International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Isolation And Screening Of Bacteria Producing Potential Lantibiotics From The Kalyana Karnataka Regions, India

Revansiddappa [1], P. Hariharan[1]*, Manikandan V[2]
[1]Department of Biotechnology, Downstream Bioprocessing Lab, Sir M Visvesvaraya Institute of Technology, Bengaluru, Karnataka, India.
[2]Department of Microbiology, Periyar University, Salem, Tamilnadu, India.
*Author for Correspondence: Prof. P. Hariharan: hariharan_biotech@sirmvit.edu

Abstract
A probiotic bacterial strains are widely used in ferment food production and it used as feed or feed additives for poultry, fish and livestock. Fermented food products are presents significant potential as alternative for probiotics that helps to enhance the health and activity of gut. There are so many fermented food products available around worldwide like kimchi, sourdough, cheese, idli, dosa so and so. All are created through the controlled action of probiotics and those products are healthy to individuals. In this study, totally 60 samples were collected from various locations of Kalyana Karnataka regions. The primary goal was to isolate, identify and analyses the microorganisms for potential probiotic nature, tolerance in different level of pH, bile, carbo and organic nitrogen in the media. 16s rRNA analysis executed for confirmation of genus and species of isolated bacterial strain.
Keywords: *traditional food, probiotics, Karnataka, 16s rRNA*

INTRODUCTION

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Derived from the Greek words "pro" (for) and "bios" (life), probiotics help restore and maintain the balance of the gut's microflora, or microbiome, especially when it has been disturbed by illness, antibiotics, or stress. The most widely used and commonly isolated probiotic bacteria from traditional foods belong to the Lactic Acid Bacteria (LAB) group, as well as the Bifidobacterium genus (1).

Lactobacillus species are vast and diverse group of bacteria, commonly found in fermented dairy, plant, and meat products. Well-known species isolated from traditional foods which includes: *Lacticaseibacillus casei* (formerly Lactobacillus casei), *Lactiplantibacillus plantarum* (formerly Lactobacillus plantarum), *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (2). Now a days, *Streptococcus thermophilus* most commonly used as starter culture for yogurt and cheese production and is frequently isolated from fermented milk. As well as *Lactococcus lactis* widely used starter culture for cheese and other fermented dairy products. Bifidobacteria are among the predominant bacteria in the gut and are commonly isolated from fermented dairy products, especially those formulated for probiotic benefits. Common species include *Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Bifidobacterium longum*.

Probiotic bacteria have long been a part of the human diet through the consumption of traditional fermented foods (3). These foods provide a rich source for isolating a diverse range of probiotic strains, which are then studied for their specific health benefits. Most common food sources are 1. Fermented dairy products, like yogurt, kefir, and buttermilk. 2. Fermented vegetables, such as kimchi, sauerkraut, and pickles. 3. Cereals and legumes, including fermented rice and soy products (4).

The maximum number of probiotics isolated from the traditional food products because microorganisms are naturally present in the food products. Even they grow without addition of microbial medium and microbial cultures (5). In worldwide, so many researchers has been reported the isolation of probiotics from the fermented food products such Ethiopian traditional food products (6), Neera drink which collected from coconut palm and naturally fermented drinks in various locations of India (7).

There are some specific components were synthesized from the probiotic bacteria namely bacteriocin, nisin and etc., from the LAB family, that used as bio preservatives in food industries and these components are generally recognized as safe (8). There are some possible mechanisms were identified while using the bacteriocin or LAB producing components against of pathogenic bacteria, it involved in controlling of food

spoilage and food borne diseases (9). Addition of probiotic bacterial culture to the fermented food, it enhances the texture, aroma and flavor of the products (10,11).

MATERIALS METHODS

Sample collection

Several traditional fermented foods that were gathered from different regions of north Karnataka were employed in this investigation. Probiotics were isolated using the serial dilution agar technique by homogenizing 10 grams of each sample in 90 ml of sterile 0.9% NaCl solution, followed by plating various dilutions on tryptone soya agar and then incubated in 35^{o}C for 24 hours.

