Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.19, 2015

Screening Potential Probiotic Bacteria as Starter Culture from Traditional Fermented Ensete (Ensete Ventricosum (WELW) Cheesman

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Abstract
Fermented Ensete (*Ensete ventricosum (*Welw) Cheesman) commonly called false banana is an important nutrient source in southern part of Ethiopia. This study aimed at screening potential probiotic bacteria from a traditional fermented Ensete. A total of 12(twelve) samples were collected from local traditional fermented Ensete. Various morphological and biochemical tests were performed for screening of the potential probiotic bacteria as starter culture. Media optimization was carried out for cultivation of the potential isolate. The potential isolate was found to be gram positive, non motile, negative for (Catalase, indole). This bacterium had broad range of pH 4.0 – 8.0 and temperature of 25-40 (^{0}c). It was tolerant to 1-4% salt concentration. High growth rate of the isolate was observed in the presence of 2 % glucose and 0.2 % peptone. The optimum temperature and pH for high biomass production was observed at 37^{0}c and pH of 7. Fermentation of Ensete with isolate increased the nutritional value and aroma of a food as well. The isolate was found to be *Lactobacilli sp. CH2* which fulfills the required criteria for a probiotic such as tolerance to such as high salt, low pH, body temperature range. The result in this study is reproducible and reliable for further processing of Ensete in the local community. Molecular study should be done for characterization of this potential strain.
Keywords: *Lactobacilli*, Prebiotic, Probiotic, Starter culture,

INTRODUCTION

The term probiotic refers to viable, non-pathogenic microorganisms when ingested confer health benefits for the consumer. They are commonly lactic acid producing and constitute a major part of the normal intestinal microflora in animals and humans (De Vrese and Schrezenmeir, 2008). These include a wide spectrum of species of *Lactobacillus*, *Bifidobacterium*, *Streptococcu*s, *Lactococcus*, some *Enterococcus* species and probiotic yeast *Saccharomyces boulardii* (Morrow *et al.,* 2012).

A number of health benefits have been claimed for probiotic bacteria and are also being recommended as a preventive approach to maintain the balance of intestinal microflora in human as well as cattle. Their beneficial effects on humans include stabilization of intestinal microflora (Denev, 2006), reduction of lactose intolerance (De Vrese and Schrezenmeir, 2008), prevention of antibiotic-induced diarrhea, and stimulation of the immune system. In addition, probiotic have been found to produce antimicrobial products during fermentation which used for safe and long storing of foods (Corgan *et al.,* 2007). They fights against pathogenic bacteria through blocking pathogenic bacteria effects by producing bactericidal substances , competing with pathogens , toxins for adherence to the intestinal epithelium, regulation of the immune responses enhancing the innate immunity and stimulating protective responses (Corcionivoschi *et al.,* 2009). The probiotic microorganisms have a characteristic tolerance to high acidity, bile and ability to adhere to intestinal surfaces to survive and colonize in gastro-intestinal tract of the host (Isolauri *et al.,* 2001).

Fermentation is one of the oldest and most common methods of food processing and preservation. Human being has known to use these probiotic microbes for preparation of food products and drinks for years. Thus practically all nations have one or more traditional type of fermented products made by the action of *Lactobacilli* and yeast alone or in combination with other microorganisms (Maiangwa *et al.,* 2013).

In Ethiopia, over 80% of the Ensete production is concentrated in the south and south-western part of the country (Taye *et al.,* (1967). Ensete products such as *Bulla* and *Kocho* are serving as the staple and co-staple food for many people in southern part of the country. The fermented *Kocho* is often stored in pits that are lined with Ensete leaves and left in a storage pit for a minimum of a month, even for several years. Fermentation played a significant role in *Kocho* preparation by reducing fermentation time and enrichment of the product through development of flavors, aromas, and textures (Yirmaga, 2013). Thus, fermentation process has been found to produce flavor enhancing compounds, useful enzymes and essential amino acids. Currently, the significance of production of functional foods containing probiotic bacteria such as *lactobacilli* and *Lactococcus* are increasing. These bacteria enhance the microbial safety and offer organoleptic, technological, nutritional, or health benefits to the consumer. One of the *lactobacilli* being used as probiotic is *lactobacilli casei.* Probiotic bacteria, the most common lactic acid producing bacteria (LAB), are becoming more important by the bacteriocins production which have been used as bio-preservatives in model food systems and shown to be effective in inhibiting the pathogenic and food spoilage microorganisms (Patil *et al.,* 2011). Thus the aim of this research was to isolate

and characterize potential probiotic bacteria from Ensete (Kocho) samples and to assess its effect on fermentation.

MATERIALS AND METHODS

Sample collection and Isolation of probiotic bacteria

A total of 12 fermented ensete samples were collected for screening of probiotic bacteria. Of which four were from Chencha, four from Sawla bulki and four from shefite districts of Gamo Gofa zone .The samples were transported to the laboratory under sample box and stored at 4^0c for further experiment.

Then after, serial dilution and spread agar techniques were used for isolation of colonies (Hoque *et al.,* 2010). Ten (10) gram of each fermented sample was dissolved into 10ml of MRS broth. After dissolving into MRS broth was homogeneously and incubated at 37^oC for 24 hrs in an aerobic condition. In serial dilution agar plate technique, suspension serial dilution up to 10^{-6} were made when appropriate and inoculated to MRS agar plates at 37^0C for 48 hrs. The plates were observed for appearance of colonies. Bacteria colony were isolated and transferred to MRS agar slants and then maintained at 4^0c for further experiments (Hoque *et al.,* 2010).

