

Original Research Article

<https://doi.org/10.20546/ijcmas.2021.1003.214>

Phenotypic Identification of Lactic Isolates Obtained from Fermented *Idli* Batter

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ABSTRACT

Keywords

Idli batter,
Fermentation,
Characterization,
Lactobacillus,
Pediococci,
Weissella,
Leuconostoc, and
Enterococci

Article Info

Accepted:
15 February 2021
Available Online:
10 March 2021

Naturally fermented foods have an array of microorganisms which play a major role in fermentation of food constituents, improve the sensory characteristics and biological value of foods. It's important to know the predominant microflora and their characteristics for thorough understanding. In this study an attempt is made to enumerate and characterize the predominant lactic cultures in fermented *idli* batter. Isolates obtained were characterized through preliminary tests, viz. Gram's staining, catalase test, CO₂ from glucose, growth in litmus milk and specific tests like dextran production on sucrose agar and specific sugar fermentation tests. All the lactic isolates were Gram positive, catalase negative, non-motile, non-spore formers with acid, reduction & coagulation in litmus milk. Out of 18 isolates, seven isolates were identified as *Leuconostoc* spp., four isolates as *Weissella* spp., four as *Pediococcus* spp., two as *Lactobacillus* spp. and one as *Enterococcus* spp.

Introduction

Fermented foods are defined as foods that had been subjected to the action of selected microorganisms, by which a biochemically and organoleptically modified substrate was produced, resulting in an acceptable product for human consumption (Tamang, 1998). It was presumed that fermented foods probably originated during 7000 - 8000 BC in area of Indus Valley. India is traditionally rich in fermented foods; their sensory characteristics differ based on local food crops and artisan

skills from region to region. *Idli* is one of the traditional fermented foods of India, which is a common breakfast in southern part of India, prepared by using rice (*Oryza sativa*) and black gram (*Phaseolus mungo*) at different proportions. These ingredients are soaked overnight in water, followed by grinding, fermentation and steaming. The current study was focussed on isolating natural microflora from fermented batter, growing them on solid substrate (black gram) and its addition as inoculum during batter preparation to hasten the fermentation process.

Ray *et al.*, (2016), studied that lactic acid fermentation was involved in *idli* batter fermentation, mediated by a mixed cultures of Lactic Acid Bacteria (LAB), which included mainly, *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus lactis* and *Enterococcus faecalis*. Other organisms were *Saccharomyces cerevisiae*, *Pediococcus cerevisiae*, *Debaryomyces hansenii*, *Hansenula anomala*, *Torulopsis candida*, and *Trichosporon beigeli*. The major bacteria involved in leavening of batter were heterofermentative LAB, *Leuconostoc mesenteroides* and later stages of fermentation yeasts produced CO₂, leading to leavening of the *idli* batter.

In the present study, typical lactic colonies from selective media plates were isolated and were maintained as pure cultures. The lactic isolates were phenotyped through preliminary tests to confirm the genus and further to species level by specific biochemical tests,

Materials and Methods

Enumeration of microflora in fermented *idli* batter

The fermented *idli* batter was serially diluted using phosphate buffer and plated for lactic counts such as leuconostoc, enterococci, pediococci and lactobacilli using sucrose agar, bile esculin agar, acetate agar, and Lactobacillus MRS Agar respectively. The media used were ready to use media from Hi-Media Pvt. Ltd. Plates were incubated at 30 °C/48 h for leuconostoc, enterococci, pediococci and at 37 °C/48 h for lactobacilli in an anaerobic jar.

Isolation and maintenance of lactic cultures

Typical lactic culture colonies were picked based on colony morphology like sucrose

agar, bile esculin agar, acetate agar and Lactobacillus MRS Agar. They were transferred to yeast glucose broth, incubated at 30 °C for 24 h. Young cultures were streaked thrice on poured plates of Yeast Glucose Agar (YGA), and purified isolates were maintained in YGA stabs. Purified isolates were maintained on YGA (0.75 %) stabs as stock cultures and while working cultures in YG broth. Sub-culturing of the isolates was done once in a month for stock cultures and 7 days for broth cultures.

