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Research Article

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM DAIRY SLUDGE SAMPLE FOR FERMENTATION OF LACTIC ACID, WOLAITA SODO, SOUTHERN ETHIOPIA

KRISHNA MOORTHY SIVALINGAM*
Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Wolaita Sodo, Post Box No.: 138, Ethiopia
*Corresponding Author: Email - skrish.microbiologist@gmail.com, skrishmicro@gmail.com

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Abstract- The aim of this study was isolation and identification of lactic acid bacteria from dairy sludge samples collected from SNNPR Bureau of Livestock and fishery resources, Wolaita Sodo dairy cattle production center. For isolation, all the samples were serially diluted and inoculated to MRS agar using pour plate technique. Well-isolated 5 different colonies were picked from each plate and marked as LAB1, LAB2, LAB3, LAB4 and LAB5 (LAB – Lactic Acid Bacteria) for further experiments. All these five isolates were identified as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum, Lactococcus lactis* and *Leuconostoc mesenteroides* by using different morphological, physiological and biochemical tests. All the isolates were Gram-positive, rods or cocci shaped, catalase negative, non-motile, able to grow at low salt concentration and ferment carbohydrate. All the isolates were fallen to two genera as follows: LAB1, LAB2 and LAB3 belong to genus *Lactobacillus* and LAB4 and LAB5 belongs to genus *Lactococcus*. Fermentation study was performed in 250ml flasks containing 100ml of sterile cow milk with 1ml of overnight active LAB isolates. All the inoculated flasks were incubated under rotary shaker incubator at 130rpm for 24h to 72hrs. The highest amount of titrable acid (TA) was observed from isolate *Lactococcus lactis* (4.74±0.21%) followed by *Lactobacillus fermentum* (4.38±0.28%), *Leuconostoc mesenteroides* (4.2±0.15%) and *Lactobacillus acidophilus* (4.05±0.32%) at temperature 37ºC and 72hours of incubations whereas the lowest TA observed from isolate *Lactobacillus brevis* (3.75±0.17%). Therefore, the present study concludes that the lactic acid bacterial isolates such *Lactobacillus acidophilus, Lactobacillus fermentum, Leuconostoc mesenteroides* and *Lactococcus lactis* can be used as inoculum to produce lactic acid by fermentation.

Keywords- *Lactic acid bacteria, Lactobacillus, Lactococcus, Dairy sludge and Fermentation*

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Introduction

Lactic acid bacteria (LAB) are diverse group of bacteria which plays an important role in a variety of food fermentation process. Lactic acid bacteria can produce lactic acid as the major product of food carbohydrates fermentations. In addition, the lactic acid bacteria can degrade different kind of proteins and lipids and produce various alcohols, aldehydes, acids, esters and sulphur compounds which contribute to the specific flavour development in different fermented food products [1]. Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, non-spore forming bacteria that are of anaerobic habit but are aero-tolerant, acid tolerant and strictly fermentative. The lactic acid bacterial group comprising of four important genera; *Lactobacillus, Streptococcus* (Lactococcus) *Leuconostoc* and *Pediococcus* [2]. Lactic acid is the most widely occurring carboxylic acid in nature and recently, it is under increasing demand in food, pharmaceutical and chemical industries and for production of poly lactic acid polymers, which possess outstanding biomedical applications. Worldwide efforts have been made for the production of lactic acid with good yield and low-cost management [3,4]. LAB could be isolated from different sources such as milk products, sugar cane plants (Liliana et al., 2006) fresh water fish [5], but studies on isolation, identification and characterization of lactic acid bacteria from dairy sludge samples remain scarce. Therefore, the present study was majorly focused on isolation and identification of lactic acid bacteria from dairy sludge sample for fermentation of lactic acid, Wolaita Sodo, Southern Ethiopia.

Materials and Methods

Sample Source and Sample Collection

The present study was used dairy sludge samples collected from SNNPR Bureau of Livestock and fishery resources, Wolaita Sodo dairy cattle production center. The dairy sludge samples were collected using sterilized plastics bottles and brought to Wolaita Sodo University Post Graduate Microbiology laboratory using ice box for further investigations. The Samples were preserved in a refrigerator at 4ºC.

Isolation of Lactic Acid Bacteria

For isolation of lactic acid bacteria each sample were taken aseptically and MRS agar and broth were used for enumeration and culture of lactic acid bacteria. The appropriate sludge sample was serially diluted and pour plated on MRS plates [7]. The MRS plates overlaid with MRS agar and incubated at 37ºC for 48 hours.
The isolated colonies with typical characteristics were picked from each plate and transferred to MRS broth. All the selected colonies were purified by streak plate methods. All the purified colonies were marked as Lab (Lactic Acid Bacteria) with serial numbers and preserved at 4ºC for further analysis and fermentation process.

Identification of Lactic Acid Bacteria

All the purified colonies were further employed for the identification process using morphological characters such as colony morphology and cell morphology, and several biochemical tests includes Catalase test, Indole test, Methyl Red test, Voges-Proskauer test, Citrate utilization, Motility test, Arginine Hydrolysis test and H_2S test. Colony morphology was observed by above said plating method and cell morphology was done by Gram's staining [8].