The white colored colonies picked from the mass of colonies and inoculated into MRS medium and Luria Bertani agar plates for purification of target isolate. All the chemicals and media components were purchased from HiMedia, Mumbai, India.

A total 60 traditional fermented foods were collected from different places in north Karnataka from Vijayapur; Raichur; Yadagir; Kalburgi and Bidar districts fermented foods. Samples were categorized into five groups: the sample collected from Vijayapur district is labeled as Group I; the sample collected from Raichur district is labeled as Group II; the sample collected from Yadagir district is labeled as Group III; the sample collected from Kalburgi district is labeled as group IV and a sample collected from Bidar district is labeled as group V.

Isolation of bacterial strain

De Man, Rogosa, and Sharpe (MRS) media is a selective and enriched medium commonly used for the isolation and cultivation of Lactobacilli. As well as, Gelatinase medium and Luria Bertani medium used for isolation of *Bacillus sp.,* form the collected food samples.

Composition of MRS Agar

Gelatin peptone: 10.0 g, Beef extract: 8.0 g, Yeast extract: 4.0 g, Dextrose (Glucose): 20.0 g, Polysorbate 80 (Tween 80): 1.0 g, Ammonium citrate: 2.0 g, Sodium acetate: 5.0 g, Dipotassium phosphate: 2.0 g, Magnesium sulfate: 0.2 g, Manganese sulfate: 0.05 g, Agar (for solid media): 10.0 g, Distilled water: 1000 ml.

Composition of LB Agar

Luria Bertani (LB) Agar is a nutrient enriched medium that used to isolate the bacterial strains from the various source of food samples. The LB medium contains 10g tryptone, 5g of yeast extract, 10g of sodium chloride and 15 of agar per liter of distilled water.

Suspend all the dry ingredients in 1000 ml of distilled water. Heat the mixture to boiling while stirring to ensure all components are completely dissolved. Adjust the pH to 6.2 ± 0.2 at 25°C. For higher selectivity, the pH can be lowered to 5.7. Sterilize the media by autoclaving at 121°C for 15 minutes. Pour the sterile media into Petri dishes for agar plates or dispense into tubes for broth.

Identification of bacterial strain

Gram staining method and Spore staining method were used to identification of morphology of isolated bacteria.

The principle of Gram staining relies on variations in the structure of bacterial cell walls, particularly the quantity of peptidoglycan and the existence of an outer lipid membrane. Gram-positive bacteria possess thick peptidoglycan layers that hold onto the crystal violet-iodine complex, resulting in a purple appearance, whereas Gram-negative bacteria, which have thinner peptidoglycan walls and an outer lipid membrane, lose this complex during the decolorization process and are subsequently stained pink or red by a counterstain.

Spore staining takes advantage of the endospore's resilient outer coat, using malachite green as a primary stain applied with heat, while water decolorizes the more susceptible vegetative cells, allowing a counterstain like safranin to turn these cells red and reveal green spores among them. Both of these staining methods used to identify the isolated bacterial strain is Gram positive or Gram negative and it able to produce spore or not.

Table 1: Groups of samples as Collected (M: Months)

Sample Collection Details:															
S. No	Group I			Group II			Group III			Group IV			Group V		
	Fresh	2M	6M	Fresh	2M	6M	Fresh	2M	6M	Fresh	2M	6M	Fresh	2M	6M
1	5	4	3	5	3	4	5	5	2	6	3	3	5	2	5

The samples were collected three different stages like fresh samples, 2 month fermented food samples and 6 month fermented food samples.

Optimization of isolates

The isolated bacterial culture used to optimize its growth on different pH and bile concentrations. Toleration to lower pH (5.5, 6.0 and 6.5) in the isolates was assayed according to the protocol mentioned by Leite *et al.* 2015 in MRS broth with 0.2% sodium thioglycolate (MRS-THIO) (12). The isolates in the agar plates were subjected to assay to check the toleration to bovine bile (Oxgall, Difco) (12). Overnight culture were inoculated in the liquid medium (1%) and cultivated for up to 9 hrs at 37°C. Each hour, absorption was measured at 620 nm.

