Invitro Evaluation Of Antibacterial Activity Of Lactic Acid Bacteria Isolated From "Ergo" And "Qotchqotcha", Ethiopian Traditional Fermented Foods, Against Some Selected Food Borne Pathogens

Lamenew Fenta, Animut Assefa

Abstracts: The prevention of pathogenic bacteria by lactic acid bacteria (LAB) isolated directly from foods is an innovative approach. With the aim of determining the anti-bacterial activity of Lactic acid bacteria isolated from "ergo" and "qotchqotcha", Ethiopian fermented food, 12 samples of each were taken from the 4 different kebeles of Assosa town. Isolation of LAB from the selected samples were carried out using MRS media. The different set of isolates were characterized using primary biochemical tests. Isolates which were gram positive, catalase negative and KOH negative were considered to be presumptive LAB and further characterized by using different biochemical tests for further identification. 16 isolates from ergo samples were isolated. Based on Bergey's manual of determinative bacteriology, the 16 isolates belonged to four (4) LAB species, namely Lactobacillus acidophilus (18.75%), Lactobacillus casei (31.25%), Streptococcus thermophiles (25%) and Lactobacillus bulgaricus (25%). Likewise, 5 isolates were isolated from Qotchqotcha and the five isolates were found to be Lactobacillus acidophilus (80%) and Pediococcus acidilactici (20%). Cell free solution from MRS broth culture of theses LAB was prepared and tested against Escherichia coli O157:H7 and Staphylococcus aureus using agar-well diffusion method. Of the 16 isolates isolated from ergo, 9 of them show antimicrobial activity against E. coli O157:H7 (with a largest inhibition zone measured about 7.33±1.20mm by EK0101) and 12 of them show antimicrobial activity against S. aureus (with a largest inhibition zone measured about 11.66 ±0.88mm by EK0201). On the basis of morphological and biochemical test done, EK0101 was found to be presumptive Lactobacillus acidophilus and EK0201 to be Streptococcus thermophiles. All of the isolates isolated from Qotchqotcha showed antimicrobial activity against the tested organisms though there was a significant difference in their activity ($P<0.05$). The isolate QK0201 showed an inhibition zone of about 6.67±0.88 mm against E. coli and 12.3±1.20 mm against S. aureus. QK0201 was also found to be presumptive Lactobacillus acidophilus. Generally, in this study, ergo and qotchqotcha were found to be a good source of LAB which have the potential to inhibit the growth of many pathogenic bacteria such as E. coli and S. aureus. So, there is a possibility to use them as an alternative therapeutic agent with no risk of antibiotic resistance and also against spoiling microorganisms.

Key words: Agar-well diffusion method, Anti-bacterial activity, Ergo, Lactic acid bacteria, Inhibition zone, Qotchqotcha

1. Introduction

Fermentation is one of the ancient methods used by man to produce and preserve foods. Microbial fermentation has played an important role in food processing for thousands of years. Fermentations provide a way to preserve food products, to enhance nutritive value, to destroy undesirable factors, to make a safe product, to improve the appearance and test of some foods, and to reduce the energy required for cooking (Parish and Davidson, 1993). These significant changes causing desirable biochemical effects involve in the development of new aroma, flavor, and taste and texture there by increasing the sensory quality, palatability and acceptability of the product. (Klaenhammer, 1993). Microbial fermentation has played an important role in food processing for thousands of years. It provides a way to preserve food products by increasing their quality as well as safety and reducing the energy required for cooking. These significant changes develop new aroma, flavor, taste and texture that increase the acceptability and the shelf life of the product.

- *Lamenew Fenta; Lecturer in the Department of Biology, Assosa University, Ethiopia. Email: lamefent21@gmail.com*
- *Animut Assefa; Lecturer in the Department of Biology, Assosa University, Ethiopia. Email: animuta2@hotmail.com*

Such activities are performed by using different metabolites produced from different microorganisms like lactic acid bacteria (LAB) (Esayas et al., 2008). Traditional methods of preparing fermented foods are not complicated and do not require expensive equipment (Eklund, 1984). Fermentation of indigenous foods is, therefore, considered by many to be an effective, inexpensive, and nutritionally beneficial household technology for communities with food scarcity and malnutrition (Jay, 1994). Traditional fermentations are those mediated by the hydrolytic influence of indigenous products derived from the microbial activity of the substrates. The process employs the entire natural microflora that could function under the varied environmental and non-sterile conditions (Klaenhammer, 1993). In Ethiopia, milk is produced in all the agricultural production systems. The bulk (98%) of the milk is produced in rural areas by subsistent farmers (Tsehay, 1998). Although milk is produced in almost every production system of Ethiopia, a minor portion of this milk enters the commercial sectors. Since most farmers live far away from major roads and have no nearby markets and for the fact that milk is relatively perishable food and a high percentage is consumed in a relatively natural state, handling of milk and its products to preserve its natural and desired characteristics is very important (Duane and Cunningham, 1991). In small-scale milk production and for those living in rural areas, pasteurization of milk is rarely practiced except smoking of containers by many rural dairy producers (Taye, 1998). Mogessie and Fekadu (1993) in their study reported that smoking was found to lower the microbial load as compared to unsmoked containers. Traditional milk containers are smoked with "Ejersa"

