The Pharma Innovation Journal 2023; 12(11): 653-660

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 653-660

www.thepharmajournal.com
Received: 11-09-2023
Accepted: 16-10-2023

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Isolation and characterization of *Lactobacillus* for induced aerobic respiration ability

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Abstract
Lactic acid bacteria (LAB) composed of a diverse group of microorganisms associated with plants, meat, vegetables and milk. Some carefully selected and characterized microorganism are used for preparation of fermented milk and food product such as *Dahi*, Acidophilus milk, Yogurt, Cheese, saurekaurt kimchi fermented sausages etc. LAB were well known for its obligate fermentative nature. In fermentation bacteria used substrate level phosphorylation and generate 2 ATP from one glucose molecule. This fermentative production of ATP is not an efficient catabolic process as it only yields 2 ATPs per glucose molecule. Some LAB species have the ability to transform itself from fermentation to aerobic respiration in presence of heme and menaquinone. *Lactobacillus* sp is the best-reported LAB species capable of carrying aerobic respiration in rich medium (MRS) supplemented with heme and menaquinone, which results in increased cell number and enhanced stress tolerance capability. Thus induced aerobic respiration could be used as a potential technique for preparation of industrially important bacterial culture. The aim of this study was to isolation and characterization of aerobic respiratory *Lactobacillus* spp for higher cell number production. The pure 39 lactobacilli isolated from milk and plant based samples. Among of these isolates, 23 have been reported the ability of aerobic respiration. The highest difference in OD600 of 0.662 was reported in isolate Gjr. The study concluded that the utility of using aerobic respiration system for lactobacilli can be used for production of higher cell number of bacteria economically with very small amount of heme and menaquinone supplementation.

Keywords: *Lactobacillus*, aerobic respiration, heme, menaquinone

Introduction
At the beginning of the twentieth century, the concept of lactic acid bacteria (LAB) gradually emerged to describe a group of bacteria with similarities in morphology, metabolism and physiology. In 1873 the first pure culture of lactic acid bacterium was obtained by J. Lister. After that in 1890 the commercial use of lactic acid bacteria was started for the production of cheese and sour milk, while fermented food has been used by man for more than 5,000 years ago. The first monograph by S. Orla-Jensen appeared in 1919 (Khalid, 2011) [6]. A distinctive lactic acid bacterium is a Gram-positive, aero-tolerant, non- spore forming, non- motile, low G+C content (less than 55%), catalase negative, acid tolerant, organotropic and strictly fermentative rods or coccus, they produce lactic acid as a major end product. The lactic acid bacteria (LAB) might be the most numerous group of bacteria linked to humans. They are naturally associated with mucosal surfaces, particularly some gastrointestinal tract of various animals like mice, rats, humans, and are also indigenous to food-related habitats, including plant (fruits, vegetables, and cereal grains), wine, milk and milk products, sea foods and meat environments. The LAB include extremely valuable nonpathogenic species that are used for industrial fermentation of dairy products, meats, and vegetables, and they are also critical for the production of wine, coffee, silage, cocoa, and sourdough. In addition, the LAB are a priceless source of antimicrobial agents, the bacteriocins (Makarova & Koonin, 2007) [8]. LAB has GRAS (Generally Recognized as Safe) status due to their ubiquitous nature in food, some LAB, notably lactobacilli, occupy important niches in the gastrointestinal tracts of humans and animals and are considered to offer a number of probiotic benefits to general health and well-being (Klaenhammer *et al*., 2002) [5]. LAB does not possess electron transport chain so LAB basically depend on the fermentation process to produce energy. In fermentation the energy is derived from organic molecules and the electrons are finally transferred to another organic molecule in the absence of oxygen. In fermentative metabolism energy is produced through only the process of breakdown of glucose i.e. Glycolysis. The degradation of six carbon molecules (glucose) to two moles of three-carbon molecule (pyruvic acid) is called Glycolysis.

In glycolysis there is only 2 ATP molecules of net energy and two NADH (from NAD+) molecules are produced. The end product of glycolysis known as pyruvic acid is reduced lactic acid in the presence of lactate dehydrogenase enzyme. This reaction is called as lactic acid fermentation. Conventional Respiration involves glycolysis, citric acid cycle and electron transport chain it completely oxidized glucose and produces 38 ATP molecule. LAB is not a traditional aerobic bacterium it lacks citric acid cycle along with non a functioning electron transport chain thus LAB undergoes fermentation before moving onto respiration to generate the necessary NADH as electron donor for electron transport chain. The cytochrome in the LABs' electron transport chain requires heme and menaquinone to function electron transport chain (Duwat *et al*., 2001) [1]. Respiratory growth with heme and menaqinone improved tolerance to oxidative stress and provide long term survival in intestine (Ianniello *et al*., 2015) [4]. From industrially important bacterial culture point of view there are many advantages to grow lactobacilli under aerobic respiratory conditions. Respiratory cultures are more robust in comparison to fermentative cells so it provides good viability and stability. Respiratory culture also exhibits enhanced long-term survival for longer time in comparison to fermentative culture (Guidone *et al*., 2013) [3]. In this work we had isolated Lactobacilli from different milk and plant based samples. Isolated culture were identified through polyphasic approach for culture purity. Induced aerobic respiration with supplementation of heme and menaquinone in MRS broth. Aerobic respiration ability of different culture was compared between their respiratory and fermentative growth condition of the culture.

