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Isolation, Identification and Characterization Lactic Acid Bacteria from Raw Cow Milk, Soil and Wastes

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Abstract

Twenty (20) isolates of lactic acid bacteria were selected from all samples (Milk, Dung, Sewage, wastes, and soil). Bacteria loads from each sample were ranging from $5x10^3$cfu/ml to $7.4x10^8$ cfu/ml especially higher in dung samples. Each isolate was characterized physiologically and biochemically based on growth temperature, pH and fermentation ability on different sugars and the suitability of isolate was tested based on a clear zone formed on skim milk medium. The dominant species found were *Lactococcus lactis* (30%), *Lactobacillus brevis* (20%), *Streptococcus thermophiles* (15%), *Enterococcus feacalis* (15%), *Lactobacillus lactis* (10%) and *Lactobacillus delbrueki* (10%). All most all isolates are suitable for starter culture and can be used in dairy food and feed industries. Thus, further characterization to the molecular level, treatment of feed with these isolates and evaluation of their capacity is needed further investigations.

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Keywords

Lactic acid bacteria, Starter Culture, Wastes

Introduction

Lactic acid bacteria are gram-positive, non-spore-forming, nonmotile, catalase-negative, nonmotile, acid-tolerant (Khalid, 2011). Cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Lactic acid bacteria are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acids, and vitamins. Recent taxonomic revisions of these genera suggest that the lactic acid bacteria comprise the following: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella* (Daeschel, 1989).

Lactic acid bacteria have been used as food and feed preservatives for centuries by bacteriocin producing in food fermentations. LAB could replace chemical preservatives for the prevention of bacterial spoilage and the outgrowth of pathogenic bacteria in the food products. They have been an effective form of natural preservation; also they strongly determine the flavor, aroma, texture of food and feeds, Industrialization of the biotechnological transformation of foodstuff has increased the economic importance of lactic acid bacteria (Stiles, 1996). The most food and feed is fermented by lactic acid fermentation, during which pH is lowered to 4, hydrogen peroxide, ethanol, diacetyl are also produced, which inhibits the growth of unwanted

microorganisms and prevents spoilage of food and feed (Goyal *et al.*, 2012).

Lactic acid fermentation also may reduce the content of natural toxins in plant food: e.g. cyanogenic glycosides in cassava (a major staple food in Africa) and soften plant tissues, enhance digestibility degradation of oligosaccharides and dietary fiber; fermentation of plant foods favors transformation of phytate by phytase and increases much fold bioavailability of iron. The consequence of lactic acid fermentation is decreased tannin content in cereals, which increases mineral absorption and protein digestibility of grains. Fermentation improves food safety and quality through the presence of probiotics that protect from *E. coli* and other pathogens and have hypocholesterolemic and anticarcinogenic effects, which is of particular significance in lactose intolerance and gastrointestinal disorders(Goyal *et al.*, 2012).

Labs are generally referred as safe (GRAS), thus they are used as starter culture in many food and feeds The raw materials traditionally used for fermentation are as diverse as fruits (vinegar, wine, etc), enset (kocho), cereals (beer, local beer, local alcohol, bread, injera, etc), honey, vegetables (soy sauce), milk (fermented milk or ergo, yogurt, buttermilk, and cheese), meat (sausages) and fish (Askal and Kebede, 2013).

Lactic acid bacteria can be isolated from different sources, especially fermented food, dairy products, vegetables or from plant (plant origin), soil and intestine of different animals, thus lactic acid bacteria diversified and isolated from differs ecologies (Sharma *et al.*, 2013; Khalid, 2011; Seema and Kumaran, 2005; Khedid *et al.*, 2009 and Goyal *et al.*, 2012). Isolation and screening of microorganisms from naturally occurring processes have always been the most powerful means for obtaining useful cultures for scientific and commercial purposes. However, the isolation and using of known lactic acid bacteria as starter culture in sub-Saharan African in general and in Ethiopia particular remain scarce

