WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Identification of Recent Methods in Isolation and Biochemical Characterization of Lactobacillus Species from Cow and Buffalo Milk Samples

Yuvabharathi Kannan[1], Ramesh babu P.B[2] and Vijayalakshmi K[1]

[1] Department of Genetic Engineering, Bharath Institute of Science and Technology, BIHER, Chennai. India.

[2] Center for Materials Engineering and Regenerative Medicine. Bharath Institute of Science and Technology, BIHER, Chennai. India.

ABSTRACT

Milk is a great source of rich nutrients with active probiotics. Biochemical and microbial analysis of raw milk is necessary for quality check in commonly available dairy products. Current methods in identifying and validating biochemical contents of milk is utmost important in dairy industries. In the present study, raw milk samples from cows and buffalos were collected in Chennai, India and reviewed the research methodology required for microbial and biochemical components quality studies, which will be beneficial to dairy industries. Several reports indicated the identification and presence of 88% of microorganism iso1ates from raw milk along with different fungus species in curd and cheese. The samples were made to culture in source appropriate MRS media. This paper describes on study of biochemical tests are performed and specifies its species of particular genes and compared with recent methods.

Keywords: Raw milk, MRS media, lactobocillus spp, milk produce and biochemical test.

Introduction:

The genomes of eubacteria are reported to be 2 to 4. 9 Mb (mega bases). Thus, the measure of protein-coding quality ranges from one, 267 to concerning four, 758 qualities (in L. sanfranciscensis and L. parakifiri, individually). Indeed, even inside one species, there is a considerable variation. For instance, strain of L. crispatus has request size beginning from one. 83 to 2.7 Mb or 1,839 to 2,688 open perusing outlines. The milk Lactobacillus could be a sort of gram-positive, facultative anaerobic or micro aerophilic, pole molded, non-spore-shaping microorganism [1]. They're a genuine piece of the carboxylic acid microorganism group

(for example they convert sugars to lactic acid). Lactobacillus could be a kind of microorganism belonging to numerous and various types of eubacterium. These are symbiotic microorganism that typically rest in our gut [2]. Urinary and sex organ frame works while they are symbiotic in nature.

The measure of protein-coding qualities ranges from 267 to concerning 758 qualities (in L. sanfranciscensis a L. parakefiri, respectively). Indeed, even inside one species, there is considerable variety. For instance, strains of L. crispatus have request sizes beginning from 83 to 2.7 Mb or 1,839 to 2,688 open perusing outlines. Lactobacillus contains an abundance of compound microsatellites inside the composition of the request and have variation themes [3].

Eubacterium is also present in a few yogurt and in dietary supplements. Lactobacillus is utilized for treating and averting detachment of the entrails, just as irresistible varieties like [4,5,6] rotavirus detachments and detachment of the guts, Its moreover acclimated thwart and treat detachment of the grits identified with exploitation antibiotics some people use eubacterium for general absorption disadvantages. Bed tempered digestive system disorder (IBS), intestinal colic in infants: Crohn's illness: aggravation of the colon; and a critical gut issue known as fiery infection (NEC) in children brought into the world less than ideal. Eubacterium it is moreover utilized for contamination with Helicobacter pylori, the kind of microorganisms that causes ulcers.

Furthermore, for various types of diseases just as tract contaminations (UTIs) eubacterium may be recorded. To thwart the abnormal growth and to hinder digestion related problems in youth is going to be take care as utmost important in prebiotic microbiota. It's also being tried to thwart genuine contaminations in people on ventilators in critically ill patients. Lactobacillus is utilized for skin diseases [7], fever rankles, ulcer, eczema (allergic dermatitis): and skin break out. It is additionally utilized for elevated cholesterol, lactose narrow mindedness Lyme ailment and to help the invulnerable framework. Women use lactobacillus suppositories to treat vaginal contaminations as a treatment to urinary tract diseases (UTIs). There are worries about the nature of some lactobacillus species, which was named to contain Lactobacillus acidophilus really contain no lactobacillus acidophilus, or they contain an alternate strain of lactobacillus such as Lactobacillus bulgaricus [8].

