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ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA IN COCONUT TODDY (*MNAZI)*

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ABSTRACT

Mnazi, like any other sugary plant sap can be processed into an alcoholic beverage through fermentation of the sugars present in the sap, yielding alcohol and carbon dioxide. It is sweet, dirty brown in color, containing 10-12% sugar, mainly sucrose (Okafor, 1975). The lactic acid bacteria isolated from mnazi were found to be Gram positive and catalase negative. A total of 86 isolates were obtained for preliminary identification. After further screening based on morphological and growth at different conditions, the number was reduced to 27. Further physiological tests led to further reduction of the isolates to 15. In addition, all the 15 strains were subjected to API 50 CHL fermentation profiling. Of the isolates 81% were identified as Lactobacillus paracaseisspparacasei. Specifically, 47% were identified as Lactobacillus paracaseisspparacasei 2, 27% Lactobacillus paracaseisspparacasei 1 while 7% were identified as Lactobacillus paracaseisspparacasei 3. Other species included Lactobacillus plantarum (13%) and Lactococusslactisssplactis 1 (6%). The presence of Lactic acid bacteria in mnazi is an indication that Lactobacilli are some of the natural microorganisms in mnazi that are responsible for its spoilage if not controlled.

Key Words: Mnazi, L. paracaseisspparacasei, L. lactisssplactis, L. plantarum

INTRODUCTION

Mnazi is commonly referred to as "Nigerian wine" in West Africa (Faparusi, 1971), Toddy (Coconut sap) in Thailand and "Tuba" in Philippines. When distilled, it is known as Lambonog or distilled wine (24-45 % alcohol) (The Coconut Committee., 1992). It is a sweet exudate from tapped unopened spathe of coconut (Banzon and Velasco, 1982) and dirty brown in color, containing 10-12 % sugar, mainly sucrose (Faparusi, 1971). *Upon storage mnazi* turns to a whitish, effervescent, acidic alcoholic beverage (Swings. and De, 1977). The wine can also be converted to syrup, crude sugar or crystallized sugar (The Coconut Committee., 1992).

Mnazi therefore is a typical tropical alcoholic beverage, produced by fermentation of sugary coconut sap. The coconut sap is tapped from palm trees grow at the coastal region of Kenya. The trees commonly used for this purpose are *Elaeisguineensis, Raphiavinifera, Cocosnucifera and Arengapinnata.* The tapping process of the toddy used in this study was as explained by Kadere *et al*. (2004). It is a product of a mixed alcoholic, lactic and acetic fermentation. As a first step, the sugar of the sap is fermented to ethanol within 8-12 h by yeasts and lactic acid bacteria, thus creating a highly suitable medium for the development of acetic acid bacteria. During fermentation, the acetic acid bacteria appear after 2-3 days.

Lactic acid bacteria (LAB) comprise a group of bacteria that are united by a constellation of morphological, metabolic and physiological characteristics. The general description of the bacteria included in the group is Gram- positive, non-spore forming, non-respiring (lack catalase) cocci or rods, which produce lactic acid as a major end product during fermentation of carbohydrates. The core group of LAB includes *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus*. Others include *Tetragenococcus* and *Vagococcus*. Members of the genus *Lactobacillus* are characterized as Gram-positive, non-motile, non-spore forming; rod shaped catalase-negative, auxotrophic, aciduric, facultative anaerobes (Batt, 2000). The lactic acid bacteria are found in foods (dairy products, fermented meat, sour dough, fermented vegetables, silage beverages- including wine), on plants, in sewage, but can also be in the genital, intestinal, and respiratory tracts of man and animals (Hames *et al*., 1991). In the food industry, lactic acid bacteria act as both beneficial organisms and spoilage organisms. They are used in the production of fermented milk products such as yoghurt, sour milk, cheese, and butter, and in the production of sausages, pickles, and sauerkraut. The result of these fermentations is more shelf-stable products with characteristic aromas and flavours, however, if the growth of lactic acid bacteria is not controlled, they can be a major cause of food spoilage.

Previous studies have shown that the presence of LAB in beers and wine is responsible for the spoilage of such alcoholic drinks (Pfenninger *et al*., 1979); however, no attempts has been made to isolate, identify and exploit the utilization of wide range of native microorganisms from *mnazi* including LAB for food and alcoholic beverage processing. This work aims at isolation and identification of common LAB species in *mnaz*i responsible for its spoilage and to avail them for future economic use.