Dilla town is the most common area that practices traditional food fermentation; like Kocho, dairy products, injera, bread, and tej, but the use of starter cultures are not available. Hence the fermentation is a spontaneous process and relies on chance inoculation (that is either natural contamination or back slopping) which results in a product of inconsistent quality. Many municipal waste products can be used as animal feeds but not used and make wasteful area e.g. Chat leftover, enset, banana, and other fruit byproducts. The uses of these municipal wastes and isolations of Lactic Acid Bacteria in and around Dilla town from raw milk, manure sewage and soil its application fermentation were not reported. Therefore the present study was focused on isolating lactic acid bacteria or starter culture from different sources of Dilla town. Therefore, the objective of this study is to isolate, identify and characterize of lactic acid bacteria (LAB) from raw cow milk, soil, sewage, manure and wastes of Dilla University

Materials and Methods

Samples

The sampling was conducted using the method of Talat *et al.*, (2009) with little modifications. A total of ten samples of raw cow milk (2 samples) from Udder of two Cows of Dilla University Farm, Manure (2 samples) of Dilla University farm site, Sewage (2 Samples) from Student Cafeteria of Dilla University, Waste (2 samples) of Dilla university surrounding and Moist garden Soil (2 Samples) at 15cm depth from agricultural farm of Dilla University were aseptically collected and packed in appropriate containers, then stored at 4◦C for further use.

Preparation of samples

The sample was aseptically weighed and homogenized. Ten grams from each sample was inoculated into 9ml of 0.85% of NaCl and vortexed thoroughly. And then a 1:10 dilution was subsequently made using 0.85% of NaCl (normal saline) followed by making 10 fold serial dilutions (Collins and Lyne, 1980).

Isolation of lactic acid bacteria

The 0.1mL from each dilution was then sub-cultured in duplicate onto the MRS agars used for isolating LAB (Badis *et al.*, 2004) to prevent the growth of yeast, the media was supplemented with 100mgL^{-1} of Cycloheximide before further used and to distinguish acid-producing bacteria from other bacteria, 1% $CaCO_3$ was added to the MRS agar plates. Incubation was carried out anaerobically at appropriate temperatures (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55◦C, and 60◦C) for 3- 5days to get an optimum temperature for growing thermophilic, mesophilic or psychrophilic. Colonies of acid-producing bacteria were differentiated by a clear zone around each colony and were randomly selected from MRS agar plates and purified by streak plating on MRS agar plates. To

perform total counts the higher dilution was used and subsequently kept two different conditions including at 4◦C for MRS plates and at -20◦C for MRS broth supplemented by 20% glycerol for further use (Mathara *et al.*, 2004).

Identification and characterization of isolates

Representative colonies were randomly selected based on their morphology, such as color, shape, and size from countable plates and purified by streaking at least three times in MRS agar. Growth characteristics were monitored daily at 10°C, 15°C and 50°C in tubes of MRS broth over 7 days period. Salt tolerance was also assessed after 3 days of incubation at the concentration of 4 and 6.5 % NaCl. All isolates were initially tested for Gram's reaction, catalase production and spore formation (Harrigan and MacCance, 1976).

Catalase test was carried out by transferring a drop of MRS broth culture on the slide, flooded with a drop of H_2O_2 and observed for the production of effervescing (Whittenbury, 1964). Only Gram's positive bacteria with catalase-negative reactions were selected (Garvie, 1986; Kandler and Weiss, 1986; Schillinger and Lücke, 1987) and the representative isolates were purified by successive streaking onto the same agar. Gas production, from glucose; Gibson semi sold medium was used, after 1-7days of incubation at optimum temperature also tested. Production of ammonia from arginine was detected using Nessler's reagent. The test for Carbohydrates was characterized at 24, 48 hr and 7 days after incubation in MRS broth, but Glucose and Meat extract replaced with 1% of different carbohydrates at a final concentration of 20gm/L, all isolates were grown overnight at optimum temperature.

Endospore test

To identify spore-forming bacteria, Gram's positive bacteria were tested. Bacterial smear was made on a microscopic slide under aseptic conditions and heat-fixed. then the slide was placed over the steaming water bath and malachite green (primary stain) was applied for 5 min.