Numerous microscopic organisms and different organisms in our body ordinarily are symbiotic in nature. For example, lactobacillus maintains gut flora, regulate immunity and provide essential nutrients. Ingest supplements and ward off "hostile" life forms that may cause illnesses, for example, looseness of the bowls [9]. Milk is supplement and basic sustenance for large growth of microbe development [10]. Milk could be a supplement rich in several valuable micronutrients and nutrients. White fluid sustenance made by the exocrine organs of well evolved creatures. It's the principal supply of nourishment for infant mammals (counting people World Health Organization region unit breastfeed) before they are prepared to process various type of sustenance. Early-lactation milk contains high fate milk in the form of colostrum. That passes the mother's antibodies to its young and might curtail the risk of the numerous illnesses [11]. It contains a diverse nutrients

including macromolecule and disaccharides, lipds, proteins. Altogether among people few consume the milk of various warm blooded animals.

MATERIAL AND METHODS

Milk samples were gathered from Chennai and Kanchipuram regions in Tamilnadu, Inda because of their wide acknowledgement among the clients of tamilnadu straight off when grouping the examples were hang on aseptically in cold chain, white products to protect from pollution and decay. In essentially examinations of the milk test of Chennai local were utilized as there contained agreeable amount of probiotic properties.

Media

The microorganism eubacterium ssp. were detached from milk test by abuse changed MRS juices and MRS agar medium for sure. 0.05% aminino alkanoic acid was side to MRS to improve the particularity of this mechanism for disconnection of eubacteria and the pH value of the medium was changed in accordance with six 5

Lactobacillus MRS Agar:

De Man Rogosa and Sharpe agar normally dissolved to MRS, which could be a specific compenent intended to support the cultivation of Lactobacilli. The above said medium was first formulated in 1960, which was named for its inventor. It contains metal acetic acid derivation, that the extension of the numerous aggressive bacteria (albeit another Lactobacillales. As Leuconostoc and Pediococcu may develop) [12] this medium incorporates a reasonable darker color, which is a typical color of MRS agar:

MRS agar is composed of tryptic digest of casein, beef extract, yeast extract, glucose and the ranges 1.0 % peptone,1.0 % meat remove, 0.4 % yeast remove, 2.0 % glucose,% sodium acetic acid derivation trihydrate, % polysorbate 80 (othcrwise called Tween 80), % dipotassium hydrogen phosphate, 0.2 % triammonium citrate, 0.02 % magnesium sulfate heptahydrate 0.005 % manganese sulfate tetrahydrate, 1.0 % agar. pH changed in accordance with 6.2 of 25°C. The yeast and meat concentrates and peptone give wellsprings of carbon, nitrogen and nutrients for general bacterial development. The yeast remove additionally contains nutrients and amino acids explicitly required by Lactobacilli.

Polysorbate 80 is a surfactant which aids supplement consumption by Lactobacilli. Magnesium sulfate and manganese sulfate give cations utilized in digestion. The milk samples were made in saline and pour plated using MRS agar and incubated at 37°C vigorously for 24-48 hrs. At the end of 48 hrs when the settlements were processed further. Morphologically particular and well-disconnected components were picked and exchanged to new MRS agar plates by streaking. Settlements indicating average qualities of lactobacilli on agar surface were gotten heedlessly and moved into MRS juices for any advancement, Further. Their virtue was kept an eye on MRS agar. The unadulterated sample preparations were exposed to distinguishing proof according to the Bergey's manual of medicinal strength through open supply programming named PIBWin and IDENTAX. A

perceptible look of the considerable number of samples was analyzed for social and morphological attributes. Estimate the shape. shading, and surface of the samples were recorded.