Phenotypic characterisation of lactic isolates

The isolates were characterised through preliminary tests such as: Gram's staining, catalase test, CO₂ from glucose, growth in litmus milk to confirm genus and specific tests included dextran from sucrose, NH₃ from arginine, growth at 10°C and sugar fermentation tests for maltose, sucrose, trehalose and arabinose as per procedures given by Harrigan, (1998) to identify the genus and species as per Bergey's Manual of Systemic Bacteriology (Vos *et al.*, 2009).

Results and Discussion

The typical lactic colonies selected were studied for their colony characteristics and cell morphology. Total 18 isolates were selected based on colony morphology. They were transferred to yeast glucose broth and incubated anaerobically at 30 °C for 24 h. Further they were streaked thrice on pre-poured plates of Yeast Glucose Agar (YGA) and purified isolates were maintained in YGA stabs as stock and yeast glucose broth as working cultures.

Identification of lactic isolates

From sucrose agar plates 7 typical colonies were creamy white to white in colour, surface

or subsurface, mucoid texture, and some were with lenticular shape. The cell morphology was cocci, with ovoid or ellipsoid shape, occurred as single or in pairs, and was coded as Leu1, Leu 2, Leu 3, Leu 4, Leu 5, Leu 6 and Leu 7. Four colonies, on acetate agar plates were white to creamy white in colour both surface and subsurface, cocci occurring in pair and tetrads. They were coded as P1, P2, P3 and P4. A surface, round edge, light white colony with black background on bile esculin agar showed the cell morphology of

round shape cocci in clusters, coded as E1. Two typical surface, round edge, white/creamy white colonies from lactobacillus MRS agar exhibited the cell morphology of rods with round ends, singly, pairs or in chains and were coded as Lb1 and Lb2. Another four colonies from Lactobacillus MRS agar, appearing small, white to creamy white, mucoid, surface and cell morphology revealed as ellipsoid with tapered rods in pairs and chains, coded as W1, W2, W3 and W4 (Table 1).

Table.1 Colony characteristics and cell morphology of isolates obtained from fermented *idli* batter

Sl. No	Genus	Isolate code	Colony Characteristics	Cell Morphology
1	<i>Leuconostoc</i>	Leu1	Cream white, small, surface	Ellipsoid, in pairs, clusters
2		Leu2	White, surface, mucoid	Ovoid, single, elongated cocci
3		Leu3	Subsurface, white	Ellipsoid, short rods
4		Leu4	Subsurface, lenticular, creamy white	Cocci, in pairs, single, straight chain, ovoid
5		Leu5	Surface, small, creamy white, entire, raised	Cocci, single or in pair
6		Leu6	Subsurface, lenticular, creamy white	Cocci, ovoid, more in pairs.
7		Leu7	Subsurface, entire, round, creamy white	Cocci, in pairs, single, straight chain, ovoid
8	<i>Pediococcus</i>	P1	Subsurface, white	Round, pairs, tetrad
9		P2	Surface, white, entire, rised	Rods, pairs, tetrad
10		P3	subsurface, white	Round, pairs, tetrad
11		P4	Subsurface, round, entire, creamy white	Circular, tetrad,
12	<i>Weissella</i>	W1	Surface, round, white, convex	Ellipsoid with tapered ends, pair, chain
13		W2	Surface, irregular, mucoid	Ellipsoid with tapered ends, pair, chain
14		W3	Surface, small, creamy white, circular	Ellipsoid with tapered ends, pair, chain
15		W4	Surface, small, entire, white colony	Ellipsoid with tapered ends, more in pairs
16	<i>Lactobacillus</i>	Lb1	surface, entire, creamy white	Rods with round ends, single or chains
17		Lb2	Surface, white, entire, rised	Rods, pairs, in chain
18	<i>Enterococcus</i>	E1	Light white, black background	Cocci, in cluster

Media used - sucrose Agar for *Leuconostoc*; Lactobacilli MRS agar for *Weissella* and Lactobacilli; acetate agar for *Pediococci* and Bile esculin agar for *Enterococci*.

All incubated at 30 °C/48 h in candle jar (anaerobic condition).