The slide was removed from the water bath and rinsed with water until water runs clear. Then the slide was flooded with the counterstain safranin for 20 seconds and rinsed with water. After these slides were blot dried, they were observed under the light microscope**.**

Data analysis

The data collected from this study were entered into MS Excel spreadsheets, Tables, and analyzed using means and Standard deviations and comparing with manual of identifications (Berge's manual 9thed) and other scientific findings

Results and Discussions

Enumeration of lactic acid bacteria

The total bacterial counts were done on the MRS agar plate to determine a load of bacteria obtained from each sample source by the manual classic method. Loads of bacteria from each sample were ranging from $5x10^3$cfu/ml to $7.4x10^8$cfu/ml. Table 1shows that the bacterial load is higher in cattle dung ($7.4x10^8$cfu/ml) followed by cafeteria sewage (6 $x10^7$cfu/ml) and lower in raw cow milk ($5x10^3$cfu/ml). The counts found in all samples were higher when compared to LAB levels reported by (Fate Chanti *et al.*, 1979; Badis, 2004) in samples isolated from Goat's milk and on another hand the present study result is slightly similar to the that of LAB counts reported Ewe's milk and in Camel milk (Khedid *et al.*, 2009).

Bacteria grow on MRS agar plate producing acid (showing clear zone) on the plate were randomly analyzed by Gram's staining; only Gram's positive isolates were recorded. The result above reveals that bacterial in Rod pattern was dominant before further characterized into Lactic acid bacteria especially in municipal's waste almost all isolates were rod-shaped (92.3%), followed by bacteria isolated from cafeteria sewage (90%). Bacteria with cocci shape were dominant in samples of milk origin (91.2 %) followed by samples isolated from cattle dung (66.66%). The results were further processed to get actual lactic acid bacterial isolate distribution.

Isolation of lactic acid bacteria

From tested samples 188 isolates were isolated, colonies were observed on the surface of MRS Agar Petri plate acid-producing, white, creamy or yellowish colonies were selected based on their color. According to Bansal *et al.*, (2013), most of the Lactic acid bacteria are producing acid from glucose; morphologically most of them were white, creamy and few of them yellowish, which is agreed with the current study.

The cultural and morphological characteristics were further resolved based on microscopic examination to identify their shape. The majority of the randomly selected microorganisms were Gram's positive bacteria. After Gram's staining the isolates were determined as representative Lactic acid cocci and lactic acid bacilli; depending on this approach 107 isolates were isolated as lactic acid bacteria from 188 isolates. The present result agreed with the findings of Rhaiem *et al.,* (2016), the microscopic observation of lactic acid bacteria isolated from cow milk and olives brine are stick form, gram's positive, immobile, catalase-negative and similar to the (Goyal *et al.,* 2012; Bansal *et al.,* 2013; Maqsood *et al.,* 2013).

Catalase, motility, and endospore tests were conducted on 107 isolates, based on this 88 isolates were nom-spore forming and only 20 isolates were catalase-negative. Generally, lactic acid bacteria are Gram's positive, non-endospore forming, catalase-negative (Harrigan and McCance, 1976; Sharpe, 1979; Schleifer *et al.,* 1985; Bansal *et al.,* 2013; Maqsood *et al.,* 2013; Rhaiem *et al.,* 2016), which is agreed with current study finding, based on morphological were categorized as Lactococci (60%) and Lactobacilli (40%).

Table shows that most of the isolates were cocci groups of isolate; D23, D34, M22, M23, S133, and W1C were referred to species *Lactococcus lactis* (30%); because, they were gram's negative cocci, catalase-negative, nonmotile, can grew at 10°C and 15°C; but, not grew at 45°C with optimum of 30°C and can grew at 4% NaCl concentration and not grew at 6.5% NaCl, this result is supported by Khedid *et al.,* (2009) *Lactococcus lactis* is dominant in humped Camel milk having morphological biochemical properties of coccus gram's positive, catalase-negative nonmotile, grew at 10°C and 15°C not at 45°C and resist 4% NaCl.

D11a, C2C, and M214 isolates were referred as *Enterococcus faecalis* (15%) that were gram's positive, catalase-negative, nonmotile, homo-fermentative, can grew at 10-45°C, and grew at 6.5%, not in 4%NaCl, that agreed with findings of (Morsi El Soda *et al.,* 2003; Khedid *et al.,* 2009; Abdelaziz and Mubarak, 2010 and Nikita and Hemagni, 2012) and whereas D223, C3D, and S33B isolates were referred to *Streptococcus thermophlus* (15%), these isolates were similar to other cocci Lactococcus species but they characterized by specific properties like, could grow at higher temperature up to 60°C (thermophilic) cant grew at 10°C and specifically grew in 6.5 % NaCl salinity (Morsi El Soda *et al.,* 2003; Khedid *et al.,* 2009; Abdelaziz and Mubarak, 2010; Nikita and Hemagni, 2012).