MATERIAL AND METHODS

Sample

Samples of *mnazi* that were used in this study were obtained from Chonyi and Kikambala areas of the Coastal region of Kenya. The samples were collected in sterile sampling tubes. The pH of the sample was determined at the sampling site using a portable pH meter. The samples were kept at 4°C and transported in cool boxes packed with dry ice to the Food Science and Technology

Laboratory at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). To ensure consistency, three tappers were selected as sources of the required samples. Their selection was based on their consistency in the way they conducted their tapping process. Another factor that was used in the selection exercise was variation in execution of the tapping technology. According to Kadere *et al.* (2004), there is little or no variation in the traditional *Mnazi* tapping, samples collected from three tappers and the raw *Mnazi* from three distillers were found to be adequate to provide conclusive results.

Isolation and Storage of LAB for Identification

Before isolation, the sample was enriched in litmus milk medium (0.5% yeast extract and 0.5% glucose). The actual isolation and identification of suspected LAB was carried out in Bromo-Crystal Purple (BCP) and De man Rogosa and Sharpe (MRS) agar. To discourage the growth of yeasts, 10ppm of cycloheximide was added in both media. Triplicate pour plates were prepared for each sample and its dilution and the plates were incubated at 25, 30 and 37°C, respectively for a period of 2-3 days. Isolates were picked from plates with less than 30 colonies. Pure colonies were obtained by transferring three times from the BCP agar into Tryptone-Yeast extract-Lactose-Glucose (TYLG) broth (yeast extract 0.5%, tryptone 1%, glucose 0.5%, lactose 0.5%, tween-80 0.1%, L-cysteine 0.01%). Thereafter one loopful of inoculums from each tube, which showed positive growth, was streaked onto plates with BCP agar for further isolation of pure cultures. After incubation for 1-2 days, the same exercise was repeated until three transfers were made. The isolates were Gram stained and tested for catalase production. Isolates that were Gram positive rods or cocci and catalase negative were stored in litmus milk medium at 4°C and transferred every 2 months or stored in skim milk medium (skim milk 10%, L-glutamic acid monosodium salt 0.1%) at -20°C until identification.

Preliminary Physiological and Biochemical Tests

Based on morphological observation of the isolated strains after Gram stain, and catalase test, a total of eighty-six (86) different colonies were picked. Gram stain was conducted using conventional methods, while catalase test was conducted by adding 3% of freshly prepared hydrogen peroxide on the colonies on MRS agar plates previously incubated at 30°C for 48 h. The presence of catalase was indicated by the production of bubbles.

Based on morphological similarities, isolates that were similar in shape and size after developing photos of the stained isolates were grouped together. A representative of each group was picked for further tests. A total of twenty seven (27) representative strains were picked out of the eight six (86) isolates for further taxonomy. The 27 isolates were identified using the following physiological and biochemical tests: The ability to grow at 10, 15 and 45°C incubation temperatures and pH 4.4 and 9.6 was conducted using TYLG broth incorporated with BCP dye (0.006%) as an indicator. Any colour change from purple to yellow was regarded as positive results, while purple colour indicated negative result. The pH of the media was adjusted using 5N

NaOH (for pH 9.6) and 5N H_2SO_4 (for pH 4.4). Growth in 6.5 and 18% saline solution was done in TYLG broth, previously sterilized by membrane filtration. After incubation at 30°C for 10 days, any bottom sedimentation was regarded as positive result. Production of ammonia from L-arginine was tested as described by Schillinger and Lüke (1987) using 1.25% L-arginine in TYLG broth. Hydrolysis of sodium hippurate with production of benzoic acid was conducted using TYLG consisting of 1% sodium hippurate. After incubation for 10 days at 30°C, the culture was centrifuged at 3000 rpm for 15min. Thereafter 1ml of 50% sulphuric acid was added to 1 ml of the super natant, followed by shaking for 30min. Any formation of benzoic acid was regarded as positive results. Homo-Hetero fermentation was conducted to establish the ability of LAB to produce gas in Gibson medium consisting of three parts mainly: broth A (distilled water-180 ml, skim milk-20 g, glucose-0.9 g and litmus indicator); broth B (distilled water- 50ml, agar- 0.75g, peptone- 0.5g, yeast extract- 0.7g and 2.5ml of 0.4% manganese sulphate) and water agar (distilled water- 50ml and agar- 0.75g). To a 9ml mixture of A and B broths in a tube, previously sterilized at 110°C for 20min. and maintained at 50°C in a hot air oven, a pure culture of the strain under investigation was inoculated. 2-4ml of agar water was then added into the inoculated mixture of A and B broths followed by incubation at 30°C for 10 days. Tubes that produced gas, hence creating a gap between the water agar and the mixture of A and B broths were regarded as hetero fermenters, while those that did not produce gas were considered as homo fermenters.