Effect of pH and Temperature

The isolated bacterial cultures were inoculated into sterile MRS broth at pH 4-10 and incubated at 37^0C for 2-3 days. Growth of the bacteria was measured at 540 nm absorbance using UV-vis spectrophotometer. The growth of probiotic bacteria on given pH was used to designate isolates as pH tolerant. Further, the selected bacterial culture was grown at varying temperatures, i.e. 25- 40^0C for 2-3 days. The Growth of the bacteria was measured. The growths of probiotic on MRS broth were used to designate isolates as temperature tolerant (Tameka and Bhutada, 2010).

Lactose utilization and Salt tolerance test

The acid producing bacterial cultures were detected by observing the change in colour of the medium and detecting the pH using digital pH meter. Sterilized fermentation medium (10g peptone, 15g NaCl, 00.18g phenol red, 5g lactose, for 1L distilled water and final pH 7.0) were inoculated with different cultures and incubated at 35^0C for 24-48 hrs. In addition, the salt tolerances of selected bacterial cultures were assessed after 24-72hrs of incubation at concentration of 1-5% NaCl in MRS broth. Growth of the bacteria was measured at 540 nm (Hoque *et al.,* 2010).

Media optimization for the growth probiotic bacteria

Bacterial cultures with good antimicrobial activity, pH tolerant, and temperature tolerant and salt tolerant and acid producing bacteria were selected for further determination of probiotic growth condition. Pure culture of the isolate was used that isolated from traditionally fermented Ensete. This probiotic was grown in standard MRS culture medium as well as our test mediums containing different concentration of carbon source and nitrogen source. The experiments were carried out in replicate. The media composition was shown in table 1.

Table 1: Media composition

Ingredients	**Grams/Liter**
Peptone	10
Meat extracts	8
Yeast extract	4
D (+)-Glucose	2
Dipotassium hydrogen phosphate	2
Sodium acetate trihydrate	5
Triammonium citrate	2
Magnesium sulfate heptahydrate	0.2
Manganous sulfate tetrahydrate	0.05

Fermentation of Ensete

The fresh harvested Ensete was taken for fermentation and biochemical characterization. The 0.1 kg Ensete sample was placed in five test tubes. Each test tube was 20 mg of sample and inoculated with the 1ml broth of the potential isolate. The nutritional value of Ensete including total carbohydrate, protein, ash and the like was biochemically determined to evaluate the efficiency of the isolate.

Proximal analysis of Ensete

Determination of total carbohydrate by anthrone method

Anthrone reagent was prepared .Weigh 100 mg of the sample was added into a boiling tube and hydrolyses by keeping it in a boiling water bath for three hrs with 5 mL of 2.5 N HCl and cool to room temperature. The hydrolyzed sample was neutralized 1N NAOH until the effervescence ceases and makeup the volume to 100 mL and centrifuge 0.5 and 1 mL aliquots supernatant was collected for analysis.

The standards was Prepared by taking 0- 1 mL and makeup the volume to 1 mL in all the tubes including the sample tubes by adding distilled water. 4mL of anthrone reagent was added and heat for 10min in a boiling water

bath Cool rapidly and read the green to dark green color at 630nm. The standard graph was plotted and calculates the amount of carbohydrate present in the sample tube (Hedge and Hofreiter, 1962).

Determination of moisture content

Moisture of the Ensete sample was determined using standard method (AOAC, 2000). A clean dried and covered flat aluminum dish was weighed and about 5gm of the sample was transferred to the dish. The dish then placed in the oven at 102 ^{0}C for 24 hrs and cooled in desiccators and re-weighed. Then, the moisture content was estimated by the formula:-

Moisture content (%) = (moisture of fresh sample –moisture of dry sample) 100
Weight of fresh sample

Determination of total ash

A dry porcelain dish containing 2g sample was placed in a muffle furnace set at 550 ^{o}c for 1hr and then allowed to cool in a desiccators and weighed. The ash content was determined using standard method (AOAC, 2000). It is calculated as follows using the formula:-

$$\text{Total } Ash(\%) = \{(W2 - W)/(W1 - W2)\} * 100$$

Where, W=weight in gram of empty dish, W1 = weight in gram of empty dish plus dry sample, W2 = weight in gram of empty dish plus ash.

Total crude fat content

A 5 g dried sample of *Kocho* was extracted with 150 ml hexane, for a minimum period of 4 hrs in the soxhlet extractor. The solvent was then evaporated and heated in a steam bath. The flask containing the extracted fat was dried on steam bath to a constant mass. The total crude fat was calculated as percentage by weight (Yirmaga, 2013).

$$\text{Crude } fat(\%) = \{(W2 - W1)/(W)\} * 100$$

Where, W1=weight of the extraction flask, W2=weight of the extraction flask plus the dried crude fat (g), W=weight of the sample

Total crude fiber content:

Crude fiber analysis was conducted using standard method (AOAC, 2000). About 15g weighed sample was transferred into a 600ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Data were recorded placing a watch glass over the mouth of the beaker. Then after, 20 ml 28% KOH was added and again boiled gently for further 30 minutes subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. Then, filtered and dried it in the electric oven at 130 ^{o}C for 2hrs. Then, it was cooled at room temperature for 30 minutes in desiccators and weighed. Thereafter the sample was transferred to crucible to muffle furnace for 30 minute ashing at 550 ^{o}C. Finally, the sample was cooled again in desiccators and re-weighed. The crude fiber content was determined by using the formula:-

$$\text{Crude } Fiber(\%) = \{(W2 - W1)/(W3)\} * 100$$

W 1= crucible weight after drying, W2 = crucible weight after ashing, W 3= dry weight, m = % moisture of the sample.

