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Isolation and characterization of lactic acid bacteria from finger millet [*Eleusine coracana (*L.) Gaertn.]

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ABSTRACT

A broad variety of cereals that yield tiny grains (seeds) from a naturally varied assortment of grass species are together referred to as "millets." Finger millet (*Eleusine coracana* L. Gaertn.) is a tropical crop that belongs to the group of major millets and is commonly called "ragi or madua". 15Lactic acid bacteria were isolated from the finger millet which five isolates recorded high titratable acidity (FMLB-2, FMLB-5, FMLB-7, FMLB-8 and FMLB-9).

Keywords: *Anti-nutritional factors, Finger millet and Lactic acid bacteria*

Among the grains, millets are unique since they are concentrated sources of calcium, fiber, protein, and polyphenols. Finger millet is a significant food crop due to its best nutritive value and better storage properties. The most common species of millet cultivated for food uses is *Eleusine coracana* where two other species namely *Eleusine indica* is a wild variety and *Eleusine africana* is a semi-wild species. Finger millet (*Eleusine coracana*) is an annual herbaceous plant widely grown as a cereal crop in arid and semiarid areas Africa and Asia (Rachie *et al*., 1996). It is a tetraploid and self-pollinating species that probably evolved from its wild relative *Eleusine africana*. Finger millet is native to the Ethiopian and Ugandan highlands. Interesting crop characteristics of finger millet are the ability to withstand cultivation at altitudes over 2,000 meters (6,600 ft) above mean sea level, high drought tolerance and long storage time.

MATERIALS AND METHODS

Sample collection

Finger millet grains (PR 10-45) were procured from ARS, Peddapuram. The grains were washed, cleaned, dried thoroughly and preserved for analysis. The *Lactobacillus acidophilus* (NCIM-2903) collected from the National Collection of Industrial Microorganisms (NCIM), Pune, was used as a reference culture during experimentation.

Isolation and purification of Lactic acid bacteria

Lactic acid bacteria were isolated from finger millet grain surface by a modified enrichment method (Teuber *et al.,* 1992). Finger millet grains (1g) were taken in a test tube and sterilized milk was added to the test tube to enrich natural lactic acid bacterial population by creating microaerophilic conditions incubated at room temperature overnight. The grain sample was transferred to 10 ml sterile water blank, mixed well and serially diluted to get 10^{-5} dilution. Aliquots (1 ml) from 10^{-4} and 10^{-5} were plated on MRS agar medium by pour plating method and plates were incubated at room temperature for 48 h.
Individual colonies were subcultured on new MRS plates to observe colony morphological characters. The isolates were observed for their colony characteristics such as size, shape, colour, position on /in agar medium, and pigmentation (Becking, 1974). Whereas, cell size, shape and arrangement under the microscope were assessed through simple staining (Barthalomew and Mittewar, 1950).

Biochemical characteristics

Different biochemical tests were performed and the protocols followed are briefly outlined below. Biochemical-based characterization was done as per Bergey's Manual of Systematic Bacteriology (Aneja, 2012)

a) Gram staining

On a clean slide a drop of distilled water was added and then bacterial smear was prepared on the slide, which was air dried and heat fixed. The smear was then flooded with crystal violet solution for one minute and then washed gently with tap water. Then the slide was flooded with iodine solution for one min, and washed gently with tap water before treating with 95 % ethanol for 10-15 seconds and again washed with water. Later, the smear was flooded with safranin solution for 1 min and then washed gently with tap water and dried in air. The slide was examined under a microscope at 100X power with oil immersion and data was recorded (Barthalomew and Mittewar, 1950).

b) Endospore staining

Endospore staining of isolates was done using 30 days old culture. The smears were prepared, dried, kept on hot water bath and flooded with malachite green (0.5% aqueous solution) frequently to avoid drying of slides. Then, slides were washed and counter stained with safranin for 30 seconds and observed under microscope (Murray *et al*., 1994).

c) Catalase activity

A loop full of bacterial isolate was placed on a sterile glass slide followed by the addition of hydrogen peroxide (3 %). The production of effervescence was recorded as positive for the test (Balazevic and Ederes, 1975).

d) Dextran production

The dextran production capacity of isolates was checked using sucrose agar medium. The isolates were streaked on sterile sucrose agar plates, incubated for three days and observed for presence of mucoid secretions on agar surface (Dolan and Morgan, 1978).

e) Starch hydrolysis

The starch agar plates were inoculated with isolates and incubated for 48 h. After incubation, plates were flooded with iodine solution wherein, the presence of a clear zone around the line of growth was confirmed as positive for the test (Aneja, 2012).

f) Lipid hydrolysis

The test was performed by inoculating isolates on tributyrin agar medium plates and the colonies with clear zones were recorded as positive (Aneja, 2012).

g) Methyl Red (MR) and Voges-Proskauer's (VP) test

The bacterial isolates were inoculated to MRVP broths and incubated at room for 48 h. After incubation, five drops of methyl red indicator was added and observed for color change. Acetoin production was tested by adding naphthol and 40 % potassium hydroxide. Development of pink colour indicates positive result (Clarke and Kirner, 1941).

h) Titratable acidity (TA)

The sample (10 ml) was taken in a 100 ml conical flask, boiled in a water bath at 70 °C, cooled and titrated against 0.1 N NaOH using three drops of phenolphthalein indicator. The appearance of a light pink colour indicated the endpoint. Total titrable acidity was expressed as % lactic acid. (Srivastava and Kumar, 1993).