Materials and Methods

Media

All the media used in the present study were prepared using distilled water and autoclaved at 15 lbs pressure (121 ºC) for 15 to 20 minutes. All the required media were procured from Himedia Laboratories Pvt. Ltd., 23, Wadhani Road, Ind. Est., LBS Marg., Mumbai-400086, India.

Fine Chemicals

Hemin (heme)

Hemin (Hi Media Laboratories Pvt. Ltd, Mumbai, India) was used to supplement MRS broth for aerobic respiration.

Menaquinone

Menaquinone (Supelco, 595, North Harrison Road, Bellefonte, USA) was also used to supplement MRS broth for aerobic respiration.

Isolation of Lactobacilli from Dairy and Vegetable Samples

Sample collections

A total number of 60 dairy and 15 vegetable and 5 fruit samples were collected from the rural and urban areas of the Chhattisgarh state. Samples were collected in sterile sample container. Dairy samples included dahi (curd), vegetable samples included cucumber, spinach, brinjal, chilli, tomato, broccoli, cabbage, ridge Gourd, bottle gourd, lady Finger.

Growth medium

For isolation of Lactobacilli cultures, MRS was used as culture medium. Vegetable and fruit samples were first given an enrichment step in MRS broth before isolation. For isolation of cultures MRS agar was used for pour plating and streak plating. Isolation of Lactobacilli from different sources

Preparation of Sample

Curd Samples

Curd samples were prepared by mixing 10 ml aseptically weighed curd to 90 ml of sterile saline (0.85% sodium chloride).

Vegetable samples

Vegetable samples were enriched by transferring to MRS broth tubes and incubated at 37 ºC for 24-48 h. Then enriched broth medium was then serially diluted in sterile saline tubes (1:10) and then pour plated using appropriate dilution.

Isolation of pure cultures

Appropriate dilutions of the samples were pour plated and streak plated on MRS agar medium and plates were incubated at 37 ºC for 24-48 h. After stipulated incubation isolated colonies were observed on the agar plates. From each plate selected colonies having translucent to whitish color and circular shape were picked up randomly and transferred to MRS broth and incubated at 37 ºC/24-48 h for further growth. Purity of the isolates was ascertained by microscopy.

Identification of *Lactobacillus* Cultures

All the randomly selected colonies were tested for morphology and purity by microscopic examination. The isolates which showed typical morphology were further subjected to biochemical identification.

Morphological characterization

The morphological characteristics i.e. shape, size and arrangement of cells were determined by Negative straining using Nigrosine stain. Gram staining was also performed to ascertain Gram reaction of the isolates.

Biochemical characterization

An array of physiological and biochemical tests was performed to identify the morphologically selected isolates. Pure cultures were grown in MRS broth at 37 ºC for 24 h. Cultures were activated 24 h before and used as an inoculum for the various physiological and biochemical tests employed to identify the isolated cultures.

Catalase test

Catalase test was performed by slide method. Using an inoculating needle culture from well isolated colony was placed on a clean glass slide. A drop of 3% H_2O_2 solution was added onto this culture and closely observed for the effervescence, indicating positive test.

Carbohydrate fermentation

Tentative identification of isolates was done mainly on the basis of sugar fermentation pattern. The ability of the cultures to ferment and produce acid from various sugars was tested in Hi- Carbo Kit (Part-A) (Hi Media Laboratories Pvt. Ltd., Mumbai, India) which contains different sugar wells. The kit includes twelve immobilized carbohydrates in wells *viz*. Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, L- Arabinose and Mannose. The sugar fermentation was carried out as per

manufacturer's instructions. Briefly, the culture was grown in 10 ml MRS broth at 37 ºC for 24 h and cells were harvested by centrifugation in refrigerated centrifuge. The supernatant was discarded carefully and pellet was washed using sterilized saline. The washed cells were suspended in 5 ml of saline, a part of which was used for assessing O.D. using spectrophotometer. The prepared inoculum was inoculated @ 50 µl in each well of the kit and incubated at 37 ºC for 24-48 h. After stipulated incubation change in color of immobilized sugar was observed and noted down.