Total crude protein content by Lowry method

Non-fermented and fermented Ensete sample was extracted by soxlet and pipette out 0.2 ml extracted protein solution was added labeled test tube. Two (2) ml of alkaline copper sulphate reagent (analytical reagent) was mixed the solutions well. This solution was incubated at room temperature for 10 mins. Two (0.2) ml of reagent Folin-Ciocalteau reagent to a tube and incubate for 30 min. The colorimeters were adjusted to zero with blank and take the optical density at 660nm. The absorbance of unknown sample and determine the concentration of the unknown sample was calculated using the standard curve plotted (Lowry *et al.,* 1951).

RESULT AND DISCUSSION

Sample collection and Isolation of probiotic bacteria

Probiotic bacteria cultures were isolated from fermented Ensete. Hundred thirty five colonies were isolated; of which 16 of them are non motile and the rest are motile. Only the six isolates of 16 were considered for morphologically and biochemically characterized. Table 2 shows the colony characteristics of the isolates, their Gram reaction, microscopic examination and biochemical characterization (Figure 1, Table 3). The isolates were grown on selective MRS agar media produced round shape, white colour and shiny colonies. Isolates when Gram stained, found to be *lactobacilli*, positive in Gram reaction those all are typical characteristics of *Lactobacilli spp*.

The goal of this research work was to isolate and characterize potential probiotic bacteri from enset samples collected and morphological, biochemical characterization and to assess their anti-bacterial activity against some common pathogenic bacteria. Based on the morphological characteristics one isolate was identified as *Lactobacilli spp* from enset samples. The potential probiotic bacteria which can be used as a starter culture were isolated from the enset that applied to examine the fermentation process, the microbial dynamics and the

physicochemical changes that occur during controlled fermentation of Ensete.

Table 2: Morphological characteristics of isolates

Isolates code	Morphological characterization						
	Microscopic characteristics				Colony characteristics		
	Gram staining	Non Spore former	AFB	Motility	Shape	Size	Color
CH2	G+	-ve	-ve	Non motile	Circular	Large	Whitish
SH	G+	-ve	-ve	Non motile	Circular	circular	Whitish
CH3	G+	-ve	-ve	Non motile	Circular	Small	Whitish
CH4	G+	-ve	-ve	Non motile	Circular	Large	Whitish
CH5	G+	-ve	-ve	Non motile	Circular	Large	Whitish
CH6	G+	-ve	-ve	non motile	Circular	Large	Whitish

Only Gram positive, non-motile, rod shaped, non acid fast and non spore former bacteria were showing phenotypic characters similar to probiotic bacteria were selected for further experiments.

The isolated bacteria were rod shaped and gram positive, morphologically large, smooth, shiny, and regular and raised, anaerobic, non-spore forming, negative for indole, VP, Citrate, and Catalase which indicates them to be the member of *Lactobacilli spp.* similar studies was observed by Ahmed and Kanwal, (2004) and Klayraung, (2008) gram's staining and Catalase test could support the characterization of *lactobacilli*. Gram-positive rod shaped, non-spore forming, and Catalase negative which indicated the typical basic characteristics of *lactobacilli*.

Table 3: Biochemical test characterization of isolate

Isolates code	Biochemical test characterization					
	Catalase	Citrate utilization	VP test	Indole Test	Starch hydrolysis	Nitrate reduction
CH2	-ve	-ve	-ve	-ve	+ve	+ve
SH	-ve	-ve	-ve	-ve	+ve	+ve
CH3	-ve	-ve	-ve	-ve	+ve	+ve
CH4	-ve	-ve	-ve	-ve	+ve	+ve
CH5	-ve	-ve	-ve	-ve	+ve	+ve
CH6	-ve	-ve	-ve	-ve	+ve	+ve

