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ISOLATION AND CHARACTERIZATION OF LACTOBACILLUS BACTERIA FROM DIFFERENT SAMPLE OF CURD

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INTRODUCTION

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod shaped, non-spore-forming bacteria. They are a major part of the lactic acid bacteria group (i.e. they convert sugars to lactic acid). In humans, they constitute a significant component of the microbiota at a number of body sites, such as the digestive system, urinary system, and genital system. In women of European ancestry, *Lactobacillus* species are normally a major part of the vaginal microbiota. *Lactobacillus* forms biofilms in the vaginal and gut microbiota, allowing them to persist during harsh environmental conditions and maintain ample populations. *Lactobacillus* exhibits a mutualistic relationship with the human body as it protects the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients. Lactobacillus is the most common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being as it can help treat diarrhea, vaginal infections and skin disorders such as eczema. Many lactobacilli operate using homofermentative metabolism (they produce only lactic acid from sugars), and some species use heterofermentative metabolism (they can produce either alcohol or lactic acid from sugars). They are aerotolerant despite the complete absence of a respiratory chain. This aerotolerance is manganese-dependent and has been explored (and explained) in *Lactobacillus plantarum*. Many species of this genus do not require iron for growth and have an extremely high hydrogen peroxide tolerance.

Probiotics

Lactobacillus species administered in combination with other probiotics benefits cases of irritable bowel syndrome (IBS), although the extent of efficacy is still uncertain. The probiotics help treat IBS by returning homeostasis when the gut microbiota experiences unusually high levels of opportunistic

bacteria. In addition, *Lactobacillus* species can be administered as probiotics. during cases of infection by the ulcer-causing bacterium Helicobacter pylori. Helicobacter pylori is linked to cancer, and antibiotic resistance impedes the success of current antibiotic-based eradication treatments. When *Lactobacillus* probiotics are administered along with the treatment as an adjuvant, its efficacy is substantially increased and side effects may be lessened. Also, *Lactobacillus* is used to help control urogenital and vaginal infections, such as bacterial vaginosis (BV). *Lactobacillus* produce bacteriocins to suppress pathogenic growth of certain bacteria, as well as lactic acid and H_2O_2 (hydrogen peroxide). Lactic acid lowers the vaginal pH to around 4.5 or less, hampering the survival of other bacteria, and H_2O_2 reestablishes the normal bacterial flora and normal vaginal pH. In children, Lactobacillus strains such as *L. rhamnosus* are associated with a reduction of atopic eczema, also known as dermatitis, due to anti-inflammatory cytokines secreted by this probiotic bacteria.

Probiotics are microorganisms that are claimed to provide health benefits when consumed. The term came into more common use after 1980. The introduction of the concept (but not the term) is generally attributed to Nobel laureate Élie Metchnikoff, who postulated that yogurt consuming Bulgarian peasants lived longer lives because of this custom. He suggested in 1907 that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". A significant expansion of the potential market for probiotics has led to higher requirements for scientific substantiation of putative benefits conferred by the microorganisms.

Although there are numerous claimed benefits of using commercial probiotics, such as reducing gastrointestinal discomfort, improving immune health, relieving constipation, or avoiding the common cold, such claims are not backed by scientific evidence and are prevented as deceptive advertisements in the United States by the Federal Trade Commission. Probiotics are considered generally safe, but may cause bacteria-host interactions and unwanted side effects in rare cases

Some *Lactobacillus* species have been associated with cases of dental caries (cavities). Lactic acid can corrode teeth, and the *Lactobacillus* count in saliva has been used as a "caries test" for many years. Lactobacilli characteristically cause existing carious lesions to progress, especially those in coronal caries. The issue is, however, complex, as recent studies show probiotics can allow beneficial lactobacilli to populate sites on teeth, preventing streptococcal pathogens from taking hold and inducing dental decay. The scientific research of lactobacilli in relation to oral health is a new field and only a few studies and results have been published. Some studies have provided evidence of certain *Lactobacilli*

which can be a probiotic for oral health. Some species, but not all, show evidence in defense to dental caries. Due to these studies, there have been applications of incorporating such probiotics in chewing gum and lozenges. There is also evidence of certain *Lactobacilli* that are beneficial in the defense of periodontal disease such as gingivitis and period.

Some *Lactobacillus* species are used as starter cultures in industry for controlled fermentation in the production of yogurt, cheese, sauerkraut, pickles, beer, cider, kimchi, cocoa, kefir, and other fermented foods, as well as animal feeds. The antibacterial and antifungal activity of *Lactobacillus* species rely on production of bacteriocins and low molecular weight compounds that inhibits these microorganisms.