Table.2 Phenotypic identity of the cultures isolated from *idli* batters

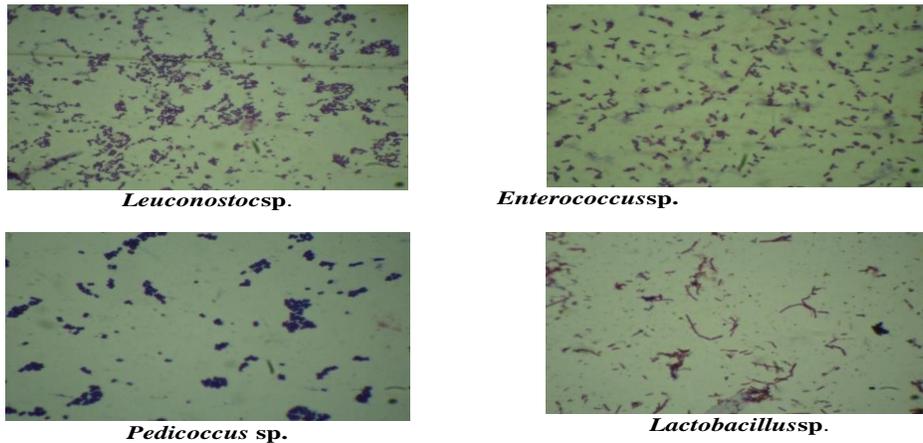
Isolate Code (numbers)	Preliminary tests		Specific tests			Sugar fermentation				Identity
	Cell morphology	CO ₂ from glucose	Dextran from Sucrose	NH ₃ from Arginine	Growth at 10°C	Maltose	Sucrose	Trehalose	Arabinose	
Leu1, Leu5, Leu7 (3)	Ellipsoid, in pairs, clusters	Positive (CO ₂ produced)	-	-	+	+	+	-	-	<i>Leuconostoc</i> sp.
Leu2, Leu3, Leu4, Leu6 (4)	Ovoid, single, elongated cocci		+	+	+	+	+	+	+	<i>Leuconostoc</i> sp.
P1, P2, P3, P4 (4)	Round, pairs, tetrad	Negative (CO ₂ not produced)	-	+	-	ND	ND	ND	+	<i>Pediococcus pentosaceus</i>
E1 (1)	Cocci in cluster	Negative	-	-	+	+	+	+	-	<i>Enterococcus</i> sp.
W1, W2, W3, W4 (4)	Ellipsoid with tapered ends, pair, chain	Positive	+	+	-	+	+	-	-	<i>Weissella confusa</i>
Lb1 (1)	Rods, with round ends	Positive	-	+	+	+	-	-	+	<i>Lactobacillus</i> sp.
Lb2 (1)	Rods, single, pairs or chains	Positive	-	ND	+	ND	-	-	-	<i>Lactobacillus</i> sp.

The results given in the table were after confirmation in 3 replicates

All the isolates were Gram positive, catalase negative, non-motile, non-spore formers with Acid, Reduction & Coagulation (ARC) in litmus milk.

Symbols: +, indicates positive, -, indicates negative, ND – No data

Fig.1 Cell morphology of lactic cultures isolated from fermented *idli* batter



Phenotypic characterisation of selected isolates

The isolates obtained were characterised through various preliminary tests, viz. Gram's staining, catalase test, CO₂ from glucose, growth in litmus milk and specific tests like dextran production on sucrose agar for sugar fermentation. Based their cell morphology and results obtained, they are identified up to genus and species level. All the 18 lactic isolates were Gram positive, catalase negative, non-motile, non-spore formers with acid, reduction and coagulation in litmus milk.

Out of 18 isolates, 3 isolates of leuconostoc Leu1, Leu5 and Leu7 were identified as *Leuconostoc* spp. did not produce dextran, no ammonia from arginine but grew at 10°C, Leu2, Leu3, Leu4 and Leu6 produced dextran, NH₃ from arginine and growth at 10°C; based on production of acid from maltose, sucrose, trehalose and arabinose, they were identified as *Leuconostoc* spp. P1, P2, P3 and P4, four isolates of pediococci were identified as *Pediococcus pentosaceus* as they did not produce CO₂ from glucose, negative for dextran from sucrose, grew at 10

°C and positive for NH₃ from arginine, negative for trehalose and positive for arabinose;

One isolate (E1) identified as *Enterococcus* sp. did not produce CO₂ from glucose, negative for dextran from sucrose, no NH₃ from arginine, grew at 10°C, produced acid from maltose, sucrose, trehalose and negative for arabinose; Four isolates W1, W2, W3, and W4 were identified as *Weissella confusa* and two isolates (Lb1, Lb2) identified as *Lactobacillus* sp., that produced CO₂ from glucose, negative for dextran from sucrose and NH₃ from arginine, whereas found growth at 10°C, produced acid from maltose, while no acid from sucrose, trehalose and arabinose but could not be placed in any species due to ambiguous test results (Fig.1 and Table 2).