Fermentation of Primary Sugar and Lactic Acid Isomer Test

After the initial identification, isolates that showed some similarities were grouped together for further tests. In the second phase of identification, fifteen (15) representative isolates were picked out of the 27 isolates and acid production from carbohydrates (amygdaline, L-arabinose, D-cellobiose, esculine, D-fructose, D-galactose, D-glucose, lactose D-mannitol, D-mannose, D-maltose, D-melezitose, D-melibiose, Na-gluconate, D-raffinose, L-rhamnose, D-ribose, salicine, D-sorbitol, sucrose, D-trehalose and D-xylose) was evaluated as explained by Parente *et al*. (1997). The lactic acid isomer test was conducted using the lactic isomer kit as previously described (Von Krusch and Lompe, 1982; Parente *et al*., 1997) using the UV-spectrophotometer at wavelength of 340nm using a cuvette size of 1.00cm light path. The reading of the absorbance was done at 20 to 25°C.

Identification Using the API 50CHL System

The selected 15 strains were further confirmed for production of acids from carbohydrates and related compounds by use of the API 50CHL system (BIOMÉRIEUX SA, France). All LAB identification procedures were conducted in accordance with manufacture's instructions. Portions of growth of each isolate were aseptically transferred from a freshly inoculated stock culture using a swab to an ampule of API 50 CHL basal medium and the emulsified to give a final turbidity equivalent to McFarland standard #2. Each tube of the API 50 CHL strip was inoculated with the bacterial suspension using a sterile pipette. The strip was placed in the incubation tray with honeycombed wells each filled with distilled or demineralised water according to the instructions

of the manufacturer. The tray with the strips in it was covered loosely with a lid, and incubated at 30°C for 72h. Reactions were visually examined after 24, 48 and 72h and determined to be positive or negative based on colour change in the tube caused by anaerobic production of acid and detected by the pH indicator present in the chosen medium. The results, which form biochemical profiles, were identified using an apiweb^{TM} software version 5.1. These profiles were then compared to those listed in the API 50 CHL Analytical Profile Index. Identifications listed in the index as excellent, very good or acceptable were accepted as correct. This together with other supplementary tests such as growth at different pH levels, incubation temperatures, and NaCl concentrations were used to confirm the strains. In contrast, further supplementary tests, such as; degradation of urea and starch, motility, VogesProskauer, growth in MRS and Acetate media were used to confirm the remaining presumptive or questionable identifications.

RESULTS

The initial isolation and identification was based on morphological appearance and catalase test. After conducting some preliminary physiological tests, a total of 15 isolates were picked for further identification. All the 15 strains were found to be Gram positive and catalase negative, non-spore forming rods or cocci. These strains were preliminarily identified for further biochemical and physiological tests as described in materials and methods. The physiological and biochemical characterization of the isolates from *mnazi* are presented in Tables 1 and 2. All the 15 isolates were considered LAB based on their positive Gram reactions, non motility, absence of catalase activity and spore formation, and their rod or coccal shape. All the strains grew at 15 and 30°C but not at 45°C (Table 1). However, there were variations in growth at 10°C. Almost 50% of the 15 isolates were able to grow at 6.5% NaCl but none was able to grow at 18% NaCl. All the 15 isolates were unable to produce carbon dioxide from glucose except TB405 (Table 1). They all showed positive growth at pH 7.0 and pH 4.4 with an exception of TB405 which was not able to grow at pH 4.4. Very few registered positive growth at pH 9.6. All the isolated strains were ADH- (negative arginine hydrolysis) while most of them were hippurate positive.