Sourdough bread is made either spontaneously, by taking advantage of the bacteria naturally present in flour, or by using a "starter culture", which is a symbiotic culture of yeast and lactic acid bacteria growing in a water and flour medium. The bacteria metabolize sugars into lactic acid, which lowers the pH of their environment, creating a signature "sourness" associated with yogurt, sauerkraut, etc. In many traditional pickling processes, vegetables are submerged in brine, and salt tolerant *Lactobacillus* species feed on natural sugars found in the vegetables. The

resulting mix of salt and lactic acid is a hostile environment for other microbes, such as fungi, and the vegetables are thus preserved—remaining edible for long periods.

Lactobacilli, especially *L. casei* and *L. brevis*, are some of the most common beer spoilage organisms. They are, however, essential to the production of sour beers such as Belgian lambics and American wild ales, giving the beer a distinct tart flavor.

MATERIAL AND METHODS

Isolation and Identification of Lactobacillus: The samples of fresh curd were collected from local market of Kalyanpur, Maharajganj, (India) in sterilized screw capped bottles. Serial dilutions (0.2 ml) of these samples were spread on sterile petri dishes of MRS agar. Inoculation was carried out at 37 ˚C for 24 – 48 hours. Colonies showing typical characteristics of morphology such as color (buff, pale yellow, white), size (less than 1 mm diameter) and shape (biconvex) on MRS agar were selected and purified by streaking at least three times in MRS agar. All colonies were maintained by weekly subculturing on MRS agar. The morphological characteristics of isolates were examined after staining by Gram stain. It was then followed by motility test, catalase test, indole test, methyl red test, voges proskauer test, citrate

test. Morphological and Biochemical test were used for identification of bacteria and all results were compared with Bergey's manual of systematic bacteriology.

SAMPLE COLLECTION- A total of 9 samples of curd were collected from local areas of Kanpur and Azamgarah.. Then all these samples were stored at 4°C. Afterwards all these samples were taken to the Microbiology laboratory of IBSBT CSJM University Kanpur for further microbiological analysis. .

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS Further identification and characterization of the isolates was done, as described in Berge's Manual of systematic bacteriology (Garrity,2005).

Morphological examination of culture- Morphological and cultural examination was carried out by using Gram's staining method described by *Hans Christian Gram* (1884). .\

GRAM STAINING- Log phase culture of isolated strains were smeared on slides and fixed. The smear was flooded by cristal violet for 45 sec and washed with distilled water . A few drop of Grams iodine was put on that smear for 45 sec , followed by washing with 95% ethyl alcohol drop by drop and than washed with distilled water. Finally smear was flooded by Safranin for 1 minute and then again washed with distilled water . Slides were then observed under oil immersion of microscope for determining coloure and shape.

CATALASE TEST - This test was used to check the production of enzyme catalase. For this test a clean microscopic slide was taken. A drop of 3% H2O2 was taken on the microscopic slide aseptically. A loopful of bacterial culture was taken and mix ed with 3% H2O2 solution on the slide and the presence of the bubble production observed.

OXIDASE TEST- Log phase culture of isolated strains was touched on the surface of oxidase disc and oserved for coloure change.

STARCH HYDROLYSIS- Starch agar plates were streaked with isolated strain incubated for 24 h. The plate were flooded with Iodine solution for 30 sec and observed for coloure change.

SUGAR FERMENTATION TEST- Tripticase soy broth was prepared and added in sterile test tube . Now durhym tube added in test tube inoculate strain in each test tube. Incubate the tube for 24 h at 37^{o}c and observed for colure change.

ANTIBIOTIC RESISTANCE TEST (Zhou et al. 2005)- Resistance to antibiotics was detected by using antibiotic discs. MRS agar media was spread with isolated strain of Lactobacillus. Antibiotic disc were placed over the surface. The sensitivity to an antibiotic was detected by inhibitory zone formed around disc after 24 h incubation at 37°c following antibiotic were used.

• 5 ml bacterial culture mixed with 0.5 ml of sterile NaCl solution and diluted by 10^{-6} then incubated at 37˚c for 48 h.

• 5 ml bacterial culture mixed with 2.2 ml of sterile sucrose solution and diluted by 10^{-6} then incubated at 37˚˚C for 48 h.