The results also show that 40% of isolates as lactic acid bacteria were Lactobacilli. Isolates C1A, W1E; and W2C were isolated as *Lactobacillus brevis* (20%) because they were grown in both 4 % and 6.5 % NaCl salinity and could grow at10°C to 40°C but not in 45°C; which is supported by several findings (Morsi El Soda *et al.,* 2003; Ammor *et al.,* 2006; Terzic-Vidojevic *et al.,* 2007). D111 and D322 isolates were referred to *Lactobacillus lactis(10%),* because they grew at 10°C and 15°C not at 45°C, could grow in 6.5%NaCL not in 4% NaCl and whereas S1A and S22 isolates were *Lactobacillus delbrueki (10%)* they were growing at 45°C not grew at 10°C and 15°C; they were growing in 4% NaCl not in 6.5% (Morsi El Soda *et al.,* 2003; Ammor *et al.,* 2006; Terzic-Vidojevic *et al.,* 2007; Abdelaziz and Mubarak, 2010).

Table.1 Bacterial distribution across the sample source

SN	Source of Sample	Total Bacterial Counts (Mean (cfu/ml))	Distribution percentage(%) of LAB	
			Rods	Cocci
1	Raw Cow Milk	$5x10^3$	8.8	91.2
2	Soil	$6.56x10^5$	68.63	31.37
3	Manure/ Cattle dung	$7.4x10^8$	33.33	66.66
4	Sewage/Cafeteria sludge	$6 x10^7$	90	10
5	Municipal wastes	$5.76 x10^7$	92.3	7.7

cfu/ml: colony-forming unit per milliliter of sample

Table.2 Morphological and Physiological Characteristics of Isolates

Isolate	Colony Color	Gram's stain	Shape	Catalase test	Spore test	Acid from glucose	Gas from glucose	Fermentation type	Motility	Growth temp				[NaCl]		Probable Species
										10°C	15°C	45°C	Optimum	4 %	6.5%	
D111	white	+	Rod	-	-	+	-	Homofermention	-	+	+	-	30	-	+	*Lactobacillus lactis*
D11a	yellowish	+	Cocci, chain	-	-	+	-	Homofermention	-	+	+	+	37	-	+	*Enterococcus faecalis*
D23	white	+	cocci	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
D223	white	+	cocci	-	-	+	-	Homofermention	-	-	+	+	45-50	-	+	*Streptococcus thermophlus*
D322	white	+	Rod	-	-	+	-	Homofermention	-	+	+	-	30	-	+	*Lactobacillus lactis*
D34	white	+	Cocci, pair	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
C1A	Creamy	+	Rod,	-	-	+	+	Heterofermention	-	+	+	-	30	+	+	*Lactobacillus brevis*
C2C	Creamy	+	Cocci, chain	-	-	+	-	Homofermention	-	+	+	-	37	-	+	*Enterococcus faecalis*
C3B	Creamy	+	Rod	-	-	+	+	Heterofermention	-	+	+	-	30	-	-	*Lactobacillus brevis*
C3D	white	+	Cocci	-	-	+	-	Homofermention	-	-	+	+	45-50	-	+	*Streptococcus thermophlus*
M214	white	+	Cocci, chain	-	-	+	-	Homofermention	-	+	+	+	37	-	+	*Enterococcus faecalis*
M22	white	+	Cocci	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
M23	white	+	Cocci	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
S1A	white	+	Rod	-	-	+	-	Homofermention	-	-	-	+	40-45	+	-	*Lactobacillus delbrueki*
S133	white	+	cocci	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
S22	white	+	Rod	-	-	+	-	Homofermention	-	-	-	+	40-45	+	-	*Lactobacillus delbrueki*
S33B	white	+	Cocci, chain	-	-	+	-	Homofermention	-	-	+	+	45-50	-	+	*Streptococcus thermophlus*
W1C	creamy	+	cocci	-	-	+	-	Homofermention	-	+	+	-	30	+	-	*Lactocuculactis*
W1E	creamy	+	Rod	-	-	+	+	Heterofermention	-	+	+	-	30	+	+	*Lactobacillus brevis*
W2C	creamy	+	Rod	-	-	+	+	Heterofermention	-	+	+	-	30	+	+	*Lactobacillus brevis*