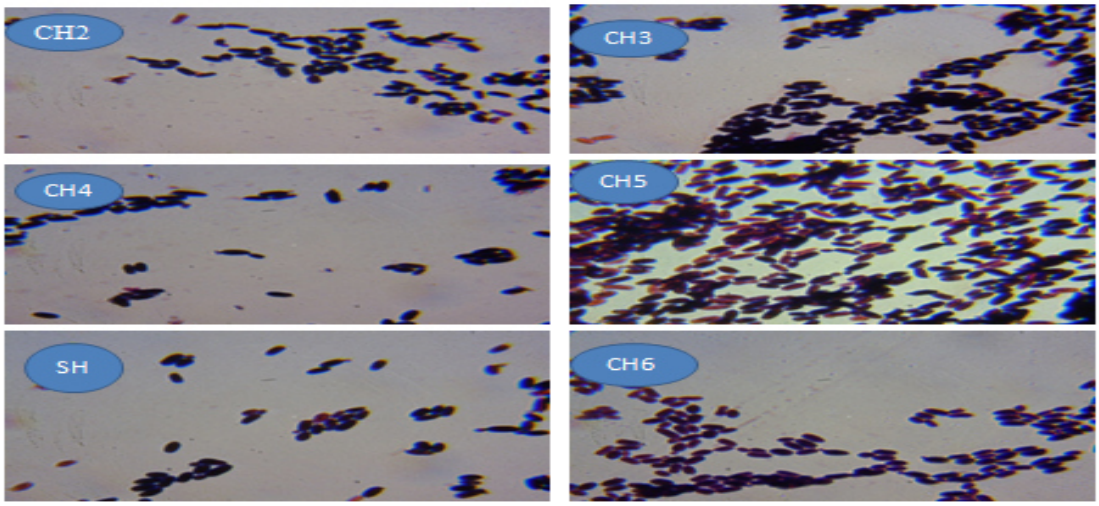

Figure 1 : Gram reaction

Antimicrobial activity of probiotic bacteria

The six isolates were found to exhibit antimicrobial activity against indicator strains as shown in Table 10. On the basis of maximum zone of inhibition and inhibition against to test microorganisms *Lactobacilli CH2* (25mm against *S.typhi* and 23mm against *E.feacalis*) was considered as potential of probiotic bacteria as compared to the other isolates and control antibiotic as shown figure 2. The isolate *Lactobacilli CH2* was bacteriostatic or bacteriocidal, have inhibit more pathogenic bacteria than ampicillin and comparable to cephalexin and Gentamycin as given in Figure 2.

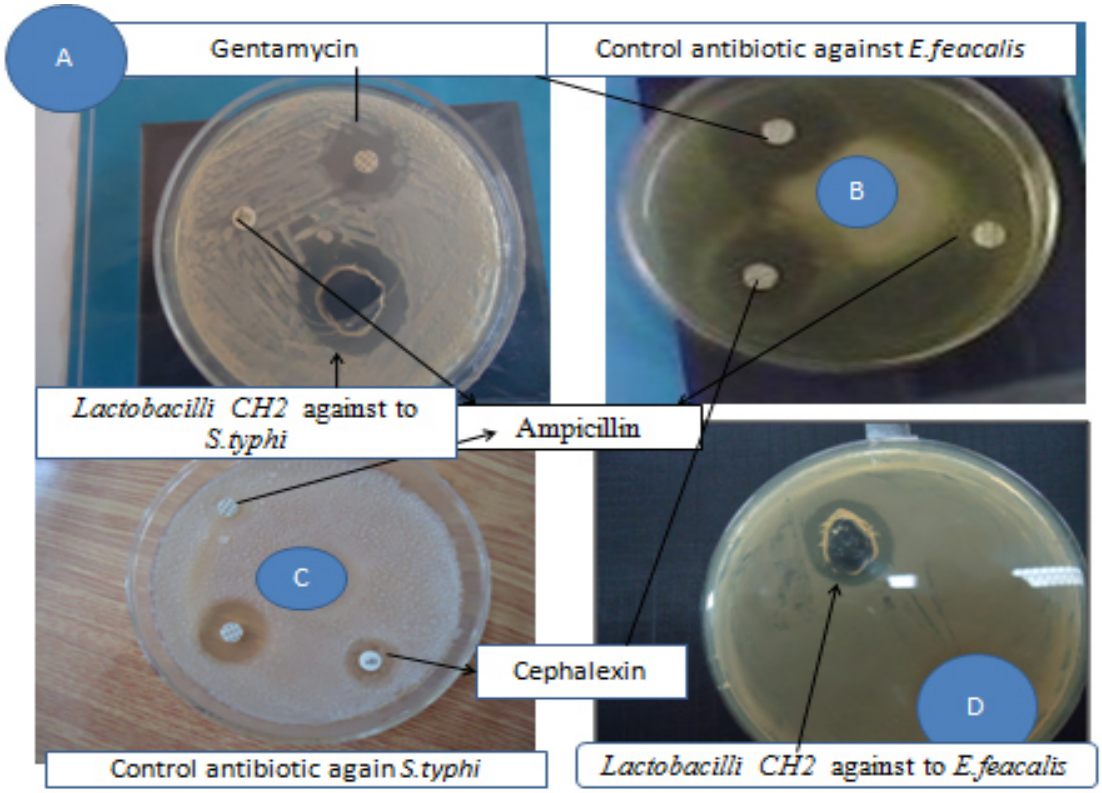

Figure 2: Antimicrobial properties of *Lactobacilli CH2 on salmonella typhi* (7A)*, entroccus faecalis* (7B) and control antibiotics *S.typhi* (7C) and control for *E.feacalis* (7D)

Effect of pH and temperature of probiotic bacteria

The growths of probiotic bacteria's were very sensitive to pH and temperature. The isolates were able to grow in pH 4, 6, 7, 8 and were survived at temperature 25, 30, 37 and 40^{0}C. However, isolate *Lactobacilli CH2* was more tolerant than others and grown maximum at given pH and temperature. Thus, isolate was considered as potential bacteria because more grow in the temperature 37 and 40^{0}c as compared to the others .The growth difference was shown in Figure 3 and 4.