A total of 15 strains were positively identified after isolation using API 50 CHL kit (BioMėrieux® SA, France). Table 3 shows the LAB species positively identified in which five different species were identified. The predominant species were *Lactobacillus paracaseisspparacasei 2* (7 strains), *Lactobacillus paracaseisspparacasei 1* (4 strains) and *Lactobacillus plantarum 1* (2 strains). As indicated in Table 3, all the 15 strains were able to ferment ribose, glucose, fructose, N-Acetyl-glucosamine, arbutine, esculine and salicine. All the strains identified as *Lactobacillus paracaseisppparacasei 2* (CM201, CM203, CB301, TB302, TB405, TB402 and TM302) showed positive reaction, in that they were able to produce acid from fermentation of the following sugars: ribose, galactose, glucose, fructose, mannose, mannitol, Sorbitol, N-Acetyl-glucosamine, amydaline, arbutine, esculine, salicine, cellibiose, maltose, Saccharose, Trehalose, inuline, Melezitose, turanose, tangarose and gluconate. Identification of these strains by API 50 CHL was

extremely good. According to analytical profile index, all the strains identified as *Lactobacillus paracaseisspparacasei2* were listed in the identification index as excellent except for CM203, which was listed as very good identification. The identification % was very high (greater or equal to 96.2%), while the T value was approximately 0.9. According to the apiweb^{TM} software version 5.1, all the strains in this category were expected to ferment α -Methyl-D-glycoside, however, the results showed that they were unable to ferment this carbohydrate hence the only test that was against the expected results.

The *Lactobacillus paracaseisspparacasei 1* (CM4081, CM4091, CB204 and CB4041) showed similar trends as those of *Lb. Paracaseisspparacasei 2* with an exception of CM4081, which was unable to ferment the following sugars: sorbitol, maltose, saccharose, inuline, melezitose and gluconate. The strain CB204 also showed a peculiar trend in that unlike other strains, it did not ferment Sorbitol. In addition, all the strains belonging to this category were able to ferment lactose except for CM4081. Strains belonging to this group were positively identified as very good with an exception of CB204 that was rated as an excellent identification. The identification percentage was over 99%, while the T value was over 0.8 for all except CM4081 that had a T value of 0.63.

Strains that were positively identified as *Lb. plantarum 1* were CB303 and CM402. The pattern of fermentation for these two strains was similar to that of *Lb. paracaseisspparacasei 2*, except that they were able to ferment raffinose, β-gentobiose, lactose and melibiose. However, they were unable to ferment inuline. CM402 did not ferment Trehalose, gluconate, and turanose. Just like *Lb. paracaseisspparacasei 1* and 2, the identification index was extremely good, in that one had excellent level of identification (CB303) while the other one (CM402) had very good index of identification. The identification % was more than 99% for both strains with a T value of approximately 0.8%.

The strain CB3021 was identified as *Lb. paracaseisspparacasei 3*. The fermentation pattern of this strain is similar to that of *Lb. paracasei app paracasei 2*; however, this strain was unable to ferment sorbitol, maltose, saccharose, inuline, melezitose and gluconate. The identification of this strain was categorized as doubtful with identification % of 96.1% and a T value of 0.61. More tests need to be done to validate this result.Finally, the strain CM303 was identified as *Lactococcuslactisssplactis 1*. This strain was able to ferment ribose, glucose, fructose, α -Methyl-D-glucoside, N-Acetyl-glucosamine, arbutine, esculine, salicine, cellibiose, maltose, saccharose, trehalose and gluconate. This stain was different from all the other 14 isolates in that it was unable to produce acid from galactose, mannose, mannitol, sorbitol, amygdaline, inuline, melezitoseturranose and tangarose. According the identification index, the strain was identified as doubtful, with identification % of 94.6% while the T value was given as 0.42.

DISCUSSION

LAB species are of paramount importance in food industry, both as beneficial organisms and as spoilage organisms. They are used in the production of fermented milk products such as yoghurt, sour cream, cheese and butter, and in the production of sausage, pickles and sauerkraut. In all these products the fermentation process gives characteristic aromas and flavours. Previous studies were able to isolate LAB species in different ecological niches such as milk, meat, vegetables as well as mouth, intestine, and vagina of mammals. Despite all these efforts there have been very few attempts targeting the isolation of LABS from traditional wines such as *mnazi*. The presence of LAB in *mnazi* is more of spoilage rather than beneficial organisms.