(Oleaafricana) splinters together with the leaves into which the raw milk is added every day and let to undergo natural fermentation at ambient temperature. The coagulated (curdled) milk called 'Ergo' with its characteristic aromas and flavors would be commonly relished alone, as part of the meal or might also be churned for butter production (Urgaet al., 1992). The rural people in Ethiopia produce fermented milk by traditional methods. The major fermented milk products produced by smallholder farmers by traditional methods include "Ergo" (fermented sour milk), "Ititu" (Fermented milk curd), "Kibe" (traditional butter), "Neterkibe" (spiced butter), "Ayib" (cottage cheese), "Arerra" (Sour defatted milk), and "Aguat" (whey). "Ergo" is traditional Ethiopian fermented milk produced by spontaneous fermentation using traditional utensils under non-hygienic environment. It has some resemblance to yogurt. It is thick, smooth and of uniform appearance and usually has a white milk color when prepared carefully. The product is semi solid and has a pleasant odor and taste. It constitutes a primary sour milk product from which other products may be processed. Depending on the storage temperature, it can be stored for 15-20 days (Almaz Gonfa, et al., 1999). "Ergo" is considered as a special food which serves as a basis for further processing and it is particularly used as a nutritional support to sick people, children and to pregnant and lactating mothers of the family, whilst in the lowland pastoral regions fresh milk is preferred (O'Connor, 1994). "Ergo" fermentation is usually natural, with no defined starter cultures used to initiate it. This is made possible only through the proliferation of the initial milk flora, with microbial succession determined by chemical changes in the fermenting milk. In most urban homes, no attempt seems to be made to control the fermentation. Raw milk is either left at ambient temperatures or kept in warmer places to ferment. In rural areas, particularly among the pastoralists raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as inoculum. Almaz Gonfa, et al. (2001) reported that "Ergo" fermentation is carried out by lactic acid bacteria belonging to the genera Lactococcus, Streptococcus, Luconostoc, and Lactobacillus. They also observed that Micrococcus sp., coliforms and spore formers were also present in fairly high numbers during the first 12-14 hrs of fermentation. Their population decreased substantial thereafter, which implies an antimicrobial activity besides low pH in the fermented milk. Qotchqotcha is a well-known fermented food item in Ethiopia especially in western part of the country, Benishangul Gumuz region. It is mostly consumed along with different foods such as ergo and raw meat. It is prepared from small pepper (Capsicumannum), garlic (Allium-ursinum) and ginger (Zingiberofficinale) with some proportions of different spices are added. Despite its availability in the region, there are very few reports done regarding qotchqotcha. Asnake and Mogese (2010) have reported that it is rich in LAB, especially, lactobacillus, pediococcus, lactococcus and leuconostoc genera are the most abundant ones. Lactic acid bacteria (LAB) comprise a diverse group of Gram positive, non-spore forming cocci, cocco-bacili or rods. In most cases they are anaerobic, Microaerophilic or aerotolerant in their oxygen demands. They are generally catalase and oxidase negative. They are chemo-organotrophic and grow in complex media. LAB in general are nonpathogenic to man and animals. They need fermentable carbohydrates for growth and produce lactic acid as a sole or main product from the energy – yielding fermentation of sugars. Lactic acid bacteria (LAB) are widespread in nature and predominate in microflora of milk and its products, many species are involved in the daily manufacturing of dairy products (Ayadet al., 2004). The lactic acid bacteria consist of several genera, which include Streptococcus, Entrococcus, Lactococcus, Luconostoc, Lactobacillu and Pediococcus. Intestinal microflora is composed of a wide diversity of bacteria that can perform important functions. Lactic acid bacteria, which are found commonly as resident microflora of the gastro-intestinal and genitor-urinary tract of vertebrates (Carr, 2002), are considered as the major probiotic organisms (Collins et al., 1998). Probiotics has been defined as "non-pathogenic microorganisms that, when ingested in certain numbers, exert a positive influence on the host physiology and health beyond inherent general nutrition" (Ouwehand et al., 2002). Several reports have indicated that probiotic lactic acid bacteria are capable of inhibiting pathogenic microorganisms. Some of the probiotic lactobacilli possess inhibitory activity against the growth of pathogenic microorganisms such as Salmonella, Escherichia coli (Dragoet al., 1997), Listeria monocytogens (Harris et al., 1989), Shigella, Pseudomonas and Helicobacter (Servin, 2004). Probiotic lactic acid bacteria have also been reported to reduce urinary tract infection, bacterial vaginosis and yeast vaginitis (Reid et al. 1995). Lactic acid bacteria improve lactose digestion and eliminate symptoms of lactose intolerance (de Vrese, et al., 2001). Some lactic acid bacteria belonging to Lactococcusand Bifidobactrium spp. are reported to metabolize cholesterol (Klaver and van der Meer, 1993). Probiotic lactic acid bacteria also play role in immune modulation in humans (Zoumpopoulouet al., 2008). Although a number of studies demonstrated the inhibitory effect of lactic acid bacteria, isolated from traditional Ethiopian foods, against some food borne pathogens, Bacha et al. (2009) and Tesfaye et al. (2010) recently evaluated the in vitro probiotic properties of lactic acid bacteria isolated from various Ethiopian traditional fermented foods and beverages. Lactic acid bacteria have an important role in the inhibition of food-born pathogenic and spoilage microorganisms with antimicrobial metabolites, including lactic acid, acetic acid, and other organic acids, hydrogen peroxide, bacteriocins and bacteriocin like substances. Most of the traditional fermented products of Ethiopia are consumed without further heating or any other form of processing; thus, they are ideal to carry probiotic bacteria into the digestive system. Therefore, the main objective of this paper is to evaluate the antimicrobial activity of lactic acid bacteria isolates from fermented milk, "Ergo'' and "Qotchqotcha", Ethiopian fermented foods. Traditional fermented foods are highly used by many peoples in Ethiopia; of which ergo and qotchkotcha are the major ones. Ergo is the most highly consumed fermented milk product all over the country including Benishangul Gumuz, while Qotchqotcha is famous in western Ethiopia, particularly Benishangul Gumuz region. Although fermentation by LAB is an integral part of their manufacture, the nature of these products is different from one region to another depending on the local indigenous microflora, handling practice and climatic conditions of the area. Thus, the microbial balance of the local food may be different from other regions. Despite the presence of high consumption of these foods in the region, there are no reports done in isolation, characterization and anti-microbial activity of LAB isolated from local fermented foods, ergo and Qotchqotcha, against some food borne pathogens. Therefore, the isolation and characterization of LAB form the local

fermented food is necessary in order to elucidate the actual LAB that is found in the food and their anti-microbial activity.

