo, Frauke Kahl, Martin Köberle, Erwin Bohn, Martin Aepfelbacher, & Ingo B. Autenrieth. (2007). Lactobacillus fermentum attenuates the proinflammatory effect of Yersinia enterocolitica on human epithelial cells. *Inflammatory Bowel Diseases,* 13(1): 83-90.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B. & Givskov, M. (2002). Food spoilage - interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78(1-2): 79-97

Hawaz, E., Guesh, T., Kebede, A., & Menkir, S. (2016). Characterization of lactic acid bacteria from camel milk and their technological properties to use as a starter culture. *East African Journal of Sciences*, 10(1): 49-60.

Hoque, M. Z., Akter, F., Hossain, K. M., Rahman, M. S. M., Billah, M. M., & Islam, K. M. D. (2010). Isolation, identification, and analysis of probiotic properties of Lactobacillus spp. from selective regional yoghurts. *World J Dairy Food Sci.*, 5(1): 39-46.

Hsieh, C., & Yang, F. C. (2004). Reusing soy residue for the solid-state fermentation of Ganoderma lucidum. *Bioresource Technology*, 91(1): 105-109.

Ismail, Y. S., Yulvizar, C., & Mazhitov, B. (2018, March). Characterization of lactic acid bacteria from local cow s milk kefir. In *IOP Conference Series: Earth and Environmental Science,* 130(1): 012019.

Kitawaki, R., Nishimura, Y., Takagi, N., Iwasaki, M., Tsuzuki, K., & Fukuda, M. (2009). Effects of Lactobacillus fermented soymilk and soy yogurt on hepatic lipid accumulation in rats fed a cholesterol-free diet. *Bioscience, biotechnology, and biochemistry*, 73(7): 1484-1488.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., & Gobbetti, M. (2000). Purification and characterization of novel antifungal compounds from the sourdough Lactobacillus plantarum strain 21B. *Applied and Environmental Microbiology*, 66(9): 4084-4090.

Marshall, S. H., & Arenas, G. (2003). Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. *Electronic Journal of Biotechnology*, 6(3): 271-284.

Masood, M. I., Qadir, M. I., Shirazi, J. H., & Khan, I. U. (2011). Beneficial effects of lactic acid bacteria on human beings. *Critical reviews in microbiology*, 37(1): 91-98.

Mazzoli, R., Bosco, F., Mizrahi, I., Bayer, E. A., & Pessione, E. (2014). Towards lactic acid bacteria-based biorefineries. *Biotechnology Advances*, 32(7): 1216-1236.

Onipede, G. O., Odah, B. C., Kolapo, A. L., Ajayi, A. A., & Fawole, A. O. Technological properties of lactic acid bacteria and yeasts isolated from ogi, a West African fermented cereal gruel.

Prescott LM, Harley JP, and Kelin DA. Microbiology, 5th ed. Boston, New York; (2002).

Roberts, D., W. Hooper, M. Greenwood & C. Struijk, (1996). Practical Food Microbiology. 3rd Edn., Blackwell Publishing, Oxford

Salminen, S., Ouwehand, A., Benno, Y., & Lee, Y. K. (1999). Probiotics: how should they be defined?. *Trends in food science & technology*, 10(3): 107-110.

Schleifer, K. H., & Ludwig, W. (1995). Phylogeny of the genus Lactobacillus and related genera. *Systematic and Applied Microbiology*, 18(4): 461-467.

Siroli, L., Patrignani, F., Serrazanetti, D. I., Tabanelli, G., Montanari, C., Gardini, F., & Lanciotti, R. (2015). Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apples and lamb's lettuce. *Food Microbiology*, 47: 74-84.

Suruga, K., Kato, A., Kadokura, K., Hiruma, W., Sekino, Y., Buffington, C. T., & Komatsu, Y. (2011). Okara: a new preparation of food material with antioxidant activity and dietary fiber from soybean. *Soybean and nutrition*, 16: 311-326.

Tambekar, D. H., & Bhutada, S. A. (2010). An evaluation of the probiotic potential of Lactobacillus sp. from the milk of domestic animals and commercially available probiotic preparations in the prevention of enteric bacterial infections. *Recent Research in Science and Technology*, 2(10): 82-88.