Glucose and malate are the preferred substrates by *B. subtilis*. MHA plates were used to evaluate the antimicrobial activity by varying the carbon sources (2%, 4% and 6%) like dextrose, lactose, maltose, starch, fructose, sucrose (13) against selected test organisms each separately by agar well diffusion method. The plates were incubated at 37°C for 24 hours and after incubation plates were observed for the antimicrobial activity. Effect of organic nitrogen source was carried out by using (2%, 4% and 6%) of Beef extract, yeast extract, Peptone, Casein and Tryptone each separately against the test organisms by agar well diffusion method. The plates were incubated at 37°C for 24 hours and after incubation plates were observed for the antimicrobial activity (14).

Molecular identification of the organisms

The identification of the selected potential strains was subjected to 16S rRNA conserved genome amplification and sequencing from Geneome Bios, Pune. 16S rRNA is an effective tool in genotypic identification of organisms. Both the16S rRNA sequences were compared with the 16S rRNA sequences of Bacterial database of NCBI with BLASTN program. BLASTN was optimized for highly similar sequences using mega blast algorithm with a linear gap cost, 28-word size and threshold of 1-6 as provided in NCBI Manual. The phylogenetic tree also created by comparative study with pervious results and interpretations.

RESULTS

The collected fermented food samples are used to isolated the bacterial strains. More over bacterial colonies were identified in all the collected samples. While the Gram staining methods, both Gram positive and Gram negative bacterial cells were identified. In this stage, Gram positive bacterial strains only selected specifically Gram positive rods. From the overall isolation, 23 Gram positive bacterial strains were identified, they are both cocci and rods shaped. From the 23 isolates, 18 isolates were identified as Gram positive rods.

MRS agar and LB agar used for the differentiation of Bacillus and Lactobacillus from the food samples. At the same time, Gram staining and spore staining methods also applied for the identification of nature of the bacterial strains.

From the over all 18 isolates, 7 bacterial strains were observed as Gram positive rods and spore forming bacteria. The selected 7 bacterial cultures were forwarded to culture optimization with the slight changes of pH, carbon and organic nitrogen in the media.