2 3.2. Sample Collection and Isolation of LAB

The samples (12 each) for the isolation of LAB were taken from 'ergo' and 'Qotchqotcha' from different kebeles of Assosa Town of Benishangul Gumuz region. After collection, the samples were brought to the laboratory and kept under refrigeration at 4^{o}C until analysis. Twenty-five (25) g of each sample were mixed with sterile 225ml of peptone water (0.1% W/V) and serially diluted according to Erdogrul and Erbilir, (2006). Isolation as well as culturing of LAB was done using the De Man Rogosa Sharpe (MRS) media. A volume of 0.1 ml of appropriate dilutions of the samples was spread-plated in triplicates on pre-dried surface of MRS (de-mann, Rogosa and sharp) agar (Oxoid) plates. The inoculated plates were incubated anaerobically, using anaerobic jars, at 30-32 for 48 hours. Colonies which were different from each other in their morphology and phenotypic appearance were picked up and streaked into fresh MRS medium repeatedly to get a pure culture. The obtained pure culture isolates were characterized using primary biochemical tests namely, gram staining, catalase test and KOH test. Isolates which were gram positive and catalase negative were considered to be presumptive lactic acid bacteria and further characterized by different biochemical test which are described below. For the purpose of our research, the pure culture isolates were also inoculated in MRS broth slant and stored in refrigerator to be used as a source of LAB.

3.3. Characterization of LAB Isolates

The presumptive LAB isolates were purified using isolation media by re-streaking on plates until only a single type of colony remained. The identification of potent isolates up to species level were done based on Bergey's Manual of Systematic Bacteriology and a descriptive table given by Nair and Surendran (2005). The cultures were subjected to a battery of biochemical tests which include fermentation of different carbon sources, acid and gas production from glucose, catalase test, KOH-test (test on lipopolysaccharide), growth at different temperatures (15^{0}C, 30 ^{0}C and 45^{0}C), gram staining and sugar utilization tests (fructose, glucose, maltose, raffinose, xylose and lactose) (Sharpe, 1979)**.**

3.4. Determination of Anti-Microbial Activity of LAB Isolates

3.4.1. Preparation of sample filtrate

The selected LAB isolates were inoculated from slants to fresh 250 ml MRS broth and incubated at 32^{0}C for 48 hrs. Culture of each isolate were killed by heating at 80 ^{0}C for 10 mins followed by centrifuged separately at 10,000 × g (Sorvall super-speed RC2-B) for 30 minutes. The supernatant was collected after centrifugation and passed through 0.2 µm sterile syringe filter (Fisher Scientific Co., Fair Lawn, NJ). To confirm antibacterial activity, the cell free neutral supernatant broths were collected for the antibacterial study against selected food borne pathogens.

3.4.2. Test food borne pathogens

The pure cultures of food borne pathogens namely E. coli O157:H7, and S. aureus, were obtained from Animal Health Laboratory of Assosa.

3.4.3. Antimicrobial activity test by agar well diffusion method

The agar well diffusion method was used to determine the antimicrobial property of the LAB isolates. Culture of the pathogens (E. coli O157:H7, S. aureus,) were suspended in saline and the density of bacterial suspension was adjusted to 0.5 Mcfarland unit spectrophotometer. Then a lawn of the indicator strain was made by spreading the cell suspension over the surface of nutrient agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter (5 mm) was used to cut uniform wells in the agar. Each well was filled with culture free filtrate obtained from the LAB isolates. After incubation at 37^{0}C for 48 hrs, the plates were observed for a zone of inhibition (ZOI) around the well. The experiment was carried out in triplicates and activity was reported as diameter of ZOI ± SD.

3.5. Data Analysis

The data were first checked for their normality. The Data which was not normally distributed was log-transformed and thereafter subjected to analysis of variance (one-way ANOVA) using SAS version 9.1. Fishers Least Significant Difference (LSD) was used to investigate statistical significance between the different anti-bacterial activities of the test microorganisms. Difference between means was considered statistically significant at $P<0.05$.

4. RESULTS AND DISCUSSION

4.1. Morphological Characteristics of the LAB Isolates Isolated from Ergo

Colonies of microorganisms were observed on the surface of MRS plates. More than one colony was observed in most of the cases. From these, twenty-four colonies were randomly picked from MRS agar and striked in to fresh MRS medium. The general properties of the isolate were determined by phenotypic characterization. From the 24 different colonies, 16 of them are found to be gram positive, KOH negative and catalase negative (Table 4.1). The remaining 8 colonies were gram negative and catalase positive. Therefore, only 16 isolates were identified as LAB and further characterized by physiological and biochemical testes. Of the 16 isolates, 5 isolates were isolated from kebele 01, 3 from kebele 02, 3 from kebele 03 and 4 from kebele 04.