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value X Normality of NaOH X 90.01 X 100}}{\text{volume of sample taken X 1000}}$$

RESULTS AND DISCUSSION

Cultural characteristics of lactic acid bacterial isolates of Finger millet

15 lactic acid bacteria were isolated, subcultured and used for studying cultural and morphological characteristics. The *Lactobacillus acidophilus* (NCIN-2903) is used as reference culture for comparison. The shape of the colonies of 15 lactic acid bacterial colonies varied from circular with varied size (small to medium) and colour varied from dull white to milky /creamy. The margins were entire /regular with elevated colonies. Majority of them were smooth or glistening surface (Table 1; Plate 1 & Plate 2). Similar observations were reported by Mannan *et al*., (2017), 25 *Lactobacilli* species were isolated from cheese and yoghurt samples. They were characterized the colonies as small to large sized, circular, regular and creamy white in colour.

Morphological characteristics of lactic acid bacterial isolates of Finger millet

Based on microscopic observations, all 15 Lactic acid bacterial isolates were Gram positive, non-motile and non-endospore formers (Table 2). The cell size differed from small to medium. However, these isolates were with varied cell arrangements from independent cocci /bacilli, in pairs, groups and short chains (Table 1; Plate 3 & Plate 4). Many reports confirmed that LA bacteria as Gram positive, non mortile, non - endospore formers (Aydin and Cebi, 2019). Ali.,(2011) tested the technological aspects of lactic acid bacteria used as starters in manufacturing

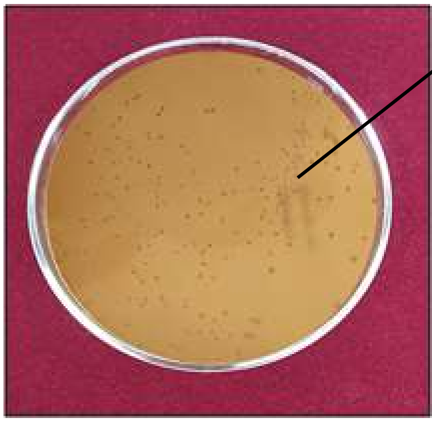


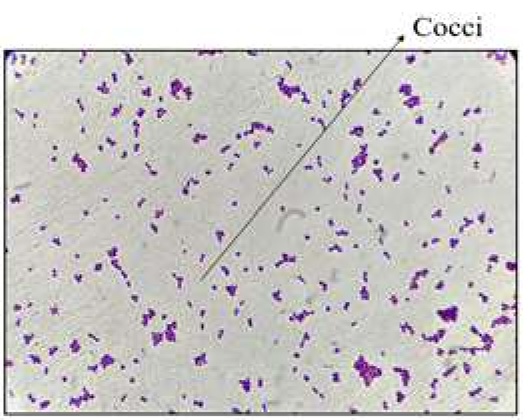
Plate .1. Isolation of lactic acid bacteria (LAB) colonies in MRS agar media

Plate .2. Pure culture of FMLB-5 on MRS agar media

Table.1.Colony and Cell morphological characteristics of lactic acid bacterial isolates of Finger Millet

Isolates	Colony shape	Colony colour	Colony margins	Colony size	Cell morphology	
					Shape	Arrangement
FMLB-1	Irregular	Milky white	Glistening	Medium	Bacilli	Chain
FMLB-2	Irregular	Pearl white	Smooth	Medium	Cocci	Group
FMLB-3	Round	Dull white	Slimy	Small	Bacilli	chain
FMLB-4	Round	Milky white	Slippery	Medium	Bacilli	Independent
FMLB-5	Round	Dull white	slimy	Small	Cocci	Diplococci
FMLB-6	Round	Milky white	Slippery	Small	Bacilli	Chain
FMLB-7	Round	Creamy	Glistening	Small	Cocci	Diplococci
FMLB-8	Round	Light Cream	Glistening	Large	Cocci	Group
FMLB-9	Round	Dull white	Glistening	Medium	Cocci	Small groups
FMLB-10	Round	Dull white	Glistening	Small	Bacilli	Short chain
FMLB-11	Round	Dull white	Slimy	Small	Cocci	Chain
FMLB-12	Circular	Creamy white	Slimy	Small	Bacilli	Groups
FMLB-13	Irregular	Milky white	Glistening	Medium	Bacilli	Chain
FMLB-14	Round	Dull white	Slimy	Small	Bacilli	chain
FMLB-15	Round	Dull white	Slimy	Small	Bacilli	Chain
Lactobacillus acidophilus	Round	Creamy	Glistening	Small	Bacilli	Short chain