Characterization of lactic acid bacteria

The following experiments were applied to characterize the lactic acid bacterial isolates.

Carbohydrate fermentation

Sugar fermentation test of all isolates were performed using glucose, sucrose, lactose, dextrose, mannitol, sorbitol and starch. At first, the sugar was dissolved in distilled water to make solution. MRS broth was prepared and added respective sugar solution. Then phenol red indicator was added in the broth solution which turns the color of broth solution into red. Then 15ml of media was dispended and Durham's tube was inserted in each of these test tubes. Then the final mixture of broth and sugar solution with Durham's tube was sterilized in autoclave for 15minutes at 121ºC. After autoclaving the solution was cooled and pure cultures of isolates were inoculated in test tubes. Then test tubes were incubated for 24hours at 37ºC where only media was used as a negative control. Results were observed by color changing and gas formation [9-11].

Growth at Different Temperatures

Temperature test media, MRS containing brome cresol purple indicator, was prepared and transferred into tubes as 15 ml. Then loop full overnight cultures inoculated to tubes and incubated for 7 days at 10°C, 15°C, 25°C, 35°C, 45°C. During these incubation time cells growth at any temperatures was observed by the change of the color, from purple to yellow [10]

Growth at Different NaCl Concentrations

In order to perform growth at different NaCl concentration, MRS containing brome cresol purple indicator and MRS containing 2%, 4% and 6.5% NaCl solution were prepared. Then 10ml of the media was dispensed in test tube and sterilized in autoclave at 121ºC for 15minutes. After autoclaving, the solutions cooled down and each bacterial sample was inoculated in test tubes containing 2%, 4% and 6.5% NaCl, broth solutions and kept for incubation at 37ºC for a week. After one week the results were observed by color changing purple to yellow [10,12].

Growth at Different pH

In order to perform growth at different pH MRS containing brome cresol purple indicator were prepared. Then 10ml of the media was dispensed in test tube and sterilized in autoclave at 121ºC for 15minutes. After autoclaving, the solutions cooled down and each bacterial sample was inoculated in test tubes containing the media with different pH 3.5, 4.5, 6, 7 and 8.5 and kept for incubation at 37ºC for a week. After one week the results were observed by color changing purple to yellow. The pH is 3.5, 4.5, 6, 7 and 8.5. The results are taken after incubation at 30°C for 24 hours, 48 hours and 72 hours [10,12].

Determination of lactic acid production

Lactic Acid production capability all the isolated lactic acid bacteria were determined by fermentation process. The cow milk was used as fermentation media and the milk fermentation is one the most important characteristics of lactic acid bacteria [13]. In order to determine lactic acid production titrimetric methods were applied. For this purpose, 5 flasks were used marked with appropriate isolated Lactic Acid Bacterial names. o.1 ml of inoculums of each isolated LAB were transferred into the appropriate flasks 100ml sterilized milk under aseptic condition. The shake flask was capped with cotton and swabbed with 70% ethanol and then incubated in a thermo-stated rotary incubator shaker for 3days at 37ºC with rotation speed of 130 rpm. At every 24hours of incubation, 3ml of samples from each flask were taken aseptically and used for titration. 3-4 drops of phenolphthalein indicators were added to the 3ml of samples and stirred. The samples were then titrated by using standardized 0.1M NaOH solutions. When the first trace of pink color was observed, titration was terminated and the final burette reading was recorded. Finally, the results of titrable acidity were calculated by using following formula [14,15]:

$$\text{Percentage LA} = \frac{(mL \times N \times 90.08) \times 100}{V \times 1000}$$

Where, mL = volume of 0.1 NaOH used, N= normality of 0.1 NaOH;
V= volume of sample used;
90.08 = molecular weight of Lactate

RESULTS

Isolation of lactic acid bacteria

Bacteria were isolated with MRS agar media at 37°C under anaerobic conditions for 24 hours. The isolated colonies from dairy sludge were named as Isolate LAB1, LAB2, LAB3, LAB4, and LAB5. The isolated colonies were then purified using streaking plate techniques for further identification.

Identification of lactic acid bacteria based on morphological examination

The complete findings of morphological examinations of the isolates are presented in [Table-1]. All the isolates such as LAB1, LAB 2, LAB 3, and LAB 4 showed white color colony except LAB 5 which showed red color colony in MRS agar (Plate 1). Among all the five isolates, all the five isolates showed Gram-positive, 3 isolates were rod shaped and other 2 isolates were cocci under microscopic examination after Gram's staining (Plate 2).

Identification of lactic acid bacteria based on biochemical tests

All the five isolates such as LAB1, LAB2, LAB3, LAB4, and LAB5 were identified as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum, Lactococcus lactis* and *Leuconostoc mesenteroides* based on the morphological, biochemical and physiological characteristics of the isolates. The biochemical test results are as follows. The results of biochemical tests of bacteria isolated from dairy sludge can be seen in [Table-1]. In case of Citrate Utilization test, the isolates such as *Lactobacillus acidophilus* and *Lactococcus lactis* showed negative and the rest of the three isolates such as *Lactobacillus brevis, Lactobacillus fermentum* and *Leuconostoc mesenteroides* showed positive in citrate utilization test. Except isolate *Lactobacillus acidophilus* (LAB1), all other isolates such as *Lactobacillus brevis* (LAB2), *Lactobacillus fermentum* (LAB3), *Lactococcus lactis* (LAB4), and *Leuconostoc mesenteroides* (LAB5) showed positive in indole test.