Table 4. 1. *Phenotypic characterization of the 16 LAB isolates isolated from 'Ergo' that were collected from the 4 kebeles of Assosa town.*

Isolate code	Samples taken	Gram staining	Catalase test	KOH test	Colony shape	Colony color
EK0101	Kebele 01	Gram+	-	-ve	Rod shaped	White
EK0102	Kebele 01	Gram+	-	-ve	Rod shaped	White
EK0103	Kebele 01	Gram+	-	-ve	Rod shaped	White
EK0104	Kebele 01	Gram+	-	-ve	Rod shaped	White
EK0105	Kebele 01	Gram+	-	-ve	Rod shaped	White
EK0201	Kebele 02	Gram+	-	-ve	Cocci	Yellowish White
EK0202	Kebele 02	Gram+	-/+	-ve	Cocci	White
EK0203	Kebele 02	Gram+	-	-ve	Rod shaped	White
EK0301	Kebele 03	Gram+	-/+	-ve	Rod shaped	Yellowish White
EK0302	Kebele 03	Gram+	-	-ve	Rod shaped	White
EK0303	Kebele 03	Gram+	-	-ve	Rod shaped	White
EK0304	Kebele 03	Gram+	-	-ve	Cocci	White
EK0401	Kebele 04	Gram+	-	-ve	Cocci	White
EK0402	Kebele 04	Gram+	-	-ve	Rod shaped	White
EK0403	Kebele 04	Gram+	-	-ve	Rod shaped	White
EK0404	Kebele 04	Gram+	-	-ve	Rod shaped	White

Among the LAB isolates, rods accounted for 75% whereas cocci accounted for 25 %. Except 2 isolates, all the lab isolates are found to be white in colour (Table 4.1). The other 2 isolates (i.e. EK0201 and EK0301) are exceptionally yellowish white.

4.2. Biochemical Test for Identification of Isolated Lactic Acid Bacteria

Biochemical test result for the identification of presumptive lab were shown in Table 4.2. Among 16 isolates, 3 of them which were found to be gram positive, catalase negative rod, capable of utilizing the sugar such as fructose, glucose, maltose, xylose and lactose but not raffinose, grew at 30 and 45 C but not at 15, grew in the presence of 3% NaCl and 6.5% NaCl. These isolates were considered as presumptive Lactobacillus acidophilus. Likewise, 4 isolates were found to be presumptive Lactobacillus casei based on the biochemical tests done. Lactobacillus casei species are gram positive, catalase negative rod shaped bacteria which are capable of utilizing sugars such as fructose, glucose, maltose but not raffinose, xylose and lactose. This group are capable of growing at 30 and 15 but not at 45 (Sharpe, 1979).

Table 4. 2. *Phenotype characteristics of Lactic Acid Bacteria Isolated from Ergo and their sugar fermentation profile*

Isolate code	Gram stain	KOH test	Catalase test	Sugar utilization test						Growth at temperature (°C)			Growth in NaCl (w/v%)		Most probable LAB
				Fru	Glu	Mal	Raf	Xyl	Lac	15	30	45	3	6.5	
EK0101	Gram+	-	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus
EK0102	Gram+	-	-ve	+	+	+	-	-	-	+	+	-	+	-	Lactobacillus casei
EK0103	Gram+	-	-ve	+	+	+	-	-	-	+	+	-	+	-	Lactobacillus casei
EK0104	Gram+	-	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus
EK0105	Gram+	-	-ve	+	+	-	-	-	+	-	+	+	+	+	Lactobacillus bulgaricus
EK0201	Gram+	-	-ve	-	+	-	-	-	+	+	+	+	+	-	Streptococcus thermophiles
EK0202	Gram+	-/+	-ve	-	+	-	-	-	+	+	+	+	+	-	Streptococcus thermophiles
EK0203	Gram+	-	-ve	+	+	-	-	-	+	-	+	+	+	+	Lactobacillus bulgaricus
EK0301	Gram+	-/+	-ve	+	+	+	-	-	-	+	+	-	+	-	Lactobacillus casei
EK0302	Gram+	-	-ve	+	+	+	-	-	-	+	+	-	+	-	Lactobacillus casei
EK0303	Gram+	-	-ve	+	+	-	-	-	+	-	+	+	+	+	Lactobacillus bulgaricus
EK0304	Gram+	-	-ve	-	+	-	-	-	+	-	+	+	+	-	Streptococcus thermophiles
EK0401	Gram+	-	-ve	-	+	-	-	-	+	-	+	+	+	-	Streptococcus thermophiles
EK0402	Gram+	-	-ve	+	+	-	-	-	+	-	+	+	+	+	Lactobacillus bulgaricus
EK0403	Gram+	-	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus
EK0404	Gram+	-	-ve	+	+	-	-	-	+	-	+	+	+	+	Lactobacillus bulgaricus

Keys: Fru: fructose utilization; Glu: Glucose utilization; Mal: Maltose utilization; Raf: Raffinose utilization; Lac: Lactose utilization

Gram positive, catalase negative, cocci, which grew at 45 and capable of utilizing the sugar glucose and lactose but not fructose, maltose, xylose and raffionse were considered as presumptive Streptococcus thermophiles. 3 of the isolates were Streptococcus thermophilus. The remaining 4 isolates were considered as presumptive Lactobacillus bulgaricus which are gram positive, catalase, negative, rods. This group are capable of utilizing the sugar fructose, glucose and lactose but no other sugars tested (Sharpe, 1979). According to the table described above, four (4) LAB species belonging to 2 genera were isolated from ergo and these include Lactobacillus acidophilus, Lactobacillus casei, Streptococcus

thermophilus and Lactobacillus bulgaricus (Table 4.2). Data in table showed that the majority of the isolates belongs to the genus Lactobacillus. Other researches such as Savadogo et al (2004) had also identified the isolation from fermented cow and lamb milk and observed that the most dominant bacteria were those from genus Lactobacillus. Generally, the species identified in the present study, were in good agreement with other studies made by Desalegn Amenu (2013) who identified Lactobacillus and Streptococcus in Ethiopian Ergo.