Preparation of heme & menaquinone supplemented broth

The screening of cultures for aerobic respiration was carried out using heme & menaquinone supplemented MRS broth (MRS-H). The heme stock solution (2.5 mg/ml) was prepared by weighing heme and dissolving in 0.05 M NaOH solution. The menaquinone stock solution (1 mg/ml) was prepared by weighing menaquinone and dissolving in ethanol. An appropriate volume of heme & menaquinone stock solution was added to MRS broth to achieve a final heme concentration of 2.5 µg/ml & menaquinone concentration of 1 µg/ml of the MRS broth. A volume of 25 ml MRS-Heme was dispensed in 250 ml volume flask. The prepared medium then was autoclaved at 121 ºC for 20 min. Menaquinone 1 µg/ml was added aseptically after sterilization of broth.

Screening of Isolates for Aerobic Respiration Ability

A total of 40 morphologically and biochemically characterized Lactobacilli isolates were screened for aerobic respiration ability. Respiration was induced by adding heme (Hi Media Laboratories Pvt. Ltd., Mumbai, India) and menaquinone (Sigma Aldrich Pvt. Ltd. USA) into MRS broth (MRS-H). A final heme concentration of 2.5 µg/ml and menaquinone concentration of 1.0 µg/ml of MRS broth was used for screening the cultures. The respiration in cultures was ascertained by increase in O.D. in MRS-H as compared with MRS as determined by spectrophotometer (Model: UV-Vis 119, Systronics Pvt. Ltd. Ahmedabad, India).

Induced Aerobic Respiration in Lactobacilli

The heme supplemented medium (MRS-H) was prepared by adding heme working solution to get a final heme concentration of 2.5 µg/ml in the broth. Lactobacilli isolates were transferred from stock culture into 10 ml of MRS broth at a constant inoculum volume of 2% (v/v) and incubated at 37 ºC for 48 h. The activated cultures were used for inoculation of MRS-H and MRS flasks @ 2%. The inoculated flasks were incubated at 37 ºC for 24 h. After incubation the growth was observed spectrophotometrically by taking culture O.D. at 600 nm.

Results and Discussion

Isolation of Lactobacilli from Dairy and Vegetable Samples

A total number of 41 dairy and 20 vegetable samples were collected from the rural and urban areas of the Chhattisgarh state. Dairy samples included dahi, vegetable samples included cucumber, spinach, brinjal, chilli, tomato, bottle Gourd, lady Finger. Samples were collected in sterile sample container brought to the laboratory and stored in refrigerated conditions till further processing.

Isolation of Lactobacilli from collected samples

Collected dairy and vegetable samples were directly submitted to isolation of Lactobacilli strains. Samples were serially diluted (10^1-10^6) in saline (0.85%) solution and then plated on MRS agar by using pour and streak Plate method (Fig. 1). The plates were incubated at 37 ºC for 48 h and then several colonies were picked randomly for identification of *Lactobacillus*. Single well separated colonies were randomly picked with a sterile toothpick and inoculated in MRS broth and incubated at 37 ºC for 24-48 h. A total of 61 samples were collected from different location of chhattisgarh in which 46 cultures of potential *Lactobacillus* were isolated in pure culture. These cultures were maintained by frequent subculturing in MRS broth and preserved for long term storage in glycerol stocks at -20ºC. All the isolates were subjected to polyphasic identification process

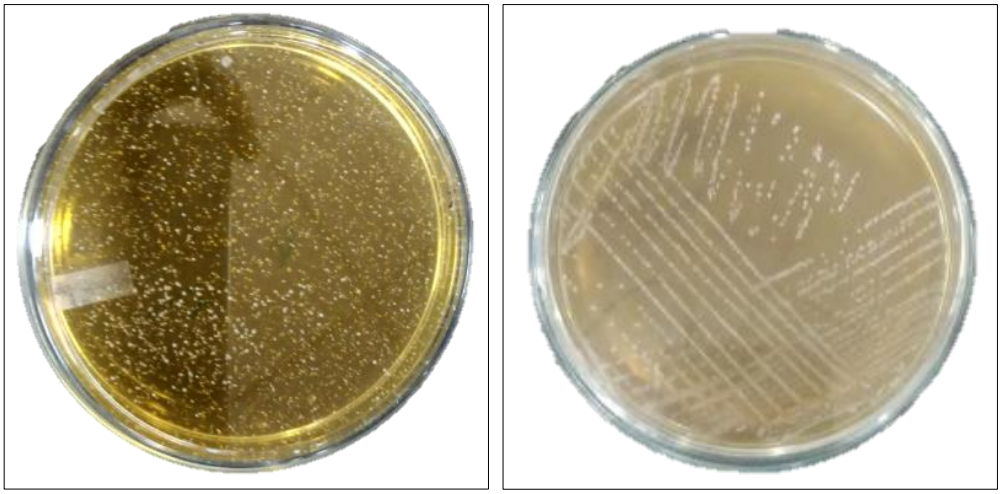

Fig 1: Pour plate and Streak plate of isolate

Identification of isolates

Morphological identification

The isolated pure cultures of tentative *Lactobacillus* strains were examined for Negative staining and Gram reaction for morphological identification. Negative staining using nigrosine was used to visualize the cells against a dark background for clear morphological viewing. The Gram Reaction of the isolates and morphological features are given in Table.1.All the tested strains were Gram positive and were rod shaped. *Lactobacillus* is a genus of the group LAB and thus it is appeared as Gram positive, rod shape bacteria.