In case of H_2O test, *Lactobacillus brevis* (LAB2), *Lactobacillus fermentum* (LAB3) showed positive in while other isolates such as *Lactobacillus acidophilus, Lactococcus lactis* and *Leuconostoc mesenteroides* showed negative. The lactic acid bacterial isolates *Lactobacillus acidophilus* and *Leuconostoc mesenteroides* showed negative results while the other isolates such as *Lactobacillus brevis, Lactobacillus fermentum* and *Lactococcus lactis* showed positive results. All the lactic acid bacterial isolates such as *Lactobacillus acidophilus, Lactobacillus brevis, Lactococcus lactis* and *Leuconostoc mesenteroides* showed negative in case of Voges-Proskauer (VP) test [Table-1].

Characterization of Lactic Acid Bacterial (LAB) isolates

Carbohydrate fermentation

[Table-2] shows that the carbohydrate fermentations status of Lactic Acid Bacterial isolates using different sugars such as Glucose, Sucrose, Lactose, Mannitol, Sorbitol, starch, Dextrose and maltose. All the five isolates fermented the Glucose, Sucrose, Lactose, Mannitol and maltose sugars. In case of sorbitol, all the *Lactobacillus* species showed negative response while the other two coccus showed positive response. All the three isolates of *Lactobacillus* groups including *Lactobacillus acidophilus, Lactobacillus brevis,* and *Lactobacillus fermentum* showed positive in fermentation. The other two isolates such as *Lactococcus lactis* and *Leuconostoc mesenteroides* showed negative. The lactic acid bacterial isolate *Lactococcus lactis* alone failed in dextrose sugar fermentation while the other four lactic acid bacterial isolates such as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum* and *Leuconostoc mesenteroides* showed positive in dextrose fermentation.

Growth of Lactic Acid Bacterial isolates at different temperatures

Temperature is one of the most important factors to characterize the Lactic acid bacterial isolates. All the isolates such as *Lactobacillus acidophilus* (Lab1) *Lactobacillus brevis* (Lab 2), *Lactobacillus fermentum* (Lab 3) *and Leuconostoc mesenteroides* (Lab 5) showed negative growth except *Lactococcus lactis* (Lab 4).

Table-1 *Morphological and biochemical characteristics features of Lactic Acid Bacteria*

Tests	Lactic Acid Bacterial Isolates (LAB)				
	Lactobacillus acidophilus (LAB1)	*Lactobacillus brevis* (LAB2)	*Lactobacillus fermentum* (LAB3)	*Lactococcus lactis* (LAB4)	*Leuconostoc mesenteroides* (LAB5)
Colony color	White	White	White	White	Red
Cell Morphology	Rods	Rods	Rods	Cocci	Cocci
Cellular Arrangement	Single, Pairs and short chains	Single, Pairs and short chains	Single and Pairs	Pairs and Short chains	Diplo-coccus/Ovoid
Gram stain	+	+	+	+	+
Catalase test	_	_	_	_	_
Motility test	_	_	_	_	_
NH_3 from arginine	_	_	_	+	_
Glucose (Acid)	+	+	+	+	+
Glucose (Gas)	_	+	+	_	_
Citrate utilization	_	+	+	_	+
Indole test	_	+	+	+	+
H_2S test	_	+	+	_	_
MR test	_	+	+	+	_
VP test	_	_	_	_	_
Fermentation type	Homo	Hetero	Hetero	Homo	Homo

+ : Positive - : Negative Homo: Homofermentative Hetero: Heterofermentative

Table-2 *Carbohydrate fermentation by Lactic Acid Bacterial isolates*

Fermentation of carbohydrate	Lactic Acid Bacterial Isolates (LAB)				
	Lactobacillus acidophilus (LAB1)	*Lactobacillus brevis* (LAB2)	*Lactobacillus fermentum* (LAB3)	*Lactococcus lactis* (LAB4)	*Leuconostoc mesenteroides* (LAB5)
Glucose	+	+	+	+	+
Sucrose	+	+	+	+	+
Lactose	+	+	+	+	+
Mannitol	+	+	+	+	+
Sorbitol	_	_	_	_	_
Starch	+	+	_	_	_
Dextrose	+	+	+	_	+
Maltose	+	+	+	+	+

Table-3 *Growth of Lactic Acid Bacterial isolates on different temperatures*

Growth at different temperature (T°)	Lactic Acid Bacterial Isolates (LAB)				
	Lactobacillus acidophilus (LAB1)	*Lactobacillus brevis* (LAB2)	*Lactobacillus fermentum* (LAB3)	*Lactococcus lactis* (LAB4)	*Leuconostoc mesenteroides* (LAB5)
10°C	-	-	-	+	_
15°C	+	+	++	+	+
25 C°	+++	+++	+++	+++	++
35 C°	+++	+++	+++	+++	+++
45 C°	++	-	++	++	+

Lactobacillus fermentum showed moderate growth while all other isolates such as *Lactobacillus acidophilus* (Lab1) *Lactobacillus brevis* (Lab 2), *Lactococcus lactis* (Lab 4) and *Leuconostoc mesenteroides* (Lab 5) showed less growth at 15°C. All the lactic acid bacterial isolates showed excellent growth except *Leuconostoc mesenteroides* at both 25°C and 35°C temperatures. The lactic acid bacterial isolate *Lactobacillus brevis* showed negative growth at 45°C while other isolates *Lactobacillus acidophilus, Lactobacillus fermentum* and *Lactobacillus lactis* at same temperature. The lactic acid bacterial isolates *Leuconostoc mesenteroides* showed less growth at 45°C [Table-3].