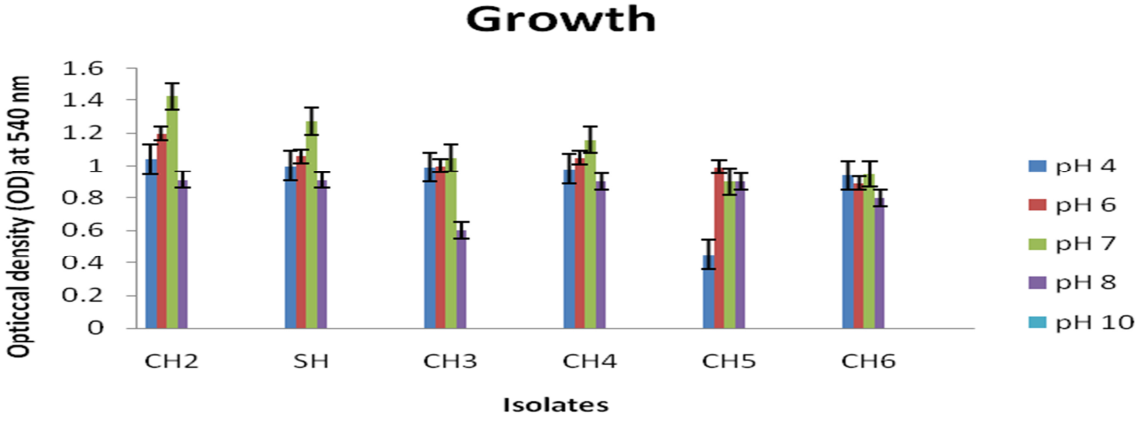

Figure 3 : Effect of pH on bacteria growth at 37^{o}c

To survive passage through the stomach and small intestine, probiotic strains must tolerate the acidic and protease-rich conditions of the stomach, and survive and grow in the presence of acids. Acid tolerance is also important for the probiotics' survival in food (Tuomola *et al*, 2001). The isolated *Lactobacilli spp* was tolerating to wide range of pH (4-8) and grow well at acidic pH (pH = 4). Similar result is reported by Chowdhury *et al.* (2012); Pundiri *et al*. (2013). Therefore, being resistant to low pH is one of the major selection criteria for probiotic strain

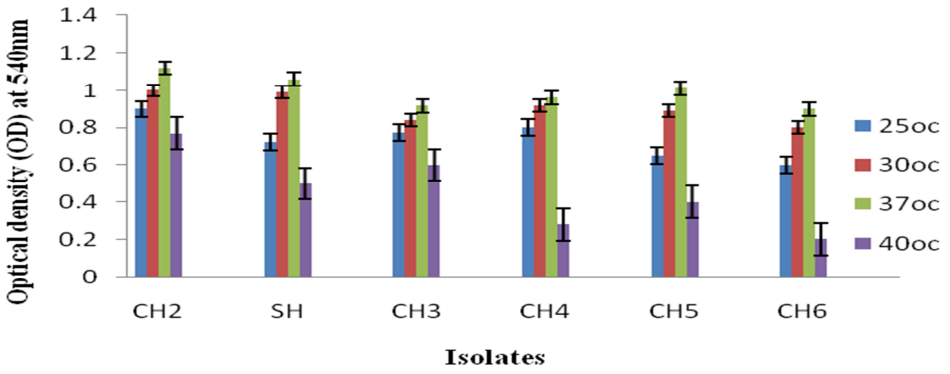

Figure 4: Effect of temprature on bacteria growth

In present study, the selected probiotic isolates were able to survive at temperature (25, 30, 37 and 40)^{0}C. The maximum growth was observed at 37^{0}C. Sathiavelu *et al.* (2011) reported similar finding. The reason for choosing this temperature range was to detect whether the isolated cultures were able to grow within range of normal body temperature or not. As if the isolates were not able to survive within the selected temperature range then they would not have been able to survive in the human gut. Therefore, the selection of optimum pH and temperature is very necessary for the optimum growth of bacteria. Therefore, the optimum pH and temperature for high growth was found to be pH 7 and 37 ^{o}c (OD=1.5) at 540 nm. Similarly finding was observed by Ali and Harsa, (2004) hight lactic acid production by *Lactobacillus casei* were investigated. The highest lactic acid productivity values were obtained at 37˚C.

Lactose utilization of probiotic bacteria

The isolate grown in fermented medium supplemented with lactose was changed in colour from red to yellow, which indicates the production of lactic acid. According to Wadher *et al.,* (2010) lactose intolerant people cannot metabolize lactose due to the lack of essential enzyme β-galactosidase. When they consume milk or lactose-containing products, symptoms including abdominal pain, cramping and diarrhoea arise. If lactose passes through from the small intestine, it is converted to gas and acid in the large intestine by the colonic microflora .Also the presence of breath hydrogen is a signal for lactose maldigestion. Therefore, the present studies provide that the addition of certain starter cultures to milk products, allows the lactose intolerant people to consume those products without any complication of lactose intolerance.

The acid producing bacterial cultures were detected by observing the change in colour of the medium. Thus, The isolates were grown in fermentation medium supplemented with lactose and were observed for change in colour from red to yellow which indicates the production of lactic acid as show below in Figure 5. It was observed that every selected probiotic isolate was able to produce lactic acid from lactose and lower the pH of the media less than 5.