Note: FMLB-finger millet Lactic acid Bacteria



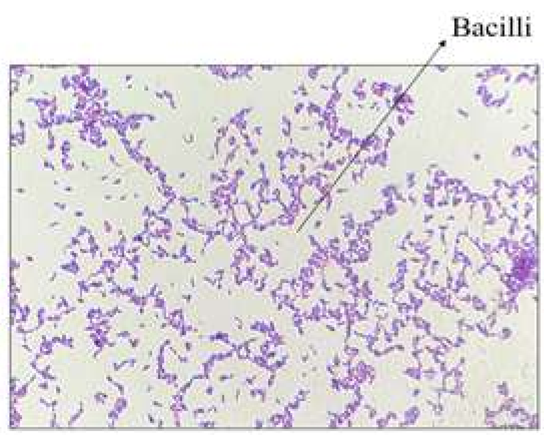


Plate .3. Cell morphology of lactic acid bacterial isolates of finger millet (*Eleusine coracana.* L) at 100 x magnification fermented dairy products that were suitable for local conditions. A total of 303 LA bacterial isolates from yoghurt were studied for morphological, cultural, physiological and biochemical characteristics and identified them as *Lactococcus* (47) and *Lactobacillus* (256) genera.

The lactic acid bacterial isolates were further studied for biochemical characteristics (Table 2). All the isolates fermented glucose with acid production and no gas formation confirming them as homo fermenters. Out of all isolates, four (FMLB-1, FMLB-3, FMLB-5 and FMLB-11) were dextran producers, four (FMLB-1, FMLB-5, FMLB-11and FMLB-12) hydrolyzed starch and three isolates hyrdrolyzed lipid. Eleven isolates were positive for Methyl red test indicating that they could perform mixed acid fermentation, while ten isolates were positive for Voges-Proskauer's test confirming the acetoin production. The best-performed isolates were screened based on titratable acidity (per cent lactic acid) for efficient fermentation of finger millet grains. During the study, five isolates recorded with high titratable acidity (FMLB-2, FMLB-5, FMLB-7, FMLB-8and FMLB-9) and were further used to standardize protocol for fermented finger millet beverage preparation (Table 2).

Table.2. Biochemical characterization of lactic acid bacterial isolates of Finger millet

Isolates	Gram reaction	Endospore Formation	Catalase activity	MR	VP	Indole test	Dextran production	Hydrolysis		Titratable Acidity (%)
								Starch	Lipid	
FMLB-1	+	-	-	+	+	-	+	+	+	0.88
FMLB-2	+	-	-	+	+	-	-	-	-	1.18
FMLB-3	+	-	+	+	+	-	+	-	-	0.90
FMLB-4	+	-	-	+	+	-	-	-	-	0.95
FMLB-5	+	-	-	+	+	-	+	+	+	1.45
FMLB-6	+	-	-	+	-	-	-	-	-	0.83
FMLB-7	+	-	-	-	-	-	-	-	-	1.39
FMLB-8	+	-	-	+	+	-	-	-	-	1.28
FMLB-9	+	-	-	-	-	-	-	-	-	1.06
FMLB-10	+	-	-	+	+	-	-	-	-	0.80
FMLB-11	+	-	-	+	+	-	+	+	-	1.22
FMLB-12	+	-	-	+	+	-	-	+	+	0.62
FMLB-13	+	-	-	+	+	-	-	-	-	0.78
FMLB-14	+	-	-	-	-	-	-	-	-	0.65
FMLB-15	+	-	-	-	-	-	-	-	-	0.70
Lactobacillus acidophilus	+	-	-	+	-	-	-	-	-	1.81

Note: +: positive results and -: negative results; MR= Methyl Red; VP = Voges-Proskauer test

CONCLUSION

It can be concluded that 15 lactic acid bacteria (LAB) strains were successfully isolated and characterized from finger millet (*Eleusine coracana* L.), demonstrating diverse cultural and morphological characteristics. The isolates exhibited colony shapes ranging from circular with small to medium sizes and colors varying from dull white to milky/creamy. Most colonies had entire margins, elevated growth, and smooth or glistening surfaces, aligning with previous studies on LAB from dairy products. Microscopic examination confirmed that all isolates were Gram-positive, non-motile, and non-endospore formers, with varied cell arrangements, including independent cocci/bacilli, pairs, groups, and short chains. These findings highlight the potential of finger millet-derived LAB as promising candidates for food fermentation and probiotic applications. To identify the most efficient strains for fermentation, titratable acidity (percent lactic acid) was assessed, and five high-performing isolates (FMLB-2, FMLB-5, FMLB-7, FMLB-8, and FMLB-9) were selected. These strains will be further utilized to standardize a protocol for fermented finger millet beverage preparation.

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