Growth of isolates in different pH level

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

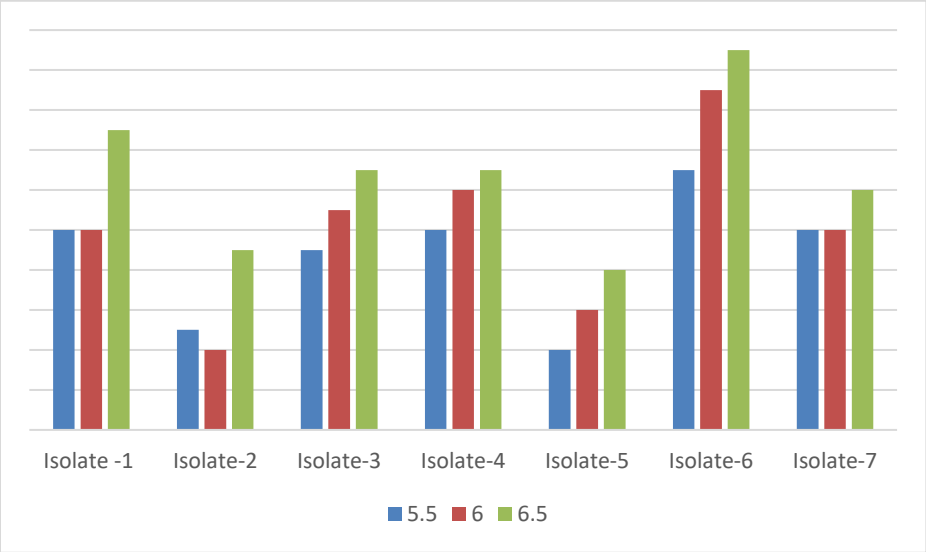

Figure 1: Growth of isolates in different pH level

Growth of isolates in different carbon level

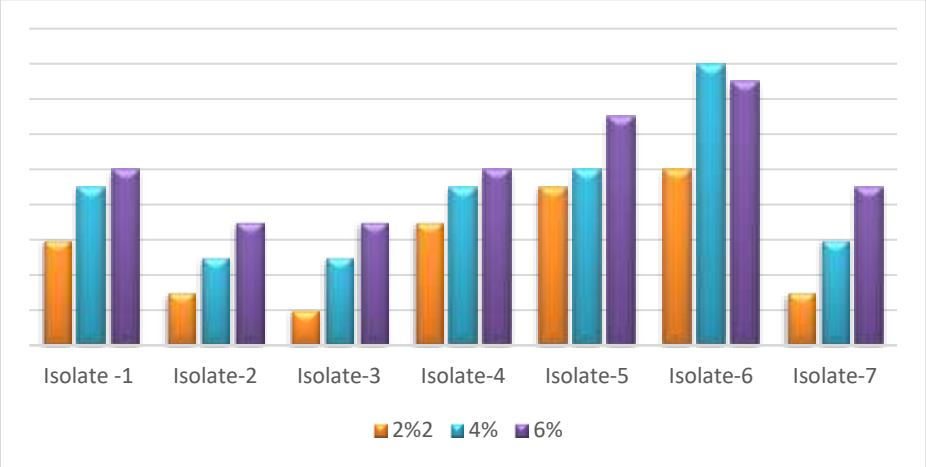

Figure 2: Growth of isolates in different carbon level

Growth of isolates in different organic Nitrogen level

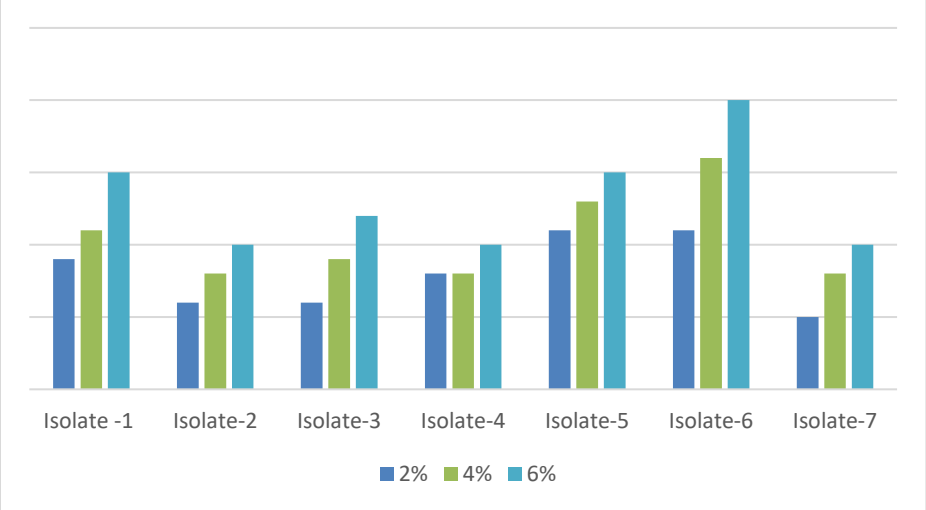

Figure 1: Growth of isolates in different organic nitrogen level

In the over all observation, isolate number 6 have potential growth in the optimization of bacterial strain. Strain number 6 has been forwarded to 16s rRNA analysis for identification of its genus and species.

Molecular analysis of isolate

16s rRNA analysis done for the identification of genus and species of the isolated bacteria. The collected database has been lasted in the NCBI BLAST portal and find it as *Bacillus pumilus*. The sequencing data and phylogenetic tree was given below.