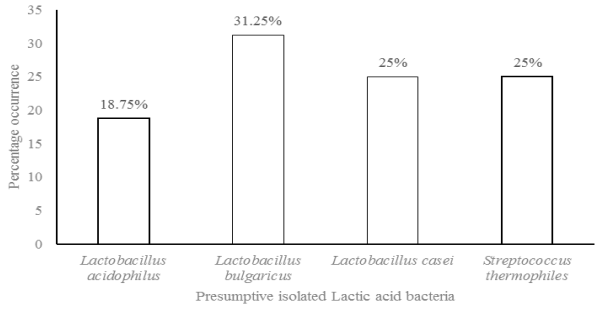

Figure 4. 1. *Percentage occurrence of lactic acid bacteria isolated from Ergo*

4.3. Antibacterial Activity of the Isolates

Of the 16 isolates, 9 of them show antimicrobial activity against Escherichia coli O157:H7 with the average diameter of the inhibition zone measured ranged from 0-7.33 mm in size. The isolate EK0101 isolated from ergo produced the maximum inhibition zone (7.33±1.20mm) against the tested microorganisms Escherichia coli O157:H7. This can be compared to the criteria of antimicrobial activity classification which is moderate (6-9 mm), strong (10-14 mm), and very strong (15-18 mm). Accordingly, the isolates EK0101, EK0104, EK0201, EK0202 and EK0304 possessed moderate activity, while the isolates EK0102, EK0203, EK0301, EK0302, EK0303, EK0402 and EK0404 did not show any activity against the tested organism. Though it is insignificant, the isolates EK0103, EK0105 and EK0403 also show an activity against the tested organisms. When we compare the antimicrobial activity of each isolates each other, the isolates showed statically significant activity ($P<0.05$). Cell free supernatant of LAB contains bacteriocin's, hydrogen peroxide, diacetyl and organic acids. The antimicrobial activity of these lactic acid bacteria may be due to various antimicrobial compounds such as organic acids by decreased pH levels, hydrogen peroxide, or presence of bacteriocins (Luo et al., 2011). Bacteriocin is an antimicrobial proteinaceous secretion of lactic acid bacteria. Abada (2008) documented that most of the bacteriocin's are purely build up with peptides as well as some are the compositions of protein, carbohydrates and lipids.

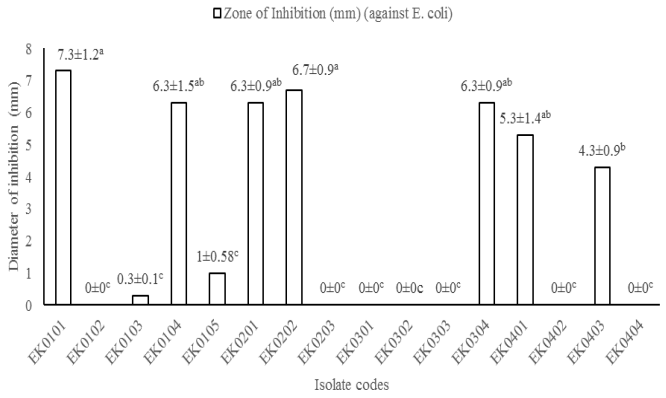

Figure 4. 2. *The diameter of inhibition of the of LAB isolates against E. coli O157:H7 (the values are mean ± SE; Means with the same letter are not significantly different).*

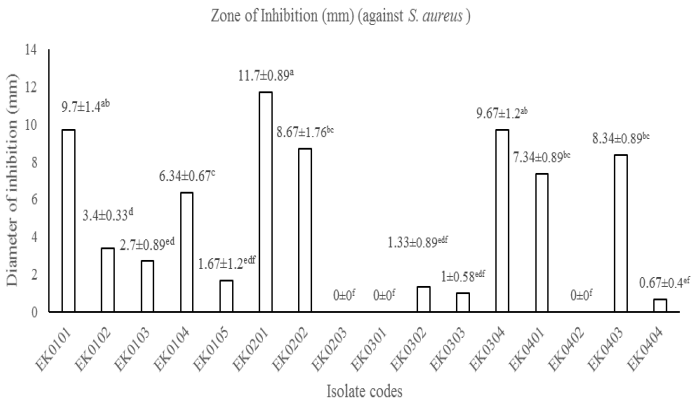

Figure 4. 3. *The diameter of inhibition of the of LAB isolates against Staphylococcus aureus (the values are mean ± SE; Means with the same letter are not significantly different).*

The antimicrobial activity of LAB isolates against Staphylococcus aureus is also shown in figure 4.3. According to the table, of the 16 isolates, 12 of them show antimicrobial activity against Escherichia coli O157:H7 with the average diameter of the inhibition zone measured ranged from 0 to 11.66 mm in size. When we compare the antimicrobial activity of each isolates each other, the isolates showed stastically significant activity ($P<0.05$). The isolate EK0201 isolated from the Ergo produced the maximum inhibition zone (11.66 ±0.88mm) against Staphylococcus aureus. On the basis of morphological, physiological and biochemical characters as well as sugar utilization pattern, the isolate is found to be presumptive Streptococcus thermophilus. The isolates namely EK0203, EK0301 and EK0402 did not show any activity against Staphylococcus aureus. It seems that the gram-negative organisms namely, E. coli O157:H7 showed less inhibition compared to the gram-positive organisms. This is in accordance with some of the earlier reports which showed that bacteriocins of LAB were more active against gram-positive organisms compared to gram-negative organisms (Jack et al., 1995; Patil et al., 2010; Savino et al., 2011). The reason for the observed activity may be due to the presence of an outer protective membrane in gram negative organisms, which covers the cytoplasmic membrane and peptidoglycan layer.

4.4. Morphological Characteristics of the LAB Isolates Isolated from Qotchqotcha

The results of phenotypic characterization of the isolates isolated from 'Qotchqotcha' that were collected from the 4 kebeles of Assosa town are shown in Table 4.3. Seven colonies were randomly picked from MRS agar and striked in to fresh MRS medium. The general properties of the isolate were determined by phenotypic characterization. Of the seven isolates, 5 of them are found to be gram positive, KOH negative and catalase negative (Table 4.4). Therefore, they were identified as presumptive LAB and further characterized by physiological and biochemical testes. The other isolates are gram negative and catalase positive so they are not considered as LAB. Among the LAB isolates, rods accounted for 80% whereas cocci accounted for 20 %. All the lab isolates are found to be white in colour.