The lactic microflora of *mnazi* sampled at the coastal region of Kenya was dominated by homofermentative*Lactobacilli*. In general, over 80% of all the isolates belonged to the species *Lactobacillus paracaseisspparacasei*. Specifically 47% were identified as *Lactobacillus paracaseissp paracasei2*, 27% *Lactobacillus paracaseissp paracasei1* while 7% were identified as *Lactobacillus paracaseissp paracasei3.* From the results it is clear that the species *Lactobacillus paracaseisspparacasei*is the most common LAB in *mnazi.* Other LAB species that were isolated include *Lactobacillus plantarum*, 13%, and *Lactococusslactisssp lactis1*, 6%.

Although all the strains were Gram positive, with *Lactococcus* cells being oval cocci commonly arranged in pairs or chain while *Lactobacillus* cells were rod-shaped arranged in chains. It is worthy pointing out that in characterizing the morphology of lactic acid bacteria, it may be difficult, at times, to distinguish a short rod from an ovoid coccus. Determining the arrangement (pairs versus tetrads of cocci, for example) can be challenging as well. It is therefore paramount for observation to be conducted carefully and repeatedly during research before making a final decision. Different LAB strains differed in their temperature requirements, but in general, the results obtained in this study agreed well with the data published earlier (Collins *et al*., 1989). As expected, all strains registered positive growth at 15°C but were not able to grow at 18% NaCl. Most of them were unable grow at 45°C and at pH 9.6. All strains were found to be L (+) lactic isomer except for CM201 that was L (D) isomer. This confirms the preliminary identification that these strains belonged to the genera *Lactobacillus.* This work confirms the fact that many of the recently described LAB species do not fit into the traditional classification scheme based on morphology and growth temperatures but instead one should rely on other method of classification such as those based on biochemical and physiological criteria(Kandler and Weiss, 1986)

API 50 CH fermentation system enables strain characterization in terms of growth and metabolism on a wide range of individual substrates and thus fulfils an important role in the taxonomic identification of *Lactobacilli*(Vandamme *et al*., 1996). Our results, based on BioMérieux software version 3 on carbohydrates fermentative pattern clearly showed that the isolates that were

positively identified as *Lactobacillus paracaseisspparacasei* were quite uniform in their biochemical and physiological characteristics. The only marked difference observed was that strain CM4081 did not produce acid from saccharose and gluconate. This phenomenon is therefore regarded as an inconsistent attribute of this species. In this study, the API 50 CHL carbohydrates fermentative profile of *Lb. paracaseisspparacasei 2* did not agree with the findings published by Charteris *et al.* (2002) in that all the strains in this study showed the ability to produce acid from dulcitol and inositol while the control of the same species published by Charteris *et al.* (2002) were unable to produce acid from these two alcohols (dulcitol and inositol). In addition, the *Lb. paracaseisspparacasei 2* in this study were able to ferment α-Methyl-D-glucoside as opposed to those published by Charteris *et al.* (2002). In this study the fermentation profile of all the strains identified as *Lb. paracaseisspparacasei*were almost identical. The major distinction between *Lb. paracaseisspparacasei 1* and *Lb. paracaseisspparacasei 2* was the ability of *Lb. paracaseisspparacasei 1* to ferment lactose and *α*-Methyl-D-glucoside as opposed to *Lb. paracaseisspparacasei 2*, which was unable to ferment these two sugars. On the other hand, the isolate that was identified as *Lb. paracaseisspparacasei 3* was significantly different from the other two species of *Lb. paracaseisspparacasei 1 and 2*, in that it was unable to ferment gluconate, melezitose, inuline, saccharose, sorbitol and maltose. However, this species was classified as doubtful, meaning more tests need to be conducted to ascertain it's really identity. The presence of a large number of *Lactobacillusparacaseisspparacasei*species calls for further research to ascertain whether *mnazi* could be having the properties of a functional food. This is because previous studies have shown the ability of *Lactobacillus paracasei* species to have probiotic properties (Gomes *et al*., 1995; Lindberg *et al.*, 1996). Previous works have shown that probiotics are able to modulate immune responses, lower biomarkers such as harmful faecal enzymes activities, show positive effects against superficial bladder cancer and cervical cancer (MacFarland, 2000). Other benefits associated with probiotics include alleviation of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) symptoms, infection control and eradication of multi drug-resistance microbes, lowering of cholesterol in the blood and antimutagenic/anticarcinogenic activities (Haenel and Bendig, 1975; Mitsuoka., 1982; Salminen *et al*., 1998). Although at the moment there is no scientific data that has been availed to prove that *mnazi* is actually a functional food, general observations has it that most people who are daily drinker of *mnazi* seem to be less susceptible to diseases such as malaria, cholera, typhoid influenza and other opportunistic diseases caused by common bacteria and viruses. This therefore calls for studies of the probiotic properties of the LAB isolates from *mnazi*.