Growth of Lactic Acid Bacterial isolates on different concentration of NaCl

Growth of lactic acid bacteria on different concentrations of Sodium chloride is also one of the important characteristics features to identify. The lactic acid bacterial isolates such as *Lactobacillus acidophilus, Lactobacillus brevis* and *Lactobacillus fermentum* grow at all the three different concentrations (2%, 4% and 6.5%) of NaCl. The coccus group of lactic acid bacterium *Lactococcus lactis* showed grow at 2% and 4% concentrations of Sodium chloride and the same isolate showed no grow at 6.5% concentrations of Sodium chloride. The lactic acid bacterium *Leuconostoc mesenteroides* showed positive growth only at 2% concentration and negative growth in the rest of the concentrations such as 4% and 6.5% [Table-4].

Growth of Lactic Acid Bacterial isolates on different pH

[Table-5] shows that the effect of different pH likes 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 on the growth of lactic acid bacterial isolates such as *Lactobacillus acidophilus* (LAB1) *Lactobacillus brevis* (LAB2), *Lactobacillus fermentum* (LAB3), *Lactococcus lactis* (LAB4) and *Leuconostoc mesenteroides* (LAB 5). All the lactic acid bacterial isolates showed moderate and excellent growth up to the pH level of 6.5 and above 6.5 showed less growth and reached negative growth at pH 9.5.

Determination of Lactic acid Production

Sterilized fresh cow milk was used as fermentation media to determine the lactic acid production capability of lactic acid bacterial isolates. Titration was done at different intervals like 24hr, 48hr and 72hrs. All the isolates fermented the milk and produced lactic acid but not significantly different at 24hrs of incubation. The lactic acid bacterial isolate *Lactobacillus acidophilus* produced maximum amount (4.74±0.21%) of titrable acid at 72hrs of shaking incubation followed by *Lactobacillus fermentum, Leuconostoc mesenteroides* and *Lactococcus lactis* with respective percentage of 4.38%, 4.20% and 4.05% [Table-6].

Discussion

Lactic acid bacteria (LAB) were isolated from dairy sludge samples collected in Wolaita Sodo town. The entire LAB was isolated using MRS agar media under anaerobic conditions. The morphological and biochemical characteristics features and sugar fermentation patterns were employed to identify lactic acid bacteria (LAB). All the isolates were identified as bacilli/rod, non-motile, Gram-positive, catalase negative. Similarly, Lactic acid bacteria were isolated and identified from pickles and curd. Most of the isolates were rod/bacilli shaped Gram-positive, non-motile and catalase negative [16]. Sugar fermentation patterns were preferred for the identification of lactic acid bacteria.

Table-4 *Growth of Lactic Acid Bacterial isolates on different concentration of NaCl*

Lactic Acid Bacterial Isolates (LAB)	Growth at different concentrations of NaCl		
	2%	4%	6.50%
Lactobacillus acidophilus (LAB1)	+	+	+
Lactobacillus brevis (LAB2)	+	+	+
Lactobacillus fermentum (LAB3)	+	+	+
Lactococcus lactis (LAB4)	+	+	-
Leuconostoc mesenteroides (LAB5)	+	-	-

Table-5 *Growth of Lactic Acid Bacterial isolates on different pH*

	Lactic Acid Bacterial Isolates (LAB)				
Growth at different pH	*Lactobacillus acidophilus* (LAB1)	*Lactobacillus brevis* (LAB2)	*Lactobacillus fermentum* (LAB3)	*Lactococcus lactis* (LAB4)	*Leuconostoc mesenteroides* (LAB5)
4.5	++	++	++	++	++
5.5	++	++	++	++	++
6.5	+++	+++	+++	+++	+++
7.5	+	+	+	+	+
8.5	+	+	+	+	+
9.5	_	_	_	_	_

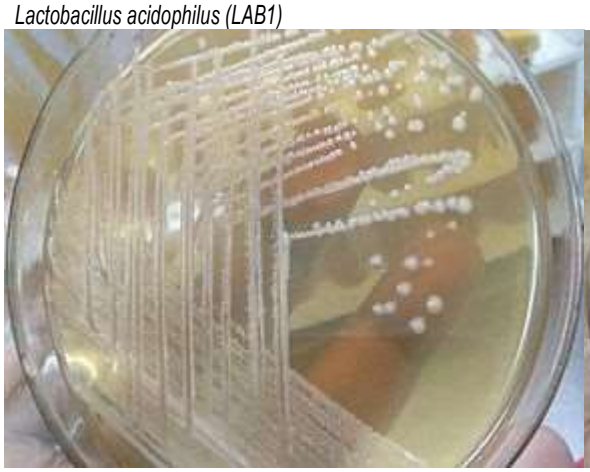
Table-6 *Percentage of titrable acid (TA) produced from lactic acid bacterial isolates*

