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Research Article

Isolation and Characterization of Probiotics Bacteria from Curd, Pickle and Fermented Rice and Screening of Antimicrobial Activity

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ABSTRACT

The live supplement of microorganisms through the dietary product is known as the probiotics. These are some special kind of bacteria they are healthy for the host organisms. These bacteria commonly found in food and dietary product. In our study, Probiotics were isolated from fermented rice, curd and pickle. Total isolated strains were studied for their characterization, and also strains were studied antibacterial also their antibiotic susceptibility quality. The antibiotic resistance of potential strain was studied using Vancomycin, gentamycin, chloromphenicol, ciprofloxacin and