Table 4. 3. *Phenotypic characterization of the isolates isolated from "QotchQotcha" that were collected from the 4 kebeles of Assosa town.*

Isolate code	Samples taken	Colony shape	Colony color	KOH test	Catalase test
QK0101	Kebele 01	Cocci	White	-	-
QK0201	Kebele 02	Rod shaped	White	-	-
QK0202	Kebele 02	Rod shaped	White	-	-
QK0301	Kebele 03	Rod shaped	White	-	-
QK0401	Kebele 04	Rod shaped	white	-	-

4.5. Biochemical Test for Identification of Isolated Lactic Acid Bacteria isolated from Qotchqotcha

Table 4. 4. *Phenotype characteristics of Lactic Acid Bacteria Isolated from Qotchqotcha and their sugar fermentation profile.*

Isolate code	Gram stain	Catalase test	Sugar utilization test						Growth at temperature (°C)			Growth in NaCl (w/v %)		Most probable LAB
			Fru	Glu	Mal	Raf	Xyl	Lac	15	30	45	3	6.5	
QK0101	Gram+	-ve	+	+	-	+/-	+	+	-	+	+	+	+	Pediococcus acidilactici
QK0201	Gram+	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus
QK0202	Gram+	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus
QK0301	Gram+	-ve	+	+	+	-	+	+	+	+	+	+	+	Lactobacillus acidophilus
QK0401	Gram+	-ve	+	+	+	-	+	+	-	+	+	+	+	Lactobacillus acidophilus

Keys: Fru: fructose utilization; Glu: Glucose utilization; Mal: Maltose utilization; Raf: Raffinose utilization; Lac: Lactose utilization

Biochemical test result for the identification of presumptive lab were shown in Table 4.4. Among 5 isolates, 4 of them are found to be gram positive, catalase negative rod, capable of utilizing the sugar such as fructose, glucose, maltose, xylose and lactose but not raffinose, grew at 30 °C and 45 °C but not at 15 °C, grew in the presence of 3% NaCl and 6.5% NaCl. These isolates are considered as presumptive Lactobacillus acidophilus. The remaining isolates are found to be presumptive Pediococci acidilactici based on the biochemical tests done. Pediococci acidilactici species are gram positive, catalase negative rod shaped bacteria which are capable of utilizing sugars such as fructose, glucose, xylose and lactose but not maltose. This group shows a variation in raffinose utilization. This group are capable of growing at 45 °C and in medium containing 3 % and 6.5 % NaCl (Sharpe, 1979). So, according to the data obtained, two (2) LAB species belonging to 2 genera were isolated from Qotchqotcha and these include Lactobacillus acidophilus and Pediococcus acidilactici. The percentage abundance of each isolates was shown in the figure below. 80 % of the isolates are presumptive Lactobacillus acidophilus and the remaining percentage is accounted by Pediococcus acidilactici

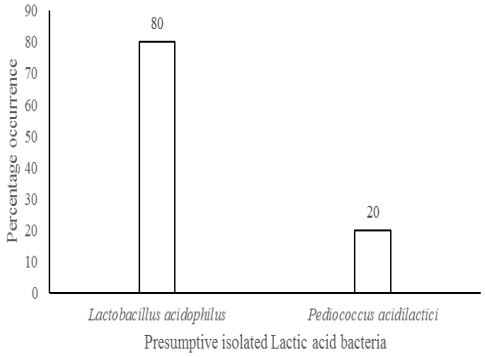

Figure 4. 4. *The percentage occurrence of the identified LAB isolates*

4.6. Antibacterial activity of the isolates isolated from Qotchqotcha

The average diameter of inhibition of the LAB isolates isolated from Qotcqotcha against E. coli O157:H7 and S. aureus are shown in the figure below.

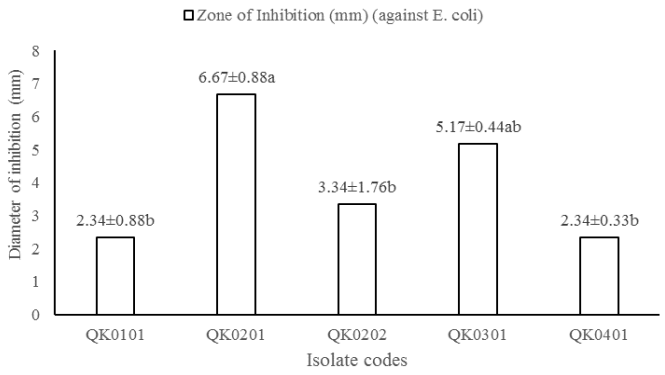

Figure 4. 5. *The diameter of inhibition of the LAB 0isolates isolated from Qotcqotcha against E. coli O157:H7 (the values are mean ± SE; Means with the same letter are not significantly different).*

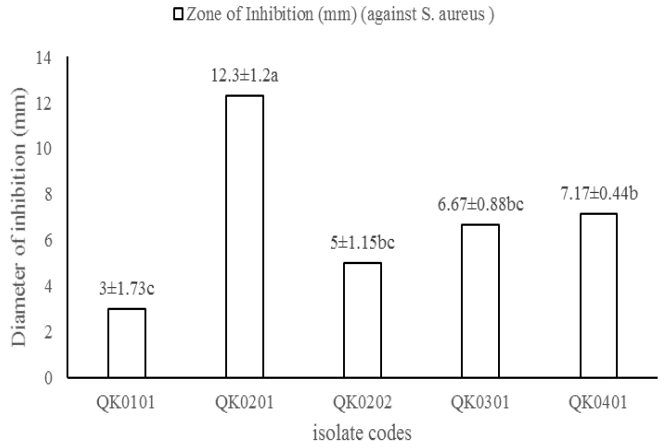

Figure 4. 6. *The diameter of inhibition of the LAB isolates isolated from Qotcqotcha against S. aureus (the values are mean ± SE; Means with the same letter are not significantly different).*