Name of the Isolates	% Titrable Acid		
	24hr	48hr	72hr
Lactobacillus acidophilus (LAB1)	1.58±0.21	3.15±0.24	4.74±0.21
Lactobacillus brevis (LAB2)	1.13±0.12	2.25±0.22	3.75±017
Lactobacillus fermentum (LAB3)	1.46±0.16	2.92±0.3	4.38±0.28
Lactococcus lactis (LAB4)	1.35±0.07	2.6±0.15	4.05±0.32
Leuconostoc mesenteroides (LAB5)	1.4±0.19	2.8±0.11	4.2±0.15

Similar observations were found in the isolation of *Lactobacillus* species from traditional cheese and yoghurts of Basmej Zone in Iran [17]. Similar to the present study, no bubble was observed indicating that the isolated bacteria are catalase negative and could not mediate the decomposition of H_2O_2 to produce oxygen [18]. Lactic acid bacteria isolated from daily sludge sample in India were Gram-positive, rods and Cocci shaped, either chained or single catalase negative, non-motile, ferment different carbohydrate and able to grow in low salt concentration and at different temperature [19]. From five isolates, three isolates *Lactobacillus acidophilus* (LAB 1), *Lactobacillus brevis* (LAB 2) and *Lactobacillus fermentum* (LAB 3) were from *Lactobacillus* genus and the rest of the two isolates were identified as *Lactococcus lactis* (LAB4) and *Leuconostoc mesenteroides* (LAB5) based on morphological, physiological and biochemical examinations. Similarly, A total of 83 lactic acid bacteria isolates were characterized and identified from twenty-five raw cow milk samples collected from the surrounding area of the city Debrezeit. All the isolates were identified based on the morphological, physiological and biochemical characteristics features. All isolates were Gram-positive, catalase negative and non-motile. The Cell morphology of all isolates was evaluated through microscopic observation and the majorities (N=61) were found to be cocci and the remaining (N=22) were rod shaped bacilli [20]. In the present study, the isolates *Lactobacillus brevis* (LAB 2), *Lactobacillus fermentum* (LAB 3) showed positive in gas production while other isolates such as *Lactobacillus acidophilus* (LAB 1), *Lactococcus lactis* and *Leuconostoc mesenteroides* showed negative in gas production. Gas production during glucose fermentation is indication of hetero-fermentation and absence of gas production is the indication of homo-fermentation. Therefore, the isolates such as *Lactobacillus acidophilus* (LAB1), *Lactococcus lactis* (LAB4) *and Leuconostoc mesenteroides* (LAB5) are homofermentative; and *Lactobacillus brevis* (LAB 2) and *Lactobacillus fermentum* (LAB 3) are hetero-fermentative types. Similarly, Lactic acid bacteria are grouped as either homofermenters or heterofermenters based on the end product of glucose fermentations. The homofermenters produce lactic acid as the major product of fermentation of glucose and carbon dioxide will be released as product. The heterofermenters produce lactic acid, carbon dioxide, acetic acid, and ethanol from the fermentation of glucose [20]. In the present study, microscopic observation showed that all strains (LAB) are Gram-positive and catalase negative which is correlated with the previous results [21-26]. Lactic acid bacteria are in coccus or rod shapes, not motile, with catalase negative, homo or hetero fermentative and growing in low acid condition [27]. In the present study, most of the lactic acid bacterial isolates especially *Lactobacillus acidophilus, Lactobacillus brevis* and *Lactobacillus fermentum* fermented all the sugars such as glucose, sucrose, lactose, mannitol, dextrose and maltose except sorbitol. In case of coccus group of isolates *Lactococcus lactis* and *Leuconostoc mesenteroides* fermented all the sugars (glucose, sucrose, lactose, mannitol, sorbitol, dextrose and maltose) except starch. Similar to present study, a total of 24 lactic acid bacterial isolates were screened for their performance regarding fermentation status in 35 sugars. All the 24 isolates were fermented lactose, fructose, galactose, trehalose, mannose, xylitol and sorbose [28]. Similarly, the identification profiles of the isolated lactobacilli based on sugar fermentations. All the isolated lactic acid bacteria were identified as *Lactobacillus delbrueckii* subsp. *Bulgaricus, Lactobacillus delbrueckii* subsp. *Lactis, Lactobacillus delbrueckii* subsp. Delbrueckii, all of these species are homo fermentative, fermenting lactose, glucose and fructose. The other species were identified as *Lactobacillus plantarum* which is homo fermentative, however, *Lactobacillus brevis* is hetero fermentative, both species have the ability to ferment glucose, fructose, galactose and lactose [29]. Temperature is one of the most important factors to characterize the Lactic acid bacterial isolates. The lactic acid bacterial isolate *Lactococcus lactis* alone found to be with positive growth at 10ºC and all other isolates such as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum* and *Leuconostoc mesenteroides* showed negative growth at 10ºC. *Lactobacillus brevis* showed negative growth at 45ºC while all other isolates showed positive growth. This result is in agreement with the experiment done on identification of Lactic acid bacteria based on the growth at different temperature ranges. LAB can generally be classified in terms of optimum growth temperatures, namely mesophilic and thermophilic LAB. Similarly, most of the lactic acid bacterial isolates showed positive growth at 15ºC. In case of 10ºC and 45ºC, less than 50% of the lactic acid bacterial isolates showed negative growth [30]. In the present study all the lactic acid bacterial isolates showed positive growth at low salt concentrations like 2% and 4% of NaCl except *Leuconostoc mesenteroides* which gave negative growth at 4% and 6.5% of NaCl. This study results were comparable with several study reports [31], in that *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Enterococcus* were isolated from raw cow milk and the identified isolates were rod or cocci shapes with Gram-positive, non-motile Catalase negative, growing in law salt Concentration and growing at different temperature. 99% of the lactic acid bacterial isolates gave negative growth at 6.5% of Sodium chloride salt concentrations.