caaagggcgg gcgtgctata catgcaagtc gagcggacag aagggagctt gctcccggat
gttagcggcg gacgggtgag taacacgtgg gtaacctgcc tgtaagactg ggataactcc
gggaaaccgg agctaatacc ggatagttcc ttgaaccgca tggttcaagg atgaaagacg
gtttcggctg tcacttacag atggacccgc ggcgcattag ctagttggtg gggtaatggc
tcaccaaggc gacgatgcgt agccgacctg agagggtgat cggccacact gggactgaga
cacggcccag actcctacgg gaggcagcag tagggaatct tccgcaatgg acgaaagtct
gacggagcaa cgccgcgtga gtgatgaagg ttttcggatc gtaaagctct gttgttaggg
aagaacaagt gcgagagtaa ctgctcgcac cttgacggta cctaaccaga aagccacggc
taactacgtg ccagcagccg cggtaatacg taggtggcaa gcgttgtccg gaattattgg
gcgtaaaggg ctcgcaggcg gtttcttaag tctgatgtga aagcccccgg ctcaaccggg
gagggtcatt ggaaactggg aaacttgagt gcagaagagg agagtggaat tccacgtgta
gcggtgaaat gcgtagagat gtggaggaac accagtggcg aaggcgactc tctggtctgt
aactgacgct gaggagcgaa agcgtgggga gcgaacagga ttagataccc tggtagtcca
cgccgtaaac gatgagtgct aagtgttagg gggtttccgc cccttagtgc tgcagctaac
gcattaagca ctccgcctgg ggagtacggt cgcaagactg aaactcaaag gaattgacgg

Phylogenic tree of isolated bacteria

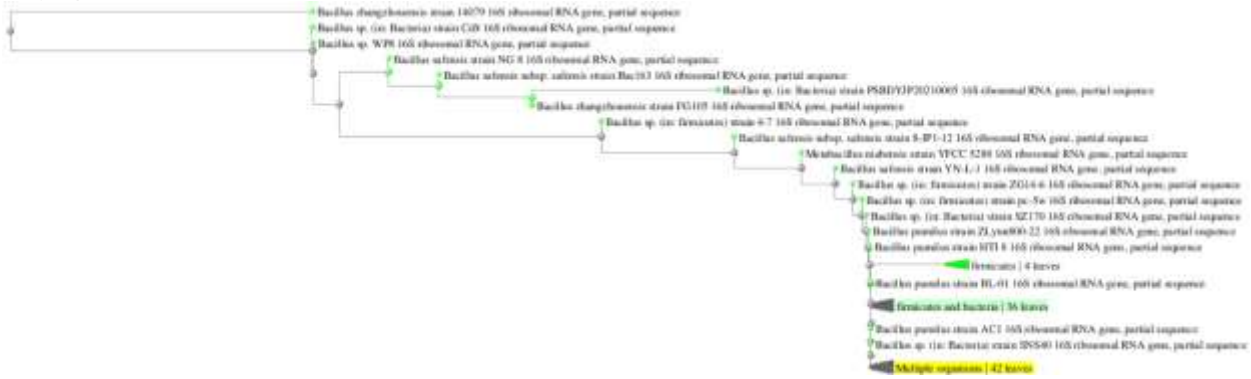

Figure : Phylogenetic tree of isolated bacteria.

DISCUSSION

In this study, 60 number of fermented food samples were collected from various locations of Karnataka. *Bacillus pumilus* has been isolated and it was confirmed by 16s rRNA analysis of isolated bacteria. The bacterial strain was optimized for identification of growth ability in various conditions of pH, carbon and organic nitrogen. Sixth isolate shows better growth in the all parameters.

Fangio et al., (15) reported that, many Bacillus sp., has been isolated from food samples. Predominantly *Bacillus pumilus* and *Peanibacillus polymyxa* together with other Bacillus strain like Bacillus subtilis, Bacillus cereus and Bacillus mycoids. These bacterial strains were isolated from wheat, rice, butternut squash and potato.

According to Bermudez-Brito (16), the presence of probiotics in the food, that may inhibit the growth of pathogens by the secretion of antimicrobial substances. Based on the previous studies, various components which produced by probiotics that can control the development the bacterial pathogens in food and food products (17).

CONCLUSION

Bacillus pumilus has isolated from collected food sample and its growth has been optimized for the compound productions. Probiotics may present in the traditional and many fermented food products. The further studies may improve the usage of probiotics in various fields like aquaculture, medicinal, food industries and pharmaceutical products.