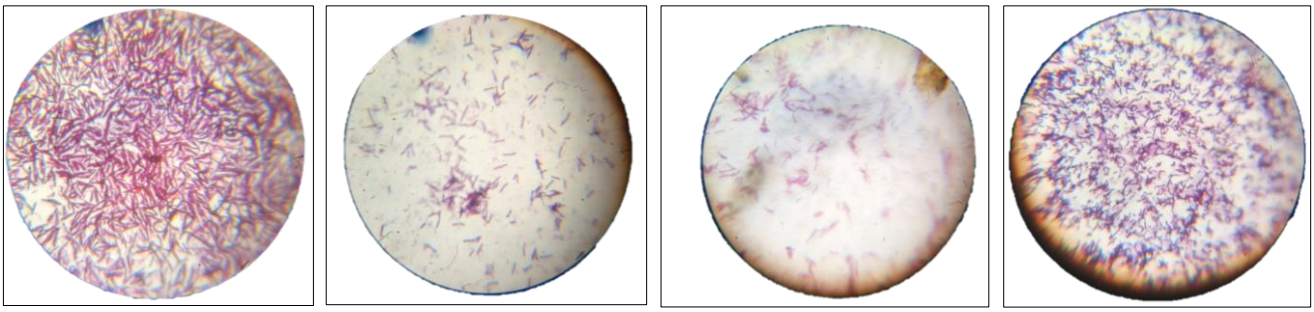

Fig 2: Gram staining of culture

Table 1: Morphological and biochemical characteristics of isolated pure cultures

S. No	Area	Code	Samples	Catalase	Grams Reaction	Morphology
1.	Tatyapara Chowk, Raipur	Dga	*Dahi*	Negative	Gram Positive	Short rods
2.	Ashwini Nagar, Raipur	Knchn	*Dahi*	Negative	Gram Positive	Long thin rods
3.	Pachpedi Naka, Raipur	Dil b	*Dahi*	Negative	Gram Positive	Long thick rods
4.	Ganjroad, Nayapara	Gjr	*Dahi*	Negative	Gram Positive	Short rods chain
5.	Ambikapur	Amb	*Dahi*	Negative	Gram Positive	Medium length rods
6.	Phool Chowk, Raipur	Hppy	*Dahi*	Negative	Gram Positive	Short rods in pairs
7.	Bilaspur	Bil	*Dahi*	Negative	Gram Positive	Long rods
8.	Gariyband	Gar-2	*Dahi*	Negative	Gram Positive	Thin rods
9.	Krishi upaj mandi Nayapara	Bknr	*Dahi*	Negative	Gram Positive	Short thin rods
10.	Gariyanband	Gar-1	*Dahi*	Negative	Gram Positive	Long thick rods
11.	Agarwal sweets, Imlidih	Na	*Dahi*	Negative	Gram Positive	Medium length rods
12.	Kawardha	K3	*Dahi*	Negative	Gram Positive	Thick rods
13.	Purani basti, Raipur	Mhrj	*Dahi*	Negative	Gram Positive	Short & thin rods
14.	Abhanpur	Th	*Dahi*	Negative	Gram Positive	Medium length rod
15.	Mukutnagar, Raipur	Grs	*Dahi*	Negative	Gram Positive	Long bacillus
16.	Avanti Vihar, Raipur	Chf	*Dahi*	Negative	Gram Positive	Diplo bacillus
17.	Janjgir Champa	Jg3	*Dahi*	Negative	Gram Positive	Medium length rod
18.	Shankar Nagar, Raipur	Nds	*Dahi*	Negative	Gram Positive	Very thin rods
19.	Baloda Bazar	J.L	*Dahi*	Negative	Gram Positive	Very thin long rods
20.	Jagdalpur	Jd5	*Dahi*	Negative	Gram Positive	Very thin rods
21.	Mungeli	Met	*Dahi*	Negative	Gram Positive	Individual short rods
22.	Korba	Krb	*Dahi*	Negative	Gram Positive	Short & thin rods
23.	Dhamtari	Dmtri	*Dahi*	Negative	Gram Positive	Long chain of thin rods
24.	Dallirajahra	Drj	*Dahi*	Negative	Gram Positive	Thin medium length rods
25.	Balod	S.L	*Dahi*	Negative	Gram Positive	Long thin rods
26.	Rajanandgaon	Rjn	*Dahi*	Negative	Gram Positive	Thin rods in chain
27.	Telibandha, Raipur	Ab3	*Dahi*	Negative	Gram Positive	Small single rods
28.	Tarry, Nayapara	Shu mis	*Dahi*	Negative	Gram Positive	Short & thin rods
29.	Raigarh	Rig	*Dahi*	Negative	Gram Positive	Thin medium length rod
30.	Durg	Drg	*Dahi*	Negative	Gram Positive	Short rod
31.	Rajim	Rchnd	*Dahi*	Negative	Gram Positive	Thick medium length rod
32.	Lakhenagar	Jmd	*Dahi*	Negative	Gram positive	Medium length rod
33.	Gatapar, Abhanpur	G1	*Dahi*	Negative	Gram Positive	Thin small rods
34.	Torala, Abhanpur	Nanl	*Dahi*	Negative	Gram Positive	Short rods
35.	Mahvir nagar, Raipur	Jtt	*Dahi*	Negative	Gram Positive	Long & thin rods
36.	Katora talab, Raipur	N.g	*Dahi*	Negative	Gram Positive	Thin medium length rod
37.	I.G.K.V. Farm	T.T.	Vegetable	Negative	Gram Positive	Long rods
38.	I.G.K.V. Farm	Ccbr	Vegetable	Negative	Gram Positive	Short individual rods
39.	I.G.K.V. Farm	R.Chil	Vegetable	Negative	Gram Positive	Thick medium length rod
40	I.G.K.V. Farm	Spi	Vegetable	Positive	Gram Positive	Thin short rods
41	Marod, Dhamtari	Lf1	Vegetable	Positive	Gram Positive	Thin short rods
42	Khartuli, Dhamtari	Gir	*Dahi*	Negative	Gram Positive	Medium length rods
43	Chatod, kurud,	Lf_2	vegetable	Negative	Gram Positive	Long rods
44	Mana camp	S.kunj	*Dahi*	Negative	Gram positive	Short rods
45	Charoda, Bhilai	Idk	*Dahi*	Negative	Gram positive	Medium length rods
46	Chatod, Kurud	Chilli n	*Dahi*	Negative	Gram positive	Individual thick road