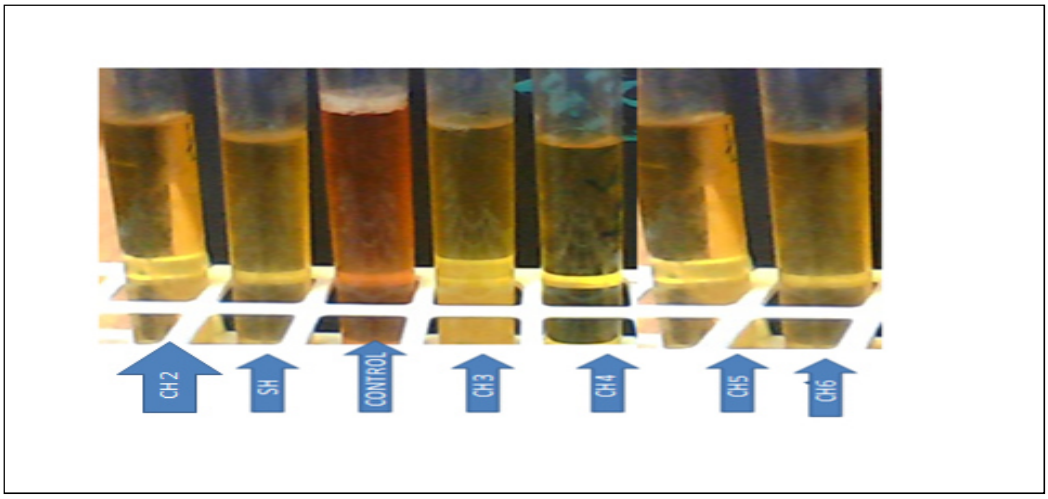

Figure 51 : Lactose utilization

NaCl tolerance of probiotic bacteria

The isolates were able to tolerate 1-4% NaCl concentration as shown in Figure 6. Isolates were tolerating 1, 2, 3 and 4% salt concentration. However, *Lactobacilli CH2* was more tolerate than the other isolates. So, the isolate *Lactobacilli CH2* was more potential than the rest isolates.

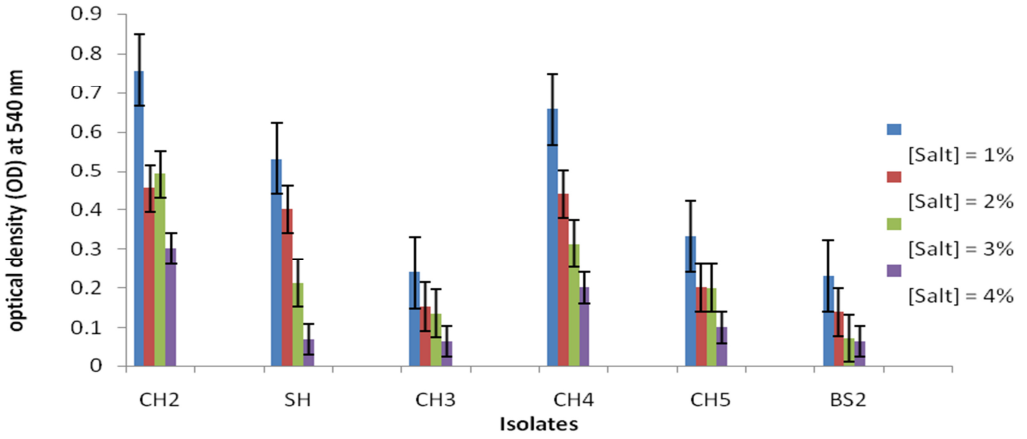

Figure 6 : Effect of different salt concentration on bacterial growth

NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria and pathogenic organisms (Pundiri *et al*., 2013). The current results showed that *Lactobacillus spp* isolated from traditionally *Kocho* was tolerant to 1-4% of NaCl. Thus, high growth was seen the salt concentration of 1-4%. Similarly, the growth of isolates in a medium containing 2%, 3% and 4% NaCl was reported by Eyassu *et al.,* (2012). The findings of Hutchins *et al.,* (1987); Togo *et al.,* (2002); and Ahmed and Kanwal, (2004) concentration of bile salts in the small intestine ranges from approximately 0.2 to 2% (wt/v), depending upon the individual and the type and amount of food ingested. Therefore, the probiotic bacteria should have to tolerate salty environment of the small intestine. Thus, the result of these studies was concomitant to the above indicated studies.

Antibiotic susceptibility of probiotic bacteria

All the isolates were tested for their antibiotic susceptibility. Thus, in this study no inhibition was measured. The isolates were resistant to antibiotics (Table 4).

Table 4: Show sensitivity of probiotic bacteria to different antibiotics

Bacteria	Antibiotic type	Unit	Zone of inhibition	Sensitivity*
	Penicillin G	10 unit	0 mm	R
	Erythromycin	15mcg	0 mm	R
	Gentamycin	10 mcg	0 mm	R
Lactobacilli spp	Nalidixic acid	30 mcg	0 mm	R
	Cephalothin	30 mcg	0 mm	R
	Ampicillin	10 mcg	0 mm	R
	Chloramphenicol	30ug	0 mm	R
	Tetracycline	30ug	0 mm	R

*R=resistance

This finding is comparable to Pundiri *et al.* (2013) who have reported the resistance of probiotic bacteria to Ampicillin (1µg), Cephalothin (30µg), Gentamycin (10µg), Nalidixic acid (30µg), Tetracycline (25µg) and amoxicillin (Flórez *et al.,* 2005). This property of the isolates was important as antimicrobial alternatives to chemical food preservatives and commonly used as antibiotics. Probiotic bacteria and their metabolites are good alternatives as a source of antimicrobial agents (Stanton *et al*., 2001). Isolate *Lactobacilli spp* were 100 % resistant to the drugs that used as control. The development of resistance among bacterial populations exposed to antibiotics enables bacteria to survive and continue to grow instead of being inhibited or destroyed by therapeutic doses of the drug. It is also known that resistance is inherent in many populations not routinely exposed to antibiotics (Apata, 2011). Thus, this property of isolate was more preferable for food industries, for fomentation of enset and therapeutic purpose.