Table.3 Biochemical characterization of isolates

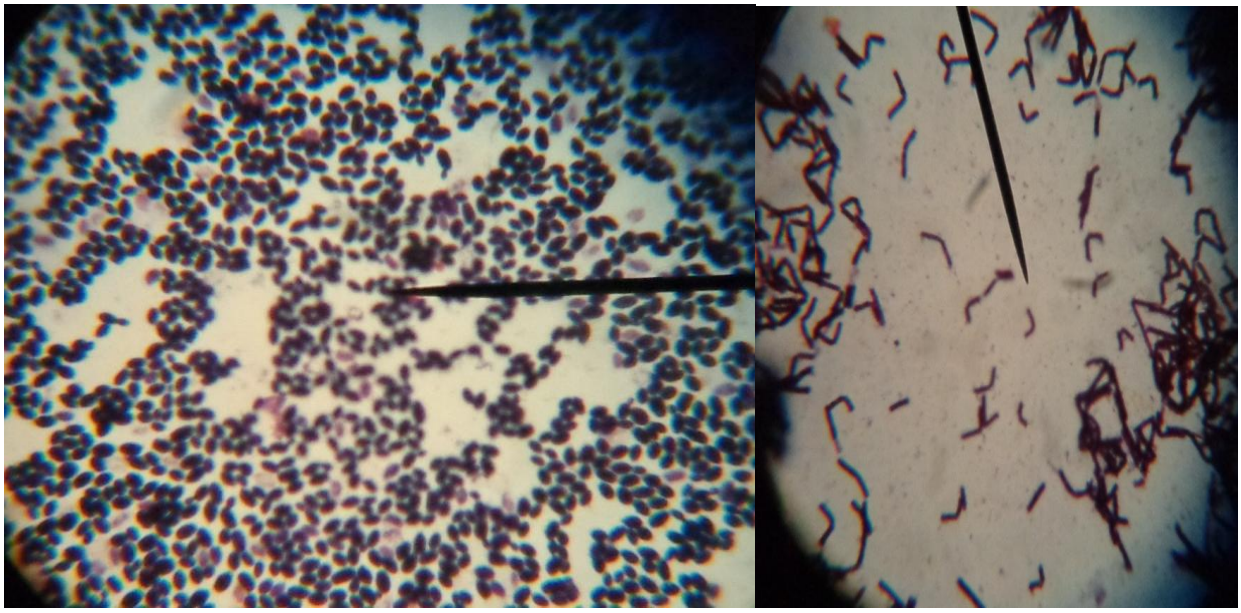
SN	Isolates	NH 3 from	Cellobiose	Lactose	Melebiose	Glucose	Galactose	Trehalose	Cellobiose	Salicin	Maltose	Sorbitol	Mannitol	Ascorbic acid	Ribose	Arabinose	Fructose	Sucrose	Starch	Raffinose	Xylose	Probable Isolates
1	D111	-	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	-	+	-	+	*Lactobacillus lactis*
2	D11a	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	-	*Enterococcus feacalis*
3	D23	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
4	D223	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Streptococcus thermophlus*
5	D322	-	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	-	+	-	+	*Lactobacillus lactis*
6	D34	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
7	C1A	+	-	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+	-	*Lactobacillus brevis*
8	C2C	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	-	*Enterococcus feacalis*
9	C3B	+	-	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+	-	*Lactobacillus brevis*
10	C3D	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Streptococcus thermophlus*
11	M214	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	-	*Enterococcus casseliflavus*
12	M22	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
14	M23	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
15	S1A	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	*Lactobacillus delbrueki*
16	S133	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
17	S22	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	*Lactobacillus delbrueki*
18	S33B	+	-	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Streptococcus thermophlus*
19	W1C	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	*Lactocucuslactis*
20	W1E	+	-	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+	-	*Lactobacillus brevis*
21	W2C	+	-	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+	-	+	-	*Lactobacillus brevis*