Catalase test

The catalase test is performed on cultures to check their ability to express the enzyme catalase, which catalyzes the degradation of hydrogen peroxide to H_2O and O_2. The oxygen released in the reaction can be seen in the form of effervescence. This test is often used for identification of LAB as they lack the catalase enzyme. The test was carried out by placing a small volume of culture on a microscopic slide and then placing a drop of 3% H_2O_2. All the tested isolates did not show any effervescence when treated with 3% H_2O_2 which indicates that the isolates belong to the LAB group. The results of catalase test are listed in table 1.

Carbohydrate utilization test (sugar profiling)

Tentative identification of isolates was done mainly on the basis of carbohydrate utilization test (sugar profiling). The ability of the cultures to ferment and produce acid from various sugars was tested in Hi-Carbo Kit (Hi Media), which contains different sugars immobilized in wells. The cultures were grown in 10 ml MRS broth at 37 ºC for 24 h and cells were harvested by centrifugation in refrigerated centrifuge. The supernatant was discarded carefully and pellet was washed using sterilized saline and re-suspended in 5 ml saline. The prepared inoculum was inoculated @ 50 µl in each well of the kit and incubated at 37 ºC for 24-48 h. The fermentation pattern obtained for different isolates and standard culture of *Lactobacillus* is shown in Table. 2 Out of 46 pure isolates 39 cultures were confirmed *Lactobacillus* on the basis of polyphasic morphological and biochemical (catalase and sugar profiling test) identification. Figure 3 shows the color change of immobilized sugar in the well from red to yellow indicative of the ability of the isolate to ferment the particular sugar (Rhaiem *et al*., 2016) [10].