Based on carbohydrates fermentation pattern two homofermentative *Lactobacilli* strains (CB303 and CM402) were identified as *Lb. plantarum.* These two strains were different from the *Lb. paracaseisspparacasei* strains in that they were able to ferment lactose, melezitose, raffinose and gentobiose while *Lb. paracaseisspparacasei*were unable to ferment these carbohydrates. Previous studies were able to isolate *Lb. planturum* from fermented sausages (Parente *et al.*, 2001), naturally fermented sicilian green olives (Randazzo *et al*., 2004). *Lactobacillus plantarum* is not only used as

a starter culture in cheese making but can as well be used as a probiotic LAB (Gomes *et al*., 1995; Vinderola *et al.*, 2000). *Lactobacillus plantarum* is distinguished by its biochemical ability to dissimilate hexoses exclusively through the Embden-Meyerhof pathway. Gluconic acid and pentoses, however are dissimilated through the oxidative pentose-phosphate pathway. This gives a total percentage of LAB in *mnazi* with strains that could be probibiotic as 94%, hence, making them the most predominant LAB species in mnazi.

Lactococcuslactis is widely used by the dairy industry for the manufacture of fermented milk products. The primary role of *L. lactis* during fermentations is to the production of lactic acid from milk sugar lactose. The presence of lactic acid bacteria in *mnazi* is an indication that LAB species are one of the major groups of microorganisms that are responsible for the spoilage of *mnazi*. However the presence of a bigger percentage of the *Lactobacillus paracaseisppparacasei, Lactobacillus plantarum*and*Lactococcuslactisspplactis* calls for further research on ability of the isolated strains to produce lactic acid using different types of substrates, ability to produce bacteriocins and volatile compounds and their potential as starter cultures of different food products.

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Table-1. Characterization of Lactic Acid Bacteria based on morphological, physiological and biochemical tests

									Growth at pH			Growth at temp. (°C)				Growth at sali sol. (%)	
S/N	Strain Ref.	Morphol	Gram Stain	Cat. Test	CO_2 from gluc.	Hippur.	NH_3 from arginine	Lactate isomer	4.4	7.0	9.6	10	15	30	45	6.5	18
1	CB303	Rods	+	-	-	+	-	L-(100%)	+	+	-	-	+	+	+	+	-
2	CM402	Rods	+	-	-	-	-	L- (D)-(79%)	+	+	-	-	+	+	-	-	-
3	CM4081	Rods	+	-	-	+	-	L-(100%)	+	+	vw	-	+	+	+	+	-
4	CM4091	Rods	+	-	-	+	-	L-(98%)	+	+	+	+	+	+	-	-	-
5	CB204	Rods	+	-	-	+	-	L-(97%)	+	+	-	w	+	+	-	-	-
6	CB4041	Rods	+	-	-	-	-	L-(95%)	+	+	-	w	+	+	-	-	-
7	CM201	Rods	+	-	-	-	-	L- (D)-71%)	+	+	-	-	+	+	-	+	-
8	CM203	Rods	+	-	-	+	-	L-(96%)	+	+	vw	-	+	+	-	-	-
9	CB301	Rods	+	-	-	-	-	L-(98%)	w	+	vw	+	+	+	-	+	-
10	TB302	Rods	+	-	-	+	-	L-(97%)	+	+	-	+	+	+	-	-	-
11	TB405	Rods	+	-	-	+	-	L-(96%)	-	+	-	w	+	+	-	+	-
12	TB402	Rods	+	-	-	+	-	L-(100%)	+	+	vw	+	+	+	+	-	-
13	TM302	Rods	+	-	-	+	-	L-(99%)	vw	+	-	w	+	+	-	+	-
14	CB3021	Rods	+	-	-	+	-	L-(99%)	+	+	w	-	+	+	-	w	-
15	CM303	Cocci	+	-	-	+	-	L-(100%)	+	+	-	-	+	+	-	-	-