Table.4 Measurements of the clear zone of isolates on skimmed milk

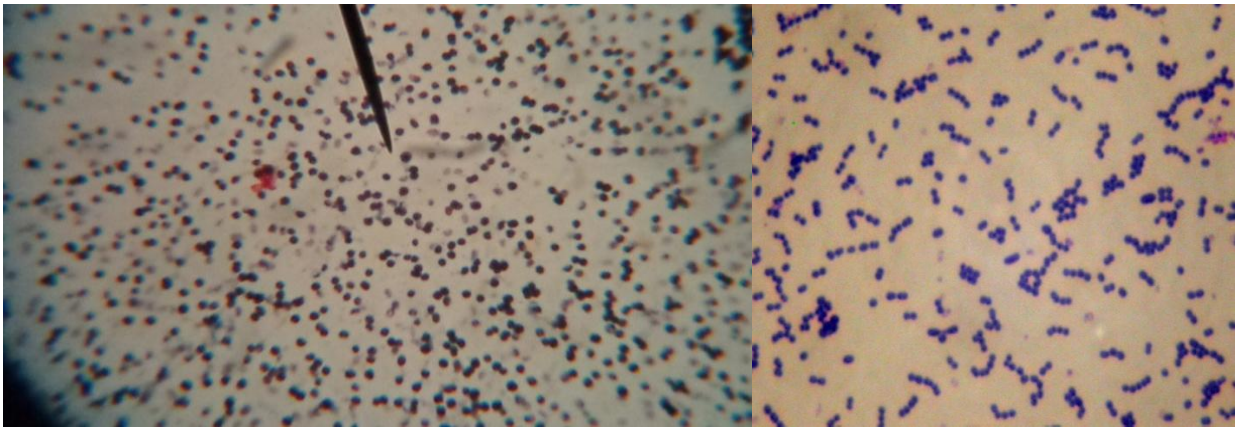
SN	Isolate	Clear Zone (mm) on skim milk (2%)		
		24 hrs	48hrs	Average
1	D111	20	23	21.5
2	D11a	11	18	14.5
3	D23	15	25	20
4	D223	20	27	23.5
5	D322	12	21	16.5
6	D34	25	33	29
7	C1A	15	17	16
8	C2C	14	21	17.5
9	C3B	10	12	11.5
10	C3D	18	23	20.5
11	M214	11	14	12.5
12	M22	10	15	12.5
13	M23	20	24	22
14	S1A	15	20	15.5
15	S133	20	21	20.5
16	S22	18	23	15.5
17	S33B	15	16	15.5
18	W1C	14	25	19.5
19	W1E	10	18	14
20	W2C	12	14	13

Figure.1 Lactic Acid bacteria growth on MRS agar supplemented with 1% $CaCO_3$ (source: present work)

Figure.2 Cellular morphology of isolated lactic acid bacteria (Source: from the present study)



Gram's positive Short Rod shape Gram's positive Rod



Gram's positive cocci, paired Gram's positive cocci, chain

The above table reveals that all isolates were Gram's positive, catalase-negative, non-spore-forming, and produce acid from glucose and Non-motile. Except for isolates C1A, C3B, W1E, and W2C all isolates were homo-fermentative and did not produce gas from glucose. These show that most of them can be used in dairy industries and those isolates producing gas from glucose and hetero-fermentative were used in food, brewery, and animal feed industries. This finding is similar to that of (Guessas and Kihal, 2004; Ammor *et al.,* 2006) *Lactobacillus brevis* isolate are mesophilic obligate hetero-fermentative used in brewery industries.

The study of bacteria growth according to parameters by Bergey's manual 9th edition; pH, temperature and salinity, For temperature, 30°C corresponds to the favorable temperature for optimal growth of lactic acid bacteria; so all these strains (D111, D11a, D23, D322, D34, C1A, C2C, C3B, M214, M22, M23, and S133) are mesophilic and the rest isolates (D223, C3D, S1A, S22, and S33B) are thermophilic. Ayad *et al.*, (2004) stated that lactic acid bacteria used in the dairy fermentation can roughly be divided into two groups of the basis of their growth optimum.

Mesophilic lactic acid bacteria have an optimum growth temperature between 20 and 30°C and the thermophilic have their optimum between 30 and 45°C. Traditional fermented products from sub-tropical countries harbor mainly thermophilic lactic acid bacteria, whereas the

products with mesophilic bacteria originated from western and northern European countries. These indicate that mesophilic isolates can be used in dairy industries and food industries.