Table 2: Carbohydrate utilization pattern (sugar profiling) of isolates

Isolates	Lact	Xyl	Malt	Fru	Dext	Gala	Raffi	Treh	Meli	Sucr	L-Arb	Man
Jg3	+	+	+	+	+	+	+	+	+	+	+	+
NDS	+	+	+	+	+	+	+	+	+	+	+	+
JL	+	+	+	+	+	+	+	+	+	+	+	+
Jd5	+	+	+	+	+	+	+	+	+	+	+	+
Met	+	+	-	+	+	+	+	-	+	+	+	+
Idk	+	+	+	+	+	+	+	+	+	+	+	+
Dmtr	+	+	+	+	+	+	+	+	+	+	+	+
Drj	+	+	-	+	+	+	+	-	+	+	+	+
S.L	+	-	+	+	+	+	+	+	+	+	-	+
Rjn	+	+	+	+	+	+	+	+	+	+	+	+
Ab3	+	+	+	+	+	+	+	+	+	+	+	+
Shu mis	+	+	+	+	+	+	+	+	+	+	+	+
Rig	+	+	+	+	+	+	+	+	+	+	+	+
Dga	+	+	+	+	+	+	+	+	+	+	+	+
Knch	+	+	+	+	+	+	+	+	+	+	+	+
Dil B	+	+	+	+	+	+	+	+	+	+	+	+
Gjr	+	+	+	+	+	+	+	+	+	+	+	+
Krb	+	+	+	+	+	+	+	+	+	+	+	+
Hppy	+	+	+	+	+	+	+	+	+	+	+	+
Bil	+	+	+	+	+	+	+	+	+	+	+	+
Gari-2	+	+	+	+	+	+	+	+	+	+	+	+
Bknr	+	+	+	+	+	+	+	+	+	+	+	+
Gari-1	+	+	+	+	+	+	+	+	+	+	+	+
K3	+	-	+	+	+	+	+	+	+	+	+	+
G1	+	+	+	+	+	+	+	+	+	+	+	+
Rchil	+	+	+	+	+	+	+	+	+	+	+	+
T.T	+	+	+	+	+	+	+	+	+	+	+	+
Lf1	+	+	+	+	+	+	+	+	+	+	+	+
Chili n	+	+	+	+	+	+	+	+	+	+	+	+
S.kunj	+	+	+	+	+	+	+	+	+	+	+	+
GG	+	+	+	+	+	+	+	+	+	+	+	+
Grs	+	+	+	+	+	+	+	+	+	+	+	+
Drg	+	+	+	+	+	+	+	+	+	+	+	+
L.acid	+	+	+	+	+	+	+	+	+	+	+	+
Ccbr	+	+	+	+	+	+	+	+	+	+	+	+
L.fmtm	+	+	-	+	+	+	+	+	+	-	+	+
Rchnd	+	+	+	+	+	+	+	+	+	+	+	+
Lf2	+	+	+	+	+	+	+	+	+	+	+	+
Jmd	+	+	+	+	+	+	+	+	+	+	+	+