Effect of carbon source on bacterial growth

The *Lactobacilli CH2* isolate was selected for media optimization as it was considered as a potential probiotic in the depending on the above isolation results Figure7 shows the effect of different carbon sources on growth rate of *Lactobacilli CH2.* Maximum growth of bacteria was obtained in media containing glucose whereas the lowest growth was recorded in media containing fructose.

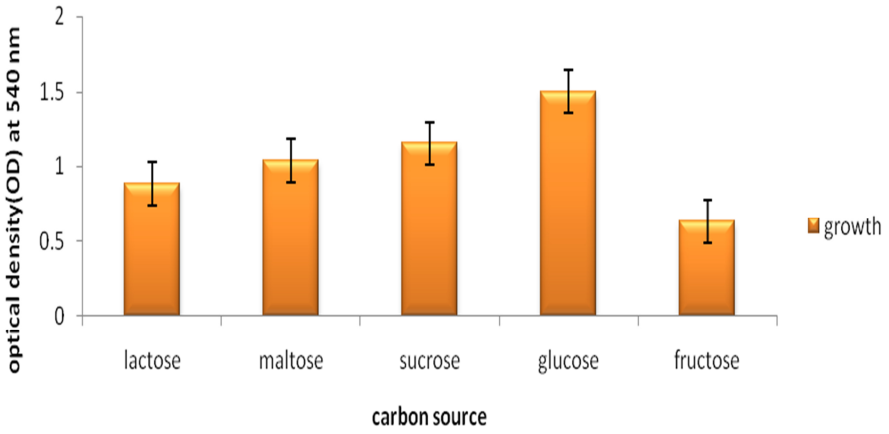

Figure7: Effect of carbon source on bacteria growth.

Effect of nitrogen source

The isolate *Lactobacilli CH2* was grown best in medium containing peptone compared to other nitrogen sources such as tryptone, ammonium sulfate, ammonium chloride and sodium nitrate (Figure 8). The lowest growth was recorded in media containing as nitrogen sources ammonium sulfate at 540 nm.

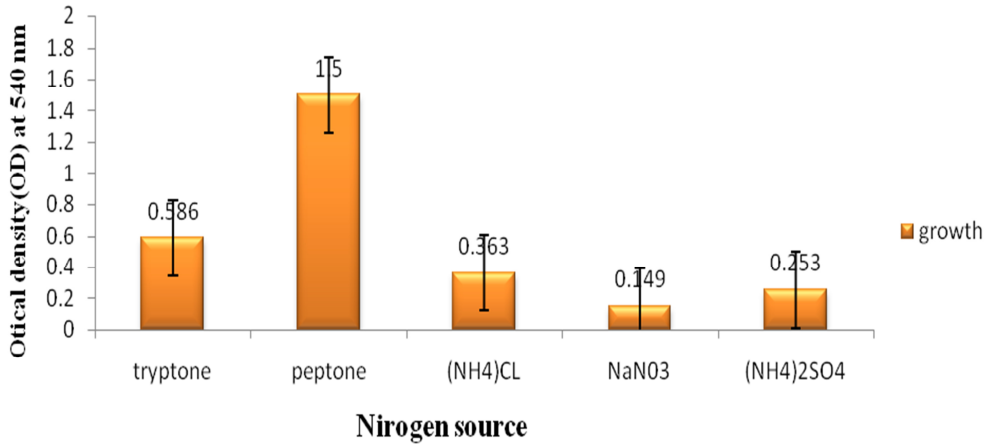

Figure 82: Effect of nitrogen source on bacterial growth

Effect of Incubation period on bacterial growth

The highest growth was obtained at 72 hrs of the incubation time at 540nm. After 3 days incubation the bacterial growth was decreased (Figure 9).

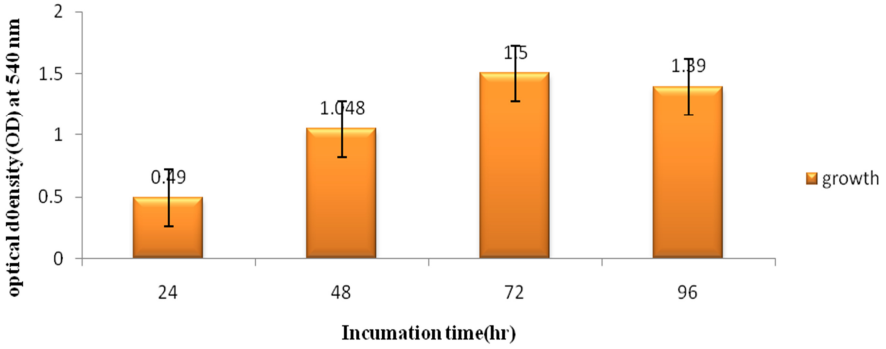

Figure 9: Effect of Incubation period

5.4.4. Effect of pH and temperature on bacterial growth

The growth of *Lactobacilli CH2* was very sensitive to pH and temperature. In this study, the optimum pH for high growth was found to be pH 7. Although the growth was vigorous in the pH range of 6-7 as shown in (Figure 10).