Biochemical characterization of lactic acid isolates

All the selected isolates were examined for their ability to ferment different sugar based on criteria suggested by Harrigan (1998), Wood and Holzapfel (1995), Holt *et al.*, (1994), and Bergey's manual 9th edition. Table 3 shows that *Lactobacillus lactis* (D111 and D322) isolates were fermented almost all test sugars, except mannitol, arabinose, sucrose, Raffinose, and Melebiose and specifically these isolates were fermented Cellobiose (Harrigan and McCance, 1976; Khedid *et al.*, 2009; Bansal *et al.*, 2013).

The current study shows that *Lactobacillus delbrueki* isolates (S1A and S22) were fermented only glucose, fructose, and Lactose, which is supported by (Hebert *et al.*, 2000; Robinson, 2002). *Lactobacillus brevis isolates* (C1A, C3B, W2C, and W1E) were able to ferment lactose, glucose,galactose, fructose, sucrose, arabinose, ribose, maltose, and raffinose; which is similar to finding of Zirnstein and Hutkins (1999), Guessas *et al.*, (2012), and Bansal *et al.*, (2013).

The results reveal that *Lactococcus lactis* (D23, D34, M22, M23, S133, and W1C) isolates were fermented lactose, glucose, galactose, fructose, sucrose, starch, and xylose; Guessas *et al.*, (2012) and sucrose indicates the specific sugar involved in maximum acid production for particular isolate isolated from buffalo milk Sharma *et al.*, (2013).

Streptococcus thermophilous (D223, C3D, and S33B) were fermented Lactose, glucose, fructose, and sucrose (Tamime and Robinson, 1985, Gobbetti; Corsetti, 1999). According to some other literature, however, lactose, glucose, fructose, and mannose are fermented but sucrose; galactose and maltose are strain-specific did not ferment ribose /pentose (Robinson, 2002).

Isolates *Enterococcus feacalis* (D11a, C2C and M214) fermented almost all test sugars except galactose and arabinose; fructose is fermented not only by *Enterococcus feacalis.* Based on their fermentation ability (they make up part of starter cultures in dairy and no dairy products) and their ability to conserve foodstuffs and animal feed (Giraffa *et al.*, 2010).

The capability of isolates as starter culture

The ability of each isolates as starter culture was tested by growing of each isolates on 2% w/w of skim milk and incubated at 38°C, whether the test organisms can ferment milk or not the clear zone formed were measured, isolate D34 (*Lactococcus lactis*) formed wide zone (29mm), followed by D223 *(Streptococcus thermophilous)* with (23.5mm) and M23 (*Lactococcus lactis*) can created (22mm) clear zone, however, all isolates were grown on skimmed milk agar.

Table 4, shows that all isolates were had proteolytic activity. The current study is agreed with the finding of Seifu *et al.*, (2002) noticed that lactic acid bacteria isolated from fermented milk (Ititu) and had proteolytic activity were *Lactobacillus plantrum, L. debrueckii subspecies bulgaricus, L. salivarium, Lactococcus lactis*, and *Enterococcus feacalis.* Therefore, all isolate can be used in a Dairy starter culture, as probiotic and or used in the food and feed industry.

In conclusion, the results of this study suggest that various Lactic acid bacteria are distributed in cattle dung, cafeteria, raw cow milk, soil and wastes, 20 LAB Isolates are isolated, identified and characterized. *Lactococcus lactis* (30%) is the most dominant LAB in all sample sources, while *Lactobacillus brevis* is the next most abundant and *Enterococcus feacalis* (15%), *Streptococcus thermophilous* (15%), *Lactobacillus lactis* (10%), and *Lactobacillus delbrueki* (10%). Almost all isolates are resistant to acidic environment, only *Enterococcus faecalis* can grow at 9.5 pH. *Streptococcus thermophilous* and *Lactobacillus delbrueki* were grown in higher temperatures, and the rest isolates are mesophilic. All isolates are grown on skimmed milk media especially isolate *Lactococcus lactis D34.*

This study is strongly recommended:

- Raw cow milk, Cattle dung, cafeteria sewage, soil, and municipal wastes are a potential source of Lactic acid Bacteria
- All isolates can be used as a starter culture, especially *Lactococcus D34*.
- Isolated LAB are needed to be further identified and characterized to the molecular level
- Further research on Animal feed treatment by isolated lactic acid bacteria should be done

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