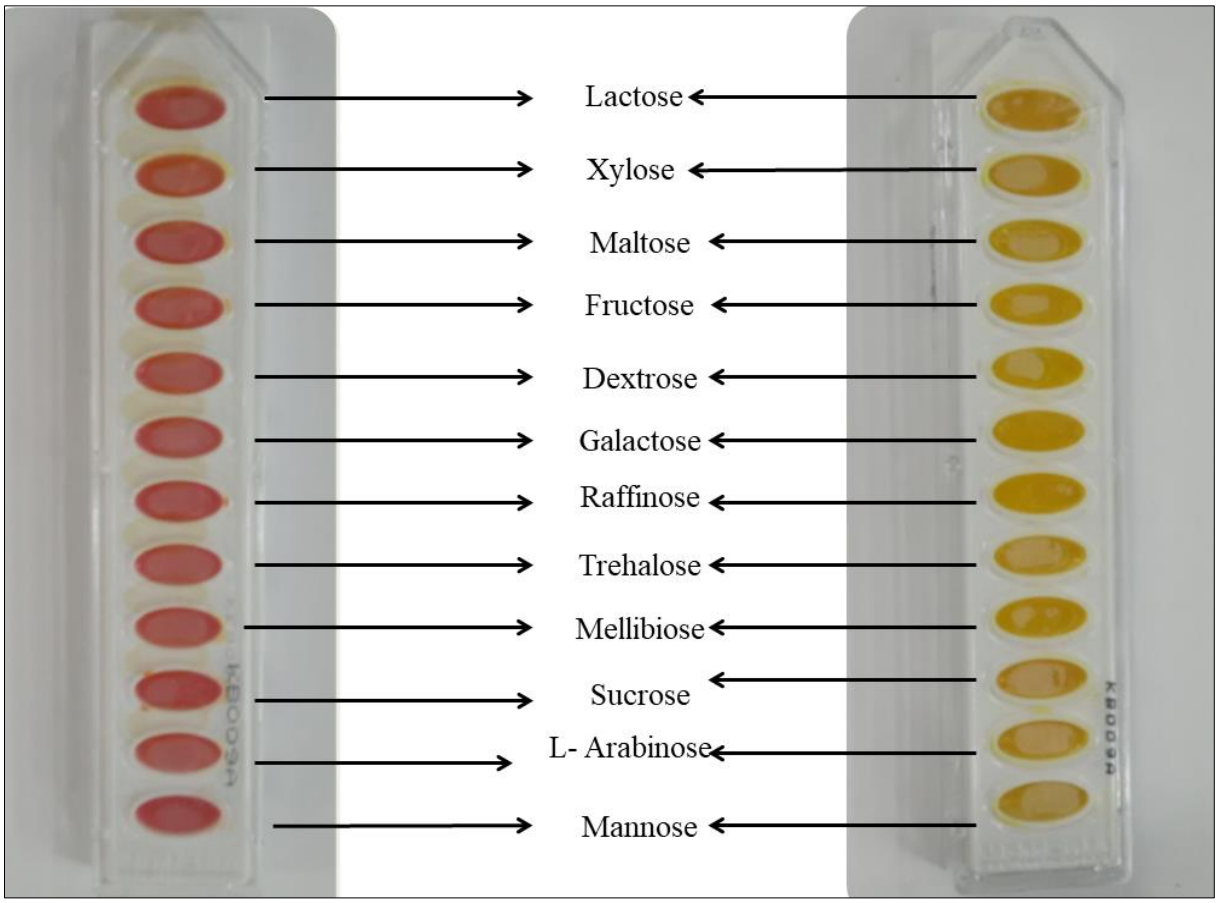

Fig 3: Hicarbo kit sugar profiling test

Morphologically and biochemically confirmed *Lactobacillus* cultures

A total number of 61 dahi, vegetable and fruit samples were collected from the different areas of the Chhattisgarh state. These 61 collections include 41 dahi, 2 cucumber, 3 chilli, 3 tomato, 1 lady finger, 3 cabbage, 2 spinach, 1 broccoli, 2 bottle guard, 1 cluster beans, 1 brinjal sample. Out of 61 pure isolates 39 cultures were confirmed *Lactobacillus* on the basis of morphological and biochemical (catalase and sugar profiling test) tests. Dahi is a natural habitat for LAB among which *Lactobacillus* cultures could be predominantly isolated. Rashid *et al*., (2006) [11] isolated *Lactobacillus* fermentum, *Lactobacillus* delbruckii subsp. bulgaricus, *Lactobacillus* delbruckii subsp. lactis from traditional dahi samples. Vegetable and fruit samples are also known to harbor *Lactobacillus*. Isolation of *Lactobacillus* from different vegetable samples have been reported by Mundt *et al*. (1968) [9]; Trias *et al*. (2008) [12].

Screening of isolates for aerobic respiration ability

A total of 36 morphologically and biochemically characterized *Lactobacillus* isolates were screened for aerobic respiration ability. Respiration was induced in *Lactobacillus* cultures using MRS-H broth. The freshly activated *Lactobacillus* isolates were inoculated in MRS-H flasks and incubated at 37 ºC for 24 h in static and shaking (120 RPM) conditions. The respiration in cultures was ascertained by increase in O.D. in MRS-H as compared with MRS as determined by spectrophotometer (Model: UV-Vis 119, Systronics Pvt. Ltd. Ahmedabad, India). The results of screening of cultures for aerobic respiration ability are shown in Table-3. Out of 36 screened *Lactobacillus* isolates, 10 cultures showed higher OD@600nm values in heme & menaquinone supplemented samples (treatment) as compared to control samples (non-heme & non-menaquinone samples) in static flask conditions. Thus these 10 cultures named-Metallic, Durg, Drj, Shumis, Jd5, Dhmtri, R. Chil, NDS, Gjr exhibited aerobic respiration. Among these 10 samples, *Lactobacillus* Gjr showed highest difference (0.662) in control and treatment samples in static conditions and thus was found to be highly respiration competent. Brooijmans *et al*. (2009) [1] analyzed genome of Lactobacilli for components of electron transport chain and checked the heme induced respiration in Lactobacilli and concluded that a cydABCD operon that encodes a bd-type cytochrome was found in the genome of *Lb. plantarum* WCFS1. However, supplementation with heme alone did not result in a respiration like phenotype (increase in biomass) in aerobic non-pH-controlled batch cultures. Indeed, the genome of *Lb. plantarum* lacks a complete menaquinone biosynthesis gene set. When aerobic cultures of Lb. plantarum were supplemented with both heme and a menaquinone source, in the form of vitamin K2, and incubated for 48 h, a greater biomass and higher final pH were observed. In their experiment the cells were grown on MRS medium supplemented with glucose (10 mM), heme (hemin) (stock solution, 0.5 mg/ml in 0.05 M NaOH) & menaquinone (2 mg/ml stock in ethanol) to a final concentration of 2.5 µg/ml & 1 µg/ml respectively. Cells were grown aerobically in 100-ml flasks with shaking at 250 rpm and anaerobically in tubes at 37 °C. After completion of 24 h of incubation they observed the optical density (at 600 n.m. O.D.) of the both conditions of the culture. They proposed

that some Lactobacilli contains a branched electron transport chain capable of using oxygen or nitrate as an extracellular electron acceptor. The electron transport chain requires activation by the addition of heme cofactor and a menaquinone pool in the form of vitamin K2. Zotta *et al.* (2014) [13] conducted an experiment on 184 strains belonging to the genus *Lactobacillus* for screening of their ability to grow under aerobic respiratory conditions, in media containing heme and menaquinone at final concentration of 2.5 µg/ml & 1 µg/ml respectively, initial pH 6.8 in order to identify respiratory and oxygen-tolerant strains. They reported the activity in 70% strains. Other tested lactobacilli showed a decrease in OD600 value as result of heme supplementation. The decrease or no significant change in OD@600 nm values of cultures in treatment as compared to control values signify that many Lactobacilli are non-respiring and respiration is a species/strain dependent phenomenon. The bacterial species unable to undergo aerobic respiration are particularly more sensitive to heme supplementation (Lechardeur *et al*., 2011) [7].