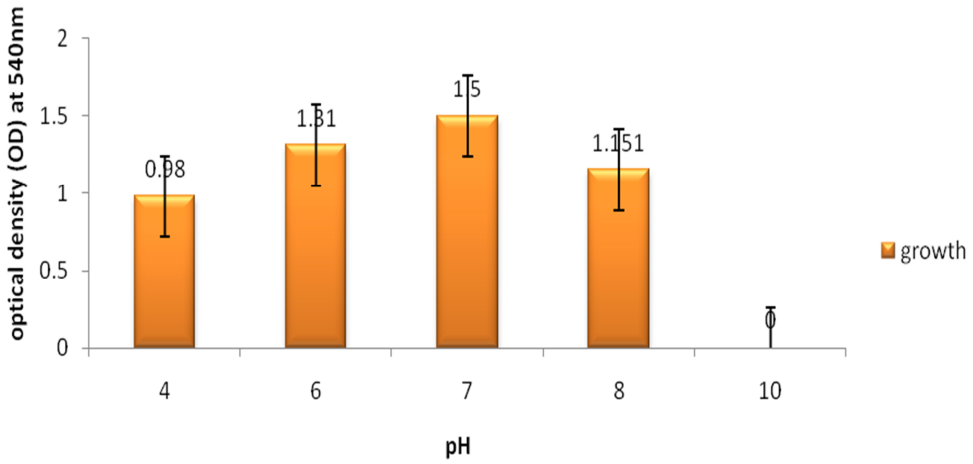

Figure 103: Effect of pH on bacterial growth

The effect of different incubation temperature on the bacteria was shown in Figure 11. The maximum growth was obtained at 37°C at 540 nm.

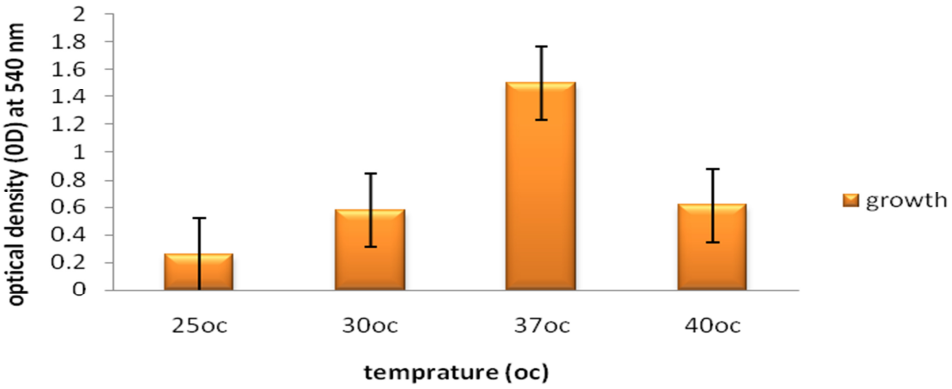

Figure 11: Effect of temperature on bacterial growth

***Fermentation of Ensete using* Lactobacilli CH2**

Fermentation time of enset in the laboratory was reducing from 2 month to fifteen days. The fermentation process was avoiding contamination of *Kocho* spoiling microorganisms. Thus, no food spoilage was observed during study.

Biochemical characterization of fermented Ensete

The absorbance of the extract was measured on a spectrophotometer at 630 nm. The value obtained used to read the concentration of water-soluble carbohydrates. The glucose concentration before and after fermentation were calculated from the give equation. The calculated result is given in Table 5. The absorbance of the protein extract was measured on a spectrophotometer at 660 nm and the value obtained used to read the concentration of protein against a standard. The calculated result was shown in Table 5.

Table 5: Result of before and after fermentation

Items	Before fermentation	After fermentation
Carbohydrate(µg/100gm)	1000	5012
Protein(µg/100gm)	499	2000
Moisture content (%)	93	96
Total Ash (%)	5.35	2.95
Crude fat (%)	4.27	3.98
Fiber content (%)	4.12	2.126

In conclusion, the isolated *Lacto bacilli sp.* fulfills the required criteria for a probiotic such as tolerance to harsh conditions such as high salt, low pH, body temperature range and can produce bacteriocin extracellular which inhibits a number of pathogenic organisms. Also biochemical, physiological and morphological tests showed that *Lactobacilli sp* were dominant in traditional fermented Ensete. We have found that the *Lactobacilli CH2* was resistant to Gentamycin, ampicillin, penicillin, nalidixic acid, cephalexin, tetracycline and chloramphenicol which are most frequently used antibiotics. This bacteria, generally regarded as potential that produce antimicrobial peptide optimally at 37°C and pH 7 in the modified MRS broth containing 2% glucose and 0.2% peptone and other media components.

It can be concluded easily from the experimental results, that the basal medium used cannot support fastidious growth, and is possibly unsuitable for use in industrial scale due to its high cost of synthesis and content in nitrogen sources, while the optimized medium can offer highly improved biomass. Since the cost of culture medium has a remarkable impact on the mass production of probiotic, the optimization of growth conditions, modification of nutrient ingredients and simplification of medium are vital for their economical production of enzymes and/or other peptides.

In the present study, the dominant lactic acid bacteria responsible for the spontaneous fermentation of the traditional fermented Ensete were identified. The isolates from the present study could be used in the development of starter cultures for the production of fermented Ensete food under controlled environment in the future. However, further research work is needed to evaluate the performance of these isolates when used in combination with other lactic acid bacteria strains in a mixed culture. Aroma production and other desirable characteristics of the isolates should also be the focus of future research, which can be used as additional criteria for screening lactic acid bacteria strains to be used as starter cultures. Further molecular study should be done for characterization of the potential strain in addition to the morphological and biochemical tests. Furthermore, the isolation of the potential microbe should be combined with other metagenomic studies.

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