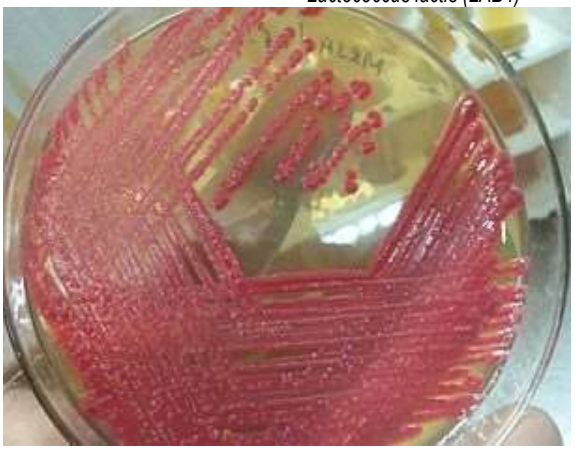
Lactobacillus acidophilus (LAB1)

Lactobacillus brevis (LAB2)



Lactobacillus fermentum (LAB3)

Lactococcus lactis (LAB4)



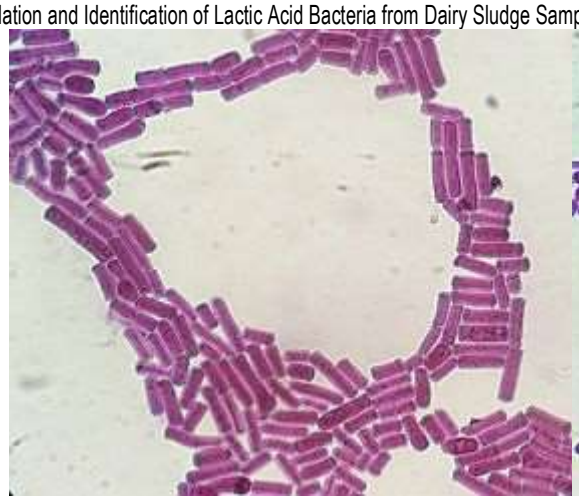
Leuconostoc mesenteroides (LAB5)

Fig-1 Colony morphology on MRS agar

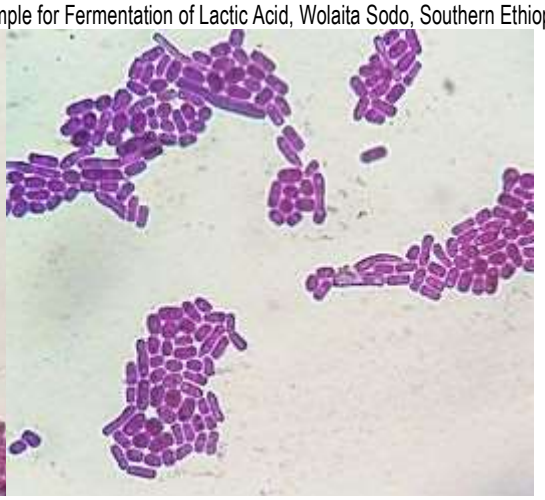
In case of titrable acidity, in the present study titrable acid amount was increased parallelly with the incubation periods from 24hrs to 72 hrs. The lactic acid bacterial isolates *Lactococcus acidophilus* (4.74%) and *Lactobacillus fermentum* (4.38%) produced highest amount of titrable acid after 72hours of incubation at temperature 37ºC. Similarly, the *Lactococcus acidophilus* produced highest amount of titrable acid was noticed at the end of the fermentation and an upward trend was kept until the 15th day. This study results were comparable with the previous study reports in which the values of titratable acidity are in the normal range of all unfermented milk samples. A significant increase in titrable acidity was noticed at the end of the fermentation and an upward trend was kept until the 15th day of incubation [32].