RESULTS

In the present study, thirty dairy samples (7 curd) were collected from the local area Kanpur . Serial dilutions of dairy samples were prepared in 100 µl sample from different dilutions were spread over the solidified MRS (de Man, Rogosa and Sharpe) medium and

incubated at 37^0C for 48 h for the isolation. Pure cultures of isolated colonies were obtained after re-streaking on MRS agar plate. Thirty bacterial isolates were initially obtained from thirty dairy samples. In all 18 bacterial isolates were isolated from curd samples.

BIOCHEMICAL CHARACTERIZATION

Catalase test

All the isolate were catalase negative as nolibration of effervescence of o2 around the bacteria colonies were seen on addition of hydrogen peroxide (h2o2).

Oxidase test -

All the isolated were oxidase negative as no change in color was noticed on oxidase disc after spot inoculation on it .

Starch hydrolysis

No clear zone was shown by the isolate and addition of iodine solution. Hence the isolate were unable to produce amylase .

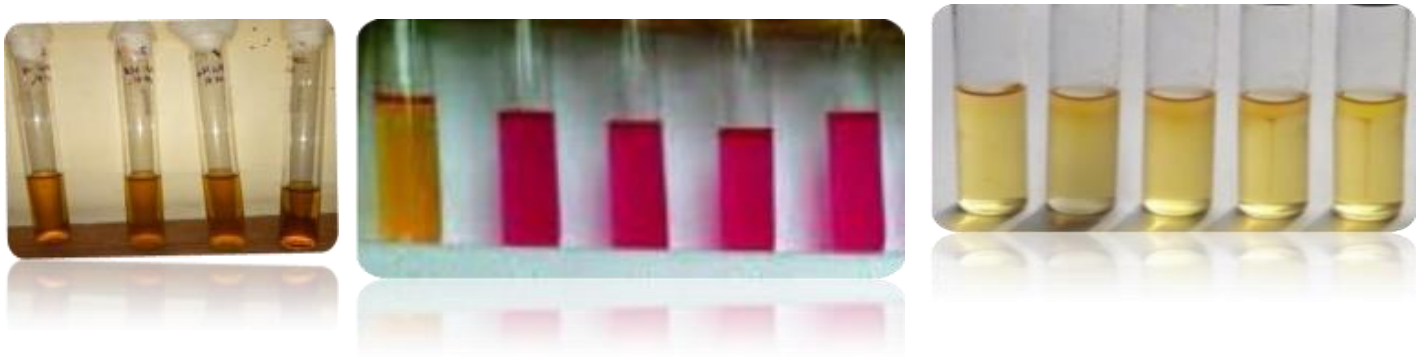
Sugar fermentation test

The isolates produced yellow color in broth and indicated that they utilized glucose ,lactose and sucrose and produced acid .

Table- Show biochemical characterization of isolates

Isolates	Gram staining	Catalase	Oxidase	M.R	V.P	Indol	Citrate	Lactose	Starch hydrolysis
LBK	+	_	_	_	_	_	+	+	+
LBG	+	_	_	_	_	_	_	+	_
LBC	+	_	_	_	_	_	_	+	_
LBP	+	_	_	_	_	_	_	+	+
LBA	+	_	_	_	-	_	_	+	+
LBKl	+	_	_	_	_	_	+	+	_
LBM	+	_	_	_	_	_	+	+	_

Figure 1-CITRATE TEST



Biochemical test

Figure 2-

ANTIBIOTIC RESISTANCE TEST-

Al the isolate of lactobacillus were resistance to the action of all the tested antibiotic . LBK was sensitive to the action of ampicillin , ,clindamycin ,erythromycin, co-trimoxazole.

Disc	LBK	LBG	LBC	LBP	LBA	LBKL	LBF	LBM
Ampicillin	_	13	20	_	20	_	_	14
penicillin	_	10	_	8	6	_	9	_
clindamycin	34	28	24	22	18	30	27	36
erythromycin	12	18	10	14	22	16	_	12
Co trimoxazole	10	_	8	14	_	9	18	20
vancomycin	8	4	_	6	_	2	5	_

Conclusion-

From a number of different 14 curd samples, 28 different strains were isolated. All the strains were characterized on the basis of their colonies morphology and other biochemical characteristics. Colonies were circular, small and large and cream white after incubation on MRS agar plate. All the strains studied were non-motile, non spore forming, Gram-positive, rod-shaped bacteria that produce lactic acid homofrementatively from glucose. In most of the strains, catalase was not produced. It was concluded that all the comparative studies including cultural, morphological and identification of the pure culture by biochemical tests showed that the strains that does not produce catalase enzyme were Lactobacillus. Only seven strains showed the positive results for Lactobacillus.

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