Similar study was carried out by Iyer *et al.*, (2013), where they used MRS agar for isolation lactic acid bacteria from *idli* batter, incubated at 37 °C for 48 h. with translucent/opaque colonies having 2-3 mm diameter. About 15 isolates were characterized by physiological and biochemical tests according to Bergey's

manual Systematic Bacteriology. All the isolates were gram positive, catalase negative, with morphology of cocci, coco-bacilli, and rods. Two isolates with code IB1 and IB2 showed antibacterial activity against pathogen were subjected to biochemical characterisation and sugar utilisation test. Both exhibited mixed acid fermentation, IB1 was heterofermentative, and IB2 was homofermentative, Both produced acid but no gas on TSI agar, and negative for citrate test. IB1 was identified as *Lactobacillus plantarum* and IB2 as *Lactococcus lactis*.

Vidhyasagar and Jeevaratnam (2012) isolated LAB from *idly* batter. From the MRS agar plates 40 elevated colonies were Grampositive, catalase negative, acid producing, cocci, were isolated from *idly* batter. They were grouped into 36 homofermentative and 4 heterofermentative, All isolates produced acid from dextrose, fructose, mannose and esculin while none of the isolates produced acid from lactose, L & D-arabinose, rhamnose, xylose, ribose, sorbose, xylitol, mannitol, inositol, dulcitol, sorbitol, melezitose, adonitol, malonate, glycerol, citrate, glucosamine and gluconate. Since they all exhibited growth at 15, 45 and 37°C while none grew at 50°C, they are confirmed as *Pediococcus pentosaceus* differing at strain level because of variation in their biochemical and physiological characteristics.

Oladipo *et al.*, (2013) conducted a study, where, 144 strains of *Enterococcus* sp. were isolated from traditional fermented vegetable condiment and West African soft cheese (*wara*). The presumptive *Enterococci* strains were gram-positive, catalase negative, oxidase negative, non-spore forming cocci in singles and pairs with ability to grow in the presence of 6.5% NaCl at pH 9.6 and at 10 and 45 °C; were able to hydrolyze esculin, pyrrolidonyl- β -naphthylamide, arginine and

were unable to hydrolyze starch. All the strains were able to ferment cellobiose, D-fructose, galactose, D-glucose, glycerol, lactose, maltose, mannitol, D-mannose, ribose, sucrose, and trehalose.

On par this study *Leuconostoc mesenteroides* (LAB1) and *Saccharomyces cerevisiae* were isolated from fermented *idli* batter and characterized phenotypically based on preliminary tests and specific tests. Preliminary tests identified the bacterial isolate LAB1 as Gram positive, catalase negative cocci and specific tests growth at various temperatures placed the bacteria as *Leuconostoc mesenteroides* (Amala, 2016).

The study was conducted to isolate and identify the lactic isolates from fermented *idli* batters. Selected colonies on selective media plates used for viable count were isolated and maintained as pure cultures. All the 18 isolates were screened by preliminary and biochemical tests and identified seven isolates as *Leuconostoc* sp., four isolates as *Pediococcus pentosaceus*, One isolate as *Enterococcus* sp., Four isolates as *Weissella confusa* and two isolates as *Lactobacillus* sp.

Acknowledgement

The author would like to thank Karnataka Veterinary Animal Science and Fishery Sciences University, Bidar for providing facilities to carry out the Ph.D. research work and University of Agricultural Sciences, Bangalore for providing in-service deputation for the study.

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How to cite this article:

Viswanatha Angadi, C. Ramkumar, Jayashri P. Hiremath and Prabha, R. 2021. Phenotypic Identification of Lactic Isolates Obtained from Fermented *Idli* Batter. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 1718-1724.
doi: <https://doi.org/10.20546/ijcmas.2021.1003.214>