Table 3: Screening of isolates for aerobic respiration ability

S. No	Culture name	Shaking		Static	
		Control	Treatment (H+M)	Control	Treatment (H+M)
1	Mettalic	0.624±0.005	0.634±0.003	0.683±0.002	0.95±0.006
2	Ccbr (she)	0.503±0.010	0.451±0.005	0.773±0.002	0.467±0.002
3	Rai	0.676±0.003	0.799±0.004	0.738±0.004	0.391±0.003
4	Durg	0.595±0.002	0.471±0.001	0.637±0.001	0.842±0.001
5	L.fmtm	0.767±0.006	0.867±0.004	0.713±0.015	0.778±0.005
6	Rjn	0.658±0.004	0.431±0.003	0.724±0.004	0.728±0.003
7	Drj	0.814±0.003	0.766±0.001	0.626±0.005	0.913±0.003
8	Korba	0.517±0.008	0.617±0.004	0.731±0.011	0.825±0.002
9	Jd5	0.664±0.007	0.653±0.009	0.785±0.011	0.917±0.006
10	IDk	0.420±0.002	0.417±0.001	0.526±0.005	0.554±0.004
11	Ab3	0.575±0.008	0.544±0.017	0.507±0.005	0.556±0.006
12	S.L	0.585±0.015	0.636±0.035	0.745±0.001	0.903±0.002
13	G1	0.652±0.002	0.735±0.003	0.788±0.003	0.969±0.002
14	Shu mis	0.899±0.005	0.731±0.004	0.595±0.003	0.943±0.010
15	Dhmtri	0.593.±006	0.709±002	0.665±0.330	0.869±0.007
16	J.L	0.485±0.001	0.496±0.002	0.718±0.001	0.776±0.002
17	Gar-2	0.328±0.005	0.456± 0.003	0.452±0.008	0.593± 0.007
18	Jmd	0.265±0.008	0.389±0.010	0.71±0.004	0.893±0.001
19	R.Chil	0.375±0.0003	0.851±0.003	0.619±0.003	0.935±0.003
20	Lf2	0.566±0.002	0.703±0.007	0.577±0.010	0.643±0.006
21	knchn	0.358±0.001	0.909±0.003	0.831±0.008	0.909±0.002
22	tt	0.677±0.006	0.617±0.004	0.894±0.005	0.923±0.012
23	GAri1	0.430±0.001	0.572±0.002	0.891±0.002	0.876±0.003
24	NDS	0.501±0.001	0.59±0.001	0.613±0.005	0.912±0.005
25	Gjr	0.558±0.002	0.847±0.026	0.485±0.002	1.147±0.006
26	Dga	0.517±0.002	0.561±0.007	0.752±0.005	0.838±0.003
27	L.acid	0.828±0.001	0.822±0.004	0.874±0.002	0.826±0.033
28	hppy	0.645±0.004	0.611±0.009	0.916±0.001	0.905±0.003
29	bil	0.715±0.003	0.724±0.010	0.814±0.002	0.912±0.006
30	Dil b	0.787±0.006	0.603±0.004	0.890±0.004	0.916±0.001
31	Gg	0.430±0.005	0.651±0.018	0.748±0.004	0.832±0.0003
32	Rchnd	0.770±0.003	0.80±0.002	0.937±0.003	0.903±0.004
33	Bknr	0.924±0.001	0.730±0.0003	0.932±0.014	0.703±0.012
34	jg3	0.592±0.002	0.643±0.011	0.722±0.002	0.871±0.002
35	k3	0.479±0.332	0.712±0.004	0.717±0.008	0.737±0.003
36	Grs	0.66±0.072	0.949±0.004	0.912±0.004	0.811±0.002
37	S.kunj	0.453±0.007	0.426±0.009	0.704±0.003	0.742±0.011
38	Chillinew	0.607±0.004	0.655±0.001	0.664±0.003	0.754±0.0006
39	lf2	0.367±0.006	0.413±0.002	0.621±0.009	0.781±0.007

Results are expressed as Mean ± SE (n=3)

Conclusion

Lactobacillus cultures were isolated from dahi, vegetable and fruits samples and out of 39 pure *Lactobacillus* isolates 23 culture shows higher OD @600nm, it means these culture are respiration competent in heme and Menaquinone supplemented conditions. *Lactobacillus* Gjr was found to exhibit highest aerobic respiration and was able to produce high biomass in heme and Menaquinone supplemented medium. The study culminated in bioprospecting of respiration competent strains. The higher cell number or biomass producer strain could be used for numerous applications such as production of starter culture, production of novel fermented products, biomolecules, vitamins etc. Aerobic Respiration thus is a useful metabolic system with many potential industrial and commercial applications.

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