Conclusion

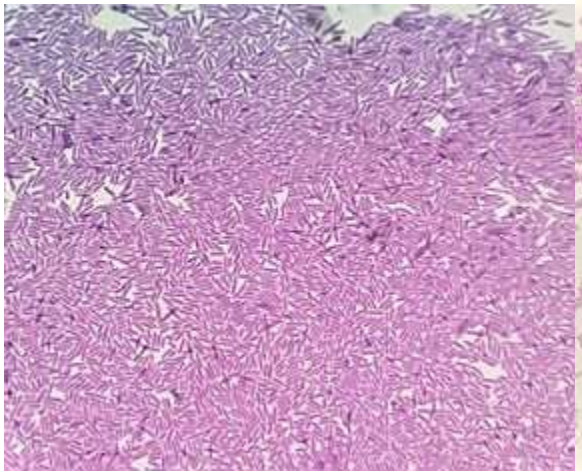
Totally five different lactic acid bacteria were isolated and named as LAB1, LAB2, LAB3, LAB4 and LAB5 from the isolation part of the present study. Further, the isolates LAB1, LAB2 and LAB3 were identified as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum*, and isolates LAB4 and LAB5 were identified as *Lactococcus lactis* and *Leuconostoc mesenteroides* based on the morphological and biochemical test results. The isolates LAB1, LAB2 and LAB3 showed Gram-positive, non-motile, rod shaped, catalase negative, positive growth with 6.5% sodium chloride (NaCl) concentrations, positive growth at 15ºC and 45ºC. The other two lactic acid bacteria were found to be with Gram-positive, non-motile, coccus shaped cell, catalase negative, positive growth with 6.5% sodium chloride (NaCl) concentrations, positive growth at 10ºC but not 45ºC. All the five isolates fermented the carbohydrates such as Glucose, Sucrose, Lactose, Mannitol and Maltose sugars.In case of fermentation study, sterilized fresh cow milk was used as fermentation media to determine the lactic acid production capability of lactic acid bacterial isolates.



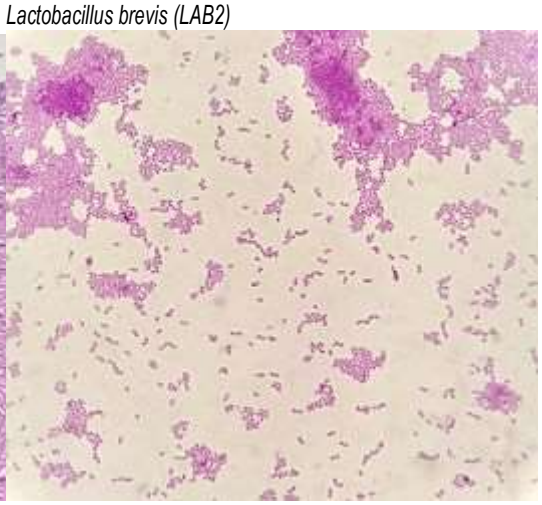
Lactobacillus acidophilus (LAB1)



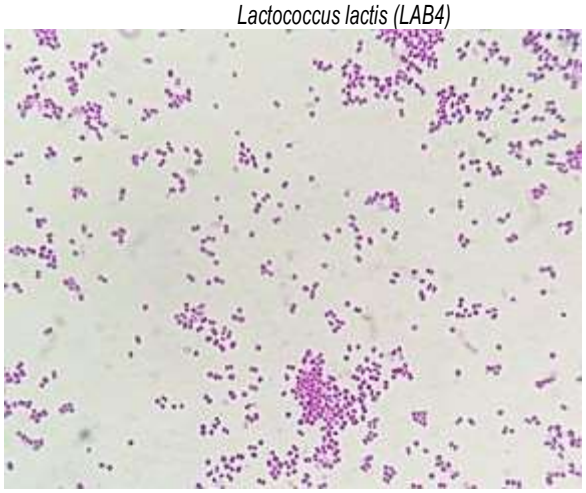
Lactobacillus brevis (LAB2)



Lactobacillus fermentum (LAB3)



Lactococcus lactis (LAB4)



Leuconostoc mesenteroides (LAB5)

Fig-2 Gram's staining

All the isolates showed positive in fermentation of milk and produced acid even at 24hours of incubation. The highest amount of titrable acid (TA) was observed from the isolates of *Lactococcus acidophilus* (4.74%) and *Lactobacillus fermentum* (4.38%) after 72hours of incubation at temperature 37°C whereas the lowest TA observed from isolate *Lactobacillus brevis* (3.75±0.17%). Therefore, the present study concluded that the dairy industry sludge can be used as source for the isolation of lactic acid bacteria and the sludge samples were dominated by the genus of *Lactobacillus* species. The lactic acid bacterial isolates *Lactobacillus acidophilus* (LAB1) and *Lactobacillus fermentum* (LAB3) can be used for the production of Lactic acid production using milk as fermentation media.

Application of research: Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits. Lactic acid bacteria are Gram-positive, non-spore forming, cocci or rods, which produce industrially important lactic acid as the major end product during the fermentation of carbohydrates.