Antibacterial activity of the isolate QK0201 showed maximum activity against pathogenic bacteria as compare to others. Table 4.4 revealed that QK0201 were Lactobacillus acidophilus. This is in line with the finding made by Zahid et al (2015). In their research, they found that L. acidophilus showed maximum activity against pathogenic bacteria such as Methicillin-Resistant-Staphylococcus-aureus, E. coli, Salmonella and Staphylococcus aureus. Even though the isolates QK0202, QK0301, QK0401 are identified as L. acidophilus, they show a relatively weaker inhibition against the tested pathogens compared to QK0201 which is also identified as L. acidophilus. This variation in activity observed for the different isolates belonged to the same species may be strain difference. The activity of QK0201 is also found to be maximum against S.auerus. Compared to its activity against E. coil the activity of Lactobacillus acidophilus is found to be higher for S. aureus. This is in accordance with some of the earlier reports which showed that bacteriocins of LAB were more active against gram-positive organisms compared to gram-negative organisms (Jack et al., 1995; Patil et al., 2010; Savino et al., 2011). The reason for the observed activity may be due to the presence of an outer protective membrane in gram negative organisms, which covers the cytoplasmic membrane and peptidoglycan layer.

5. Conclusion

Generally, in this study, Ergo and Qotchqotcha were found to be a good source of lactic acid bacteria which have the potential to inhibit the growth of many pathogenic bacteria such as E. coli O157:H7 and staphylococcus aureus. So, there is a possibility to use them as an alternative therapeutic agent with no risk of antibiotic resistance and also against spoiling microorganisms.

6. Acknowledgments

Firstly, we would like to express our deepest gratitude to Asossa University for financing this research. We are also very thankful for Assosa Animal Health Laboratory for their positive cooperation and permission to undertake our laboratory work. We also thank Assosa university Biology and Chemistry departments for their assistance both ideally and materially

REFERENCES

[1] Abada EA, (2008) Isolation and characterization of a antimicrobial compound from Bacillus coagulans, Anim. Cell Syst, 1: 41-46.

[2] Almaz Gonfa, Alemu Fite, Kelbesa Urga and Berhanu Abegaz Gashe.1999. Microbiological aspects of Ergo (Ititu) fermentation. Ethiopian Journal of Science .22(2): 283-290.

[3] Almaz Gonfa, Foster, H A., Holzapfel, W. H. 2001. Field survey and literature review of Ethiopian traditional fermented milk products. International Journal of Food Microbiology. 68: 173-186.

[4] Anteneh Tesfaye, Tetemke Mehari and Mogessie Ashenafi. 2011. Antagonism of lactic acid bacteria against foodborne pathogens during fermentation and storage of borde and shamita, traditional Ethiopian fermented beverages. Int. Food Res. J. 18(3): 1189-1194

[5] Asnake Dessalegn and Mogessie Ashenafi. 2010. Evaluation of the Probiotic Properties and Antibiotic Resistance of Lactic Acid Bacteria Isolated from Awaze, Qotchqotcha and Tef dough, traditional Ethiopian fermented foods. Internet Journal of Food Safety.12:187-191

[6] Axelsson, L.T., Chung, T.C, Dobrogosz, W.J and S.E.Lindgren. 1989. Production of a broad spectrum antimicrobial substance by Lactobacillus reuteri. Microb. Ecol. Health Dis. 2: 131–136.

[7] Bauer, R and L.M. Dicks. 2005. Mode of action of lipid II-targeting lantibiotics. Int. J. Food Microbiol.101: 201–216.

[8] Chang, B.L., Sheik, Y.H., Wang, L.H, Liao, C.K and H.S. Gill, 2000. Enhancing Immunity by Dietary Consumption of a Probiotic Lactic Acid Bacterium (Bifidobacterium lactis HN019): Optimization and Definition of Cellular Immune Responses. Eur J ClinNutr. 54:849-855

[9] Chung, T.C., Axelsson, L.T., Lindgren, S.E. and W.J.

Dobrogosz.1989. In vitro studies on reuterin synthesis by Lactobacillus reuteri. Microb. Ecol. Health Dis.2: 137–144.

[10] Collins, J.K., Thornton, G and G.O.Sullivan. 1998. Selection of probiotic strains for human applications. Int. Dairy J. 8: 487-490.

[11] CSA (Central Statistical Agency). 2007.Agricultural Sample Survey 2007/08. Volume II. Report on livestock and livestock characteristics (private peasant holdings). CSA, Addis Ababa, Ethiopia.

[12] De Vrese, M., A. Stegelmann, B. Richter, S. Fenselau, C. Laue and J. Schrezenmeir. 2001. Probiotics compensation for lactase insufficiency. Am.J.Clin. Nutr. 73:421–429.

[13] Drago, L., Gismondo, M.R., Lombardi, A., de Haen, C. and L. Gozzini. 1997. Inhibition of in vitro growth of entero pathogens by new Lactobacillus isolates of human intestinal origin. FEMS Microbiol. Lett**.** 153:455-463.

[14] Duane, A. and M. Cunningham.1991. Animal science and Industry. 4th ed. Prentice Hall, Engle Wood Clifts, New Jersey.

[15] Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E. and D.A, Relman. 2005. Diversity of the human intestinal microbial flora. Science **308**, 1635 – 1638.

[16] Eklund, T.1984. The effect of carbon dioxide on bacterial growth and on uptake processes in the bacterial membrane vesicles. International Journal of Food Microbiology. 1: 179- 185.

[17] Gibson, G.R., J.M. Saavedra, S. McFarlane and G.T. McFarlane.1997. Probiotics and intestinal infections. In: Probiotics2: Applications and Practical Aspects, Fuller, R., ed., New York: Chapman and Hall, pp10–38.