REFERENCE

1.Russell DA, Ross RP, Fitzgerald GF, Stanton C, Metabolic activities and probiotic potential of bifidobacteria. Int J Food Microbiol, 2011, 149(1):88–105.
2. Saad M, Abdelsamei H, Ibrahim E, Abdou A, El Sohaimy S, Effect of pH, heat treatments and proteinase K enzyme on the activity of Lactobacillus Acidophilus bacteriocin. Benha Vet Med J, 2015, 28(1):210–5.
3. Shehata MG, El Sohaimy SA, El-Sahn MA, Youssef MM, Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. Ann Agricult Sci, 2016, 61(1):65–75
4. Monika, S., Kumar, V., Kumari, A., Angmo, K., and Bhalla, T. C., Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India. *J. Food Sci. Technol.* 2017, 54, 1945–1952. doi: 10.1007/s13197-017-2629-1
5. Anggraini, L., Marlida, Y., Mirzah, M., Wizna, W., Jamsari, A. and Huda, N., Isolation and characterization of lactic acid bacteria producing GABA from indigenous West Sumatera fermented food. Int. J. Adv. Sci. Eng. Inf. Technol., 2019, 9(3): 855-860.
6. Mulaw, G., Tessema, T.S., Muleta, D. and Tesfaye, A., In vitro eval uation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. Int. J. Microbiol., 2019: 7179514.
7. Somashekaraiah, R., Deepthi, B.V. and Sreenivasa, M.Y, Probiotic properties of lactic acid bacteria isolated from neera: A naturally fermenting coconut palm nectar. Front. Microbiol., 2019, 10: 1382.
8. Mokoena MP, Mutanda T, Olaniran AO, Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. Food Nutr Res, 2016, 60:29630.
9. Hoelzer K, Moreno Switt AI, Wiedmann M, Boor KJ, Emerging needs and opportunities in foodborne disease detection and prevention: From tools to people. Food Microbiol, 2018, 75:65–71.
10. O'Bryan CA, Crandall PG, Ricke SC, & Ndahetuye JB, Lactic acid bacteria (LAB) as antimicrobials in food products: analytical methods and applications. In: Handbook of Natural Antimicrobials for Food Safety and Quality, 2015, https://doi.org/10.1016/B978-1-78242-034-7.00007-4
11. Ricci A, Cirlini M, Maoloni A, Del Rio D, Calani L, Bernini V, Galaverna G, Neviani E, Lazzi C, Use of dairy and plant-derived lactobacilli as starters for cherry juice fermentation. Nutrients, 2019, 11(2):213. https://doi.org/10.3390/nu11020213
12. Leite AMO, Migue MAL, Peixoto RS, Ruas-Madiedo R, Paschoalin VMF, Mayo B, Delgado S. Probiotic Potential of selected Lactic Acid Bacteria strains isolated from Brazilian Kefir grains. J Dairy Sci. 2015; 98(6):3622-32.
13. Kumar P, Nagarajan A, Uchil PD. Analysis of Cell Viability by the Lactate Dehydrogenase Assay. Cold Spring Harb Protoc. 2018 Jun 1;2018(6).
14. Thakur S, Sharma NK, Thakur N, Savitri, Bhalla TC. Organic solvent tolerant metallo protease of novel isolate Serratia marcescens PPB-26: production and characterization. 3 Biotech. 2016; 6(2): 180.
15. Fangio, Maria & Roura, Sara & Fritz, Rosalía, Isolation and Identification of Bacillus spp. and Related Genera from Different Starchy Foods. Journal of food science, 2010, 75. M218-21. 10.1111/j.1750-3841.2010.01566.x.
16. Bermudez-Brito M., Plaza-Diaz J., Munoz-Quezada S., Gomez-Llorente C., Gil A. Probiotic Mechanisms of Action. Ann. Nutr. Metab. 2012;61:160–174. doi: 10.1159/000342079.
17. Shi W.P., Zeng H., Wan C.X., Zhou Z.B. Amicoumacins from a desert bacterium: Quorum sensing inhibitor against Chromobacterium violaceum. Nat. Prod. Res. 2021;35:5508–5512. doi: 10.1080/14786419.2020.1788554.