W, Weak growth; vw , Very weak; +, All strains positive; -, All strains negative;

Table-2. Fermentation profiles of LAB isolated from *nnazi* by convectional method

Strain Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Strain reference		CB 303	CM 402	CM 4081	CM 4091	CB 204	CB 4041	CM 201	CM 203	CB 301	TB 302	TB 405	TB 402	TM 302	CB 3021	CM 303
S/N	Substrates (Tests)															
1	Amylagdine	+	+	-	+	+	+	+	-	+/-	+	-	+	-	-	+
2	L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Cellibiose	+	+	-	+	+	+	+	+	+/-	-	+/-	+	-	-	-
4	Esculine	+	+	+	+	+	+	+	-	+/-	+	+	+	-	+	+
5	D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Galactose	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+
7	D- Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Lactose	-	+	+	+	+	-	+	-	-	-	-	+	-	-	-
9	Maltose	+	+	-	-	-	-	+	-	+/-	-	-	+	+	-	-
10	Mannitol	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+
11	D-Mannose	-	-	-	+	+	-	+	+	+	+	-	+	+	+	+
12	Melezitose	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+
13	Melibiose	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
14	N- Acetyl - glucosamine	+	-	-	-	+	-	+/-	+	+	+	+	+	+	-	+
15	D-Raffinose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Ribose	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
18	Salicine	+	+	+/-	+	+	+	+	+/-	+	+	+	+	+	+	+/-
19	Sorbitol	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+/-
20	Saccharose	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-
21	Trehalose	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
22	D-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, All strains positive; -, All strains negative; +/-, varible results

Table-3. Fermentation profiles of LAB isolated from *mnazi* by API 50 CH kit grown 30 °C

Strain Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Strain reference		CB 303	CM 402	CM 4081	CM 4091	CB 204	CB 4041	CM 201	CM 203	CB 301	TB 302	TB 405	TB 402	TM 302	CB 3021	CM 303
S/N	Substrates (Tests)															
0	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	L-Arabinose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	D-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	L-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Adonitol	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
9	β- Methyl-xyloside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
11	D- Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
14	L-Sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
19	Sorbitol	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-
20	α-Methyl-D-mannoside	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	α-Methyl-D-glucoside	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+
22	N- Acetyl - glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	Amygdaline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
24	Arbutine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	Esculine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	Salicine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27	Cellibiose	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
28	Maltose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
29	Lactose	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
30	Melibiose	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Saccharose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
32	Trehalose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
33	Inuline	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
34	Melezitose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-
35	D-Raffinose	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36	Amidon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	Glycogene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	β- Gentiobiose	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
40	D-Turanose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
41	D-Lyxose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	D-Tagatose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
43	D-Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	L-Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	D-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	L-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	Gluconate	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
48	2 ceto-gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	5 ceto-gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Level of Identification		E.I.	V.G.I.	V.G.I.	V.G.I.	E.I.	V.G.I.	E.I.	V.G.I.	E.I.	E.I.	E.I.	E.I.	E.I.	D.F.	D.F.
Identification %		99.9	99.3	99.2	99.9	99.9	99.0	96.2	99.3	96.2	96.2	96.2	96.2	96.2	96.1	94.6
Test		0.86	0.78	0.63	0.86	0.81	0.8	0.9	0.88	0.9	0.9	0.9	0.9	0.9	0.61	0.42
Test against		TAG 7%	TRE 96%	AMY 98%,	NON	SOR 86%	ADO 13%,	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MAL 80%,	GAL 90%,
Identified as:		**Lb. plantarum 1**		**Lb. paracasei ssp paracasei 1**				**Lb. paracasei ssp paracasei 2**							**LPSP 3**	**LLSL 1**

+, All strains positive; -, All strains negative; E.I, Excellent identification; V.G.I, Very god identification; D.F., Doubtful identification; Lb.- *Lactobacillus*; LPSP 3- *Lb. paracasei ssp paracasei* 3; LLSL 1- *Lactococcus lactis ssp lactis* 1