[18] Gilliland, S.E. and M.L. Speck. 1977. Deconjugaton of bile acids by intestinal lactobacilli. Appl. Environ. Microbiol 33:15–18.

[19] Girum Tadesse, Eden Ephraim and Mogessie Ashenafi (2005)a. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shameta, traditional Ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. Int. J. food Safety, **5:**13-20.

[20] Gregor, R. 2010. The potential role for probiotic yogurt for people living with HIV/AIDS. Gut Microbes.1:411-414.

[21] Harris, L.J., Daechsel, M.A., Stiles, M.E and T.R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against Listeria monocytogens. J. Food Protect. 52:384-387.

[22] Jay, J.M. 1994. Modern Food Microbiology, 4th ed. Van Nostrand Rainhold, New York.

[23] John, L.1998. Laboratory Manual for Food Microbiology Laboratory. University of Wisconsin, Madison.

[24] Kendall, P. 2012, Bacterial Foodborne Illness. Food and Nutrition Series, Colorado state university. Factsheet No.9.300

[25] Ketema Bacha, Tetemke Mehari and Mogessie Ashenafi. 2009. In-vitro probiotic potential of lactic acid bacteria isolated from 'Wakalim', a traditional Ethiopian fermented beef sausage. Ethiop J Health Sci. 19(1**):** 21-29.

[26] Klaenhammer, T.R. 1993.Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12: 39–85.

[27] Klaver, F.A.M and R. van der Meer. 1993. The assumed assimilation of cholesterol due to their bile deconjugation activity. Appl. Environ. Microbiol. 59:1120.

[28] Leroy, F and L. deVuyst .2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. TrendsFood Sci Technol.15, 67–78.

[29] Lindgren, S.E and W.J. Dobrogosz.1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87: 149–163.

[30] Linscott, A. J. 2011. Food-Borne Illnesses. Clinical Microbiology Newsletter, 33: 41-45.

[31] Luo, F., Feng, S., Sun, Q., Xiang, W., Zhao, J., Zhang, J., Yang, Z. 2011. Screening for bacteriocin-producing lactic acid bacteria from kurut, a traditional naturally-fermented yak milk from Qinghai Tibet plateau. Food Control, 22: 50 53.

[32] Luo, F., Feng, S., Sun, Q., Xiang, W., Zhao, J., Zhang, J., Yang, Z. 2011. Screening for bacteriocin-producing lactic acid bacteria from kurut, a traditional naturally-fermented yak milk from Qinghai Tibet plateau. Food Control, 22: 50 53.

[33] Macfarlane, G.T. and G.R. Gibson. 1995. Bacterial infections and diarrhea. In: Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology, Gibson, G.R. and G.T. Macfarlane,eds., Boca Raton, FL: CRC Press, pp 201–226.

[34] Marteau, P., P. Pochart, Y. Bouhnik and J.C. Rambaud. 1993. Fate and effects of some transiting microorganisms in the human gastrointestinal tract. World Rev. Nutr. Diet. 74:1–21.

[35] Muhammad Zahid, Muhammad Ashraf, Muhammad

Arshad, Ghulam Muhammad, Aqeela Yasmin and Hafiz Muhammad Adnan Hameed (2015) Antimicrobial Activity of Bacteriocins Isolated from Lactic Acid Bacteria Against Resistant Pathogenic Strains, international Journal of Nutrition and Food Sciences, 4(3): 326-331

[36] Nair, P. S. and P.K. Surendran. 2005. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. J. Culture Coll. **4:** 48-52.

[37] O'Connor, C.B. 1994. Rural Dairy technology. ILRI training manual No.1. International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. 133pp.

[38] Ouwehand, A.C, Salminen, S. and E. Isolauri. 2002. Probiotics: an over view of beneficial effects. Antonie Van Leeuwenhoek. 82:279-289.

[39] Parvez, S, Malik, K.A, Ah Kang, S. and H.Y. Kim. 2006. Probiotics and their fermented food products are beneficial for health. J. Appl. Microbiol. 100:1171-1185.

[40] Saulat, J. 2012. Epidemiology of foodborne illness, Scientific, Health and Social aspects of the food industry, Dr. Benjamin Valdez (Ed), ISBN 978-953-307-916-5, In Tech, Available at: http://www.itechopen.com/books/scientific-health-and socialaspects-of-the-food-industry/epidemiology-of-foodborneillness. Accessed 13 October 2014.

[41] Sharpe, M.E., 1979. Identification of the Lactic Acid Bacteria. In: Identification Methods for Microbiologists, Skinner, F.A and D.W. Lovelock (Eds.). Academic Press, London, pp: 233-259.

[42] Sikes, M and J.M. Bruno-Barcena. 2011. The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer. Am J Physiol Gastrointest Liver Physiol. 301:401-24.

[43] Simango, C. 1997. Potential use of traditional fermented foods for weaning in Zimbabwe. Journal of Social Science and Medicine, 44, 1065–1068.

[44] Taye T.1998. Qualities of Cow milk and the effect of Lactoperoxidase system on preservation of milk at Arsi, Ethiopia. Msc. Thesis, Alemayal University, Ethiopia.

[45] Trenev, N.1998. Probiotics: Nature's Internal Healers. Garden City Park, NY: Avery Publishing Group.

[46] Tsehay, R. 1998. Milk processing and marketing options for rural small-scale producers. In: proceedings of the 5th Annual conference of Ethiopian Society of Animal Production (ESAP). 15-17 May, 1997. Addis Ababa, Ethiopia. Pp. 61-71.

[47] Urga, K., B.A. Gashe, A. Fite and A. Nigatu.1992. Changes in acidity and lactic acid production during Ititu fermentation. Ethiopian Journal of Agricultural Science. 9:91-95