Gram Stain:

Staining strategy for the fundamental identification of bacterium, amid which a violet color is connected, trailed by a decolorizing operator thus a red color. The cell dividers of bound bacterium (indicated Gram-constructive) hold the essential color and appear to be violet, though individuals who separate (meant Gram-negative) appear to be red, furthermore known as Gram's strategy.

Technique:

The performance of the Gram Stain on any sample requires some basic steps that include applying [13-18]. Get ready and warm the fix spreads and stain the slides as procedure, treat the antifungal for one moment. Pour off abundance color and wash gently in water and channel the slide against a towel. Open the smears to grain's iodine for one moment by clothing with iodine at that point including extra. Iodine and exertion it on the smear till the moment is finished. Wash with water and channel demandingly (try not to blotch). Wash with 95% ethanol for 30 seconds, then wash with water at the highest point of the 30 seconds to forestall the decolonization, channel recolor with zero 25% saffranine for 30 seconds Wash. Channel, smudge, and analyzed glass slide underneath drenching oil at 100X goals optical magnifier. **Result:** Gram stain is performed and seen that bacteria is grain positive bacteria

Biochemical Test

Biochemical reactions used in biochemical tests [19] depend on the presence of such bacteria. Such biochemical tests have been designed to measure the levels of bacterial enzymes which can be interpreted to accurately identify the species of bacteria they have been produced by Indole test, Methyl red test, Bromothymol blue test, Tube Catalase test

Indolc test:

The indole test is a biochemical test performed on bacterial species to determine the ability of the organism to convert tryptophan into indole [20]. This division is performed by a chain of a number of different intracellular enzymes, a system generally referred to as "tryptophanase."Add 0.5 ml of Kovac's reagent to the juices culture positive:pink hued ring after the expansion of suitable reagent, negative: No shading change of sample noticed after the expansion of proper reagent

Methyl red test:

By using sterile inoculates being into the contemporary, first clean the medium by sterilization and leave the contras' stock–uninoculated (control). Incubate the inoculation cylinder at 35-37ºC for 2 to 5 days, **o**nce cultured, obtain the juices from the culture and include with a drops of methyl bunch red compound operator

to the juices**.** Observe the shading and development of red shading is considered as positive. Development of yellow shading (No shading change) alien the expansion of synthetic specialist is taken as negative [21]. Bromothymol Blue test. This 0.1% watery bromothymol blue arrangement (otherwise called Bromthymol Blue) is a generally utilized It is a decent marker of disintegrated carbon dioxide (CO2) and other feebly acidic solutions. BromothymoI is likewise used to obstetrics.

Identifying untimely crack of layers.

Fig. 1. 2 ml of lactobacillus bacterial broth culture in test tube media color is yellow before test

Fig.2. 2ml of lactobacillus bacterial broth culture with Bromothymol blue in test tube in test tube. Medium color changed to orange. Before test.

Tube Catalase Test:

The catalase test is a particularly important test used to determine whether a gram-positive bacteria is a staphylococci or a streptococci. Add four to five drops of three H_2O_2 (Hydrogen peroxide) to in a very tubing employing a wood applier stick, collect a tiny low quantity of organism from wells isolated after 18 to 24 hour colony culture and place into the tubing note: observe out to not acquire agar (esp if victimization Blood Agar) - rationalization in precaution below). Place the tube against a dark background and observe for immediate bubble formation the tip of the wood applier stick. Observe positive reactions as evident by immediate effervescence (bubble formation) [22]. Enzyme Negative reaction: No bubble formation (no enzyme accelerator to -change the gas peroxide) [23].

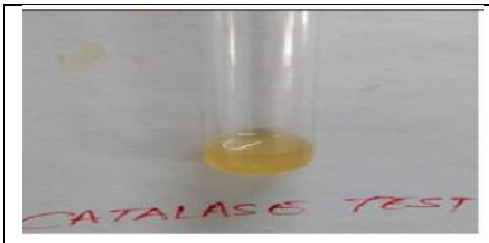

Fig.3. 2ml of Lactobacillus‘ bacterial broth culture in test tube. media color is yellow’ without bubble. Beforetest

Fig. 4. 2 ml of lactobacillus bacterial broth culture in test tube. Media color is yellow with bubble formation. Before test

Table1: Biochemical characteristics of isolated lactobacilli

S.no	Biochemical Test	Result
1	Indole test	-
2	Methyl red test	+
3	Bromothymol blue test	-
4	Tube Catalase test	+

Table 2: Current methods in isolating microorganisms from milk products

S. no	Method	Detected Organisms	Reference
1	Ferment lactose to lactic acid	Streptococcus lactis	Lu, M & etal., 2013. Food Nutr. Sci. 4, 113–123.
2	Produce heat-stable extracellular enzymes	Pseudomonas fluorescens	Ledenbach, L.H and etal., 2009. Springer, pp. 41–67.
3	Antibiotic treatments	Clostridium difficile	Van Dissel, J. T & et al. (2005). Journal of Medical Microbiology, 54, 197–205.
4	Effect of ultrasound on cell wall permeability	Lactobacillus acidophilus	Tabatabaie, F., and A. Mortazavi. 2008. World Applied Sciences Journal 3:119–21

Grams test results demonstrated that the microscopic organisms are bar shape gram positive microorganisms [24, 25]. Indole test demonstrated that microorganisms can't part amino corrosive tryptophan to shape the compound indole. Which implies microorganisms does not amalgamation tryptophanase compound. Methyl test demonstrates that microbes have capacity to deliver stable acids end items (Mixed-corrosive aging) from provided glucose [26,27]. Bromothymol blue test demonstrates negative outcome. which implies there is no untimely burst of layers. Tube cause test result is sure which demonstrates that catalase chemical present in microbes which breakdown of hydrogen peroxide into oxygen and water [28].

Biochemical on lactobacillius microscopic organisms has been performed [29, 30] and reasons that gram recoloring is sure. Indole test result demonstrates that microscopic organisms isn't delivering tryptophanase catalyst [31]. Methyl red test outcome demonstrated that microscopic organisms have capacity to deliver stable acidic final result from provided glucose, Bromothymol blue test demonstrates that bacterial films isn't burst.

Cylinder catalase test result demonstrates that catalyse compound present in microscopic organisms which breaks down of hydrogen peroxide into oxygen and water. The methods in detecting and isolation milk microorganisms are shown in table 2.

Summary:

Bacterial species present in milk, fills in as great mode for microbial development. Four crude milk tests from two cow and two buffaloes were gathered in Chennai. They were examination for 85% of pathogen's microorganism separates have observed to be from crude milk with diverse parasite species were found in curd and cheddar the examples were made to culture in source MRS media as reported in previous literatures (32,33). Lyophilization test and biochemical test are performed [34,35], and biochemical tests on lactobacillius microbes has previously been done and found that grain recoloring was sure. Indole test results demonstrated that microscopic organisms isn't creating tryptophanas protein methy1 red test outcome demonstrates that microscopic organisms lost the potential to create stable acidic growth results from glucose. Bromothymol blue test demonstrated that bacterial films subtyping and catalase test results demonstrated that enzymes present in microorganisms breaks down hydrogen peroxide into oxygen and water.

Reference

- The lactic acid bacteria: a literature sum ej . Carr FJ. Chill D. Maida N. Crit Re Microbiol 2002.28(4):281-370. Clinical Laboraton' Consulting, 1tl7 Ootsima Way. Loudon. TN 37774. USA
- Lactic acid bacteria of foods and their current taxonomy. Stiles ME'. Holzapfel WH. 1'397 Apr 29.36(1):1-29.Department of Food Science and Nutrition. Unit ersitv of Alberta. Edmonton.
- Canadalnt I Food Microbiol Gene expression s› steins for lactic acid bacteria deVos WM CurropinMicrobiol. 1999 Jun.2(3): 259-95 Wngeningen Centre for Food Science. Wageningen. The Netherlands. u illein.devos?ijalgemeen.inicr.u au nl
- Lnctic acid bactcria of foods and thTir cnrrcnt taxonoin› . Stiles ME1. Holzapfel WH. hit I Food Microbiol l'?'27 Apr 29.36(1): 1-29.Department of Food Science and Nutrition. Unit ersit› of Alberta. Edmonton. Canada.
- Genomic fingerprinting of Bartonella species b\ repetitn'e element PCR for distinguishmg species and isolates Rodriguez-Barradas MC1, Hainill RJ. Houston ED. Georghiou PR. Clarridge JE. Regneo RL. Koehler JE J Clin M icrobiol. 1995 Maj ,33(5): 1089-93. Section of Infections Diseases. Medical Service. Veterans Affairs Medical Center, Houston, TX 77030. USA.
- Blazer ic DJ. Schr0ck0nbcrg0r PC. Mntscn JM. Evaluation of the PathoTcc "Rapid I-D Sjstem" AppIMicrobiol. 1973 Dec,26(6):88fi—S8°J. [PMC free article] I ubMed]
- Brooks KA. Jcns M. Sodeman TM, A clinical ei'aliiation of the API inicrotube s¿ stein for identification of Enterobacteriaceae. Am J Med Technol. 1974 Feb,40(2) 55—C 1. {PubMed]

- CLARKE PH, COWAN ST. Biochemical methods for bacteriologꜛ . J Gen Microbiol 1952 Fcb:6(1-2): I 87-197, {PubMcd]

- Fay GD. Barrv AL. Methods for detecting indole production by graiti -ne gative nonsporefonning anaerobcs ApplMicrobiol 1974 Mar:27(3):562-5C5. {PMC frec articl* I [ubMed{ISEN BERG HD, SUN DHE IM LH. Indole reactions in bacteria. I Bacteriol. 1'258 Jun.7S(G):CS2 690, [PMC free article]

- Milk and Parity Affected Serum Biochemical Parameters and Hormone Profiles in Mid-Lactation Holstein Cows. Xuehui Wu [1], Hui-Zeng Sun [2 3], Mingyuan Xue [4], Dimin Wang [5], Leluo Guan [6], Jianxin Liu [7.] Days-in-Milk and Parity Affected Serum Biochemical Parameters and Hormone Profiles in Mid-Lactation Holstein Cows. Animals. 2019. 10;9(5):230.

- Lu, M., Shiau, Y., Wong, J., Lin, R., Kravis, H., Blackmon, T., Pakzad, T., Jen, T., Cheng, A., Chang, J., Ong, E., Sarfaraz, N., Wang, N.S., 2013. Food Nutr. Sci. 4, 113–123.

- Ledenbach, L.H., Marshall, R.T., 2009. In: Sperber, W.H., Doyle M.P. (Eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety, Springer, pp. 41–67. Lehotova, V., Petrulakova, M., Valík

- Van Dissel, J. T., de Groot, N., Hensgens, C. M., Numan, S., Kuijper, E. J., Veldkamp, P., et al. (2005). Bovine antibody-enriched whey to aid in the prevention of a relapse of Clostridium difficile-associated diarrhoea: preclinical and preliminary clinical data. Journal of Medical Microbiology, 54, 197–205.

- Tabatabaie, F., and A. Mortazavi. 2008. Studying the effects of ultrasound shock on cell wall permeability and survival of some LAB in milk. World Applied Sciences Journal 3:119–21

- Bahmani M, Shirzad H, Rafieian S, Rafieian-Kopaei M. *Silybum marianum*: Beyond hepatoprotection. J Evid Based Complementary Altern Med 2015;20:292-301.

- Ebrahimie M, Bahmani M, Shirzad H, Rafieian-Kopaei M, Saki K. A review study on the effect of Iranian herbal medicines on opioid withdrawal syndrome. J Evid Based Complementary Altern Med 2015;20:302-9.

- Asadi-Samani M, Rafieian-Kopaei M, Azimi N. Gundelia: A systematic review of medicinal and molecular perspective. Pak J Biol Sci 2013;16:1238-47.

- Bahmani M, Banihabib E, Rafieian-Kopaei M, Gholami-Ahangaran M. Comparison of disinfection activities of nicotine with copper sulphate in water containing *Limnatis nilotica*. Kafkas Univ Vet Fak Derg 2015;21:9-11.

- Nasri H, Behradmanesh S, Ahmadi A, Rafieian-Kopaei M. Impact of oral Vitamin D (cholecalciferol) replacement therapy on blood pressure in type 2 diabetes patients; a randomized, double-blind, placebo controlled clinical trial. J Nephropathol 2014;3:29-33.

- Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci 2012;17:621-5.

- Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, *et al.* Oxford-MEST classification in IgA nephropathy patients: A report from Iran. J Nephropathol 2012;1:31-42.

- Rafieian-Kopaei M, Asgary S, Adelnia A, Setorki M, Khazaei M, Kazemi S, *et al*. The effects of cornelian cherry on atherosclerosis and atherogenic factors in hypercholesterolemic rabbits. J Med Plants Res 2011;5:2670-6.

- Aroutcheva AA, Simoes JA, Faro S. Antimicrobial protein produced by vaginal *Lactobacillus acidophilus* that inhibits *Gardnerella vaginalis*. Infect Dis Obstet Gynecol 2001;9:33-9.

- Boris S, Jiménez-Díaz R, Caso JL, Barbés C. Partial characterization of a bacteriocin produced by *Lactobacillus delbrueckii* subsp. *lactis*UO004, an intestinal isolate with probiotic potential. J Appl Microbiol 2001;91:328-33.

- Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr J Biotechnol 2003;2:219-27.

- Chaillou S, Christieans S, Rivollier M, Lucquin I, Champomier-Verges MC, Zagorec M. Quantification and efficiency of Lactobacillus sakei strain mixtures used as protective cultures in ground beef. Meat Sci 2014;97:332–8.

- Davoodabadi A, Soltan Dallal MM, Lashani E, Tajabadi Ebrahimi M. Antimicrobial activity of Lactobacillus spp. isolated from fecal flora of healthy breast-fed infants against diarrheagenic Escherichia coli. Jundishapur J Microbiol 2015a;8: e27852.

- Davoodabadi A, Dallal MM, Foroushani AR, Douraghi M, Harati FA. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Anaerobe 2015b;34:53–8.

- Delcaru C, Alexandru I, Podgoreanu P, Cristea VC, Bleotu C, Chifiriuc MC, et al. Antagonistic activities of some Bifidobacterium sp. strains isolated from resident infant gastrointestinal microbiota on Gram-negative enteric pathogens. Anaerobe 2016;39:39–44.

- Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 2014;11:4745–67.

- Fijan S, Šulc D, Steyer A. Study of the in vitro antagonistic activity of various single-strain and
- multi-strain probiotics against Escherichia coli. Int J Environ Res Public Health 2018;15:1539.

- Fooladi AA, Forooshai MC, Saffarian P, Mehrab R. Antimicrobial effects of four lactobacilli strains isolated from yoghurt against Escherichia coli O157: H7. J Food Saf 2014;34:150–60.

- Halimi S, Mirsalehian A. Assessment and comparison of probiotic potential of four Lactobacillus species isolated from feces samples of Iranian infants. Microbiol immunol 2016;60:73–81.

- Hashemi SM, Shahidi F, Mortazavi SA, Milani E, Eshaghi Z. Potentially probiotic Lactobacillus strains from traditional Kurdish cheese. Probiotics Antimicrob Proteins 2014;6:22–31.