Research Category: Industrial Microbiology/Microbial Biotechnology

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****Principal Investigator or Chairperson of research: Dr S. Krishna Moorthy**
University: Wolaita Sodo University, Wolaita Sodo, Post Box No.: 138, Ethiopia
Research project name or number: Research station study

Author Contributions: Sole author

Author statement: Author read, reviewed, agreed and approved the final manuscript. Note-Author agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: SNNPR Bureau of Livestock and fishery resources, Wolaita Sodo dairy cattle production center

Strain name: *Lactobacillus acidophilus, Lactobacillus fermentum, Leuconostoc mesenteroides and Lactococcus lactis*

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Moulay M., Benlancen K., Aggad H. and Kihal M. (2013) Adv. Environ. Biol., 7(6), 999-1007.
[2] Šuškoviæ J., Kos B., Beganoviæ J., Pavunc A.L., Habjaniè K. and Matošiæ S. (2010) Food Technology and Biotechnology, 48, 296-307.
[3] Young-Jung Wee., Jin-Nam Kim. and Hwa Won Ryu. (2006) Food Technol. Biotechnol., 44(2),163-172.
[4] Vijayakumar J., Aravindan R., Viruthagiri T. (2008) Chemical and Biochemical Engineering Quarterly, 22, 245-264.
[5] Liliana S.C. (2006) Electron.J.Biotech, 9(1),152-156.
[6] Gonzales C.J., Encinas J.P., Gracia-Lopez M.L. and Otero A. (2000) Food Microbiol., 17(1), 383-391.
[7] De Man J.C., Rogosa M. and Sharpe M.E. (1960) Journal of Applied Bacteriology, 23, 130-135.
[8] Cunning G., Seghrouchni K., Ruffieux E., Vaudaux P., Gaye-Ageron A., Cherkaoui A., Godinho E., Lew D., Hoffmeyer P. and Uckay I. (2014) Int. Orthop., 38 (6), 1283-90.
[9] Robert froid M.B. (2000) American Journal of Clin, nutrition, 71, 1682-1687.
[10] Charteris W.P., Kelly P.M., Morelli L. and Collins, J.K. (2001) Journal of Basic Microbiology, 41, 241-251.
[11] Tserovska L., Stefanova S. and Yordanova T. (2002) Journal of Culture Collection, 3, 48-52.
[12] Harrigan W.F. and McCance M.E. (1976) Laboratory Methods in Food and Dairy Microbiology (second ed), Academic Press, London (1976).
[13] Herresos M.A., Fresno J.M., Priteto M.J. and Tornadijo M.E. (2003) International Dairy Journal, 13, 469-479.
[14] Sarantinopoulos P., Kalantzopoulous G. and Tsakalıdou E. (2001) Applied and Environmental Microbiology, 5482-5487.
[15] Caric M., Milanovic S. and Vucelja D. (2000) Standard Methods for Milk and Milk Products Analysis. Novi Sad, Srbija.
[16] Mamta Thakur., Deshpande H.W. and Bhate M.A. (2017) Int. J. Curr. Microbiol. App. Sci., 6 (4), 1023-1030.
[17] Forouhandeh H. (2010) Curr. Res. Bacteriol., 3 (2), 84-88.
[18] Mithun S., Dipak V. and Sheela S. (2015) Int. J. Scientific and Res. Publications, 5 (4), 1-5.
[19] Choksi Nikita. and Desai Hemangi. (2012) Isolation, Identification and characterization of Lactic acid bacteria from dairy sludge sample, 7, 1A.
[20] Misganaw Wassie. and Teketay Wassie. (2016) Int. J. Adv. Res. Biol. Sci., 3 (8), 44-49.
[21] Scatassa M.L., Gaglio R., Macaluso G., Francesca N., Randazzo W., Cardamone C., Di Grigoli A., Moschetti G. and Settanni L. (2015) Food Microbiology, 52, 31-41.
[22] Zhang S., Zhang L., Jiao Y., Luo X., Li H., Xin L., Xue C., Zhang Y., Yi H. and Han X. (2014) Journal of Food Quality, 37, 395-402.
[23] Mchiouer K., Bennani S., El-Gendy N.S. and Meziane M. (2016) Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 38, 3572-3577.
[24] Lairini S., Beqqali N., Bouslamti R., Belkhou R. and Zerrouq F. (2014) Revue Internationale des Sciences et Technologie, 10, 267-277.
[25] Asfour H.A., Gamal I.M., and Darwish S.F. (2016) Assiut Veterinary Medical Journal, 62, 47-59.
[26] Caplice E. and Fitzgerald G.F. (1999) International Journal of Food Microbiology, 50, 131-149.
[27] Holzapfel E.H., Haberer P., Geisen R., Björkroth J. and Schillinger U. (2001) American Journal of Clinical Nutrition, 73(2), 365S-373S.
[28] Ayman Ashmaig., AlaaHasan. and Eisa EL Gaali. (2009) Afr. J. Microbiol. Res., 3 (8), 451-457.
[29] Bennani S., Mchiouer K., Rokni Y. and Meziane M. (2017) J. Mater. Environ. Sci., 8 (S), 4934-4944.
[30] Badis A., Guetarni D., Boudjema B.M. Henni D. and Kihal M. (2004) Food Microbiology, 21, 579-588.
[31] Khedid K., Faid M., Mokhtari A., Soulaymani A. and Zinedine A. (2009) Microbiological Research, 164, 81-91.
[32] Julijana Tomovska., Nikola Gjorgievski. and Borche Makarijoski. (2016) Journal of Materials Science and Engineering A 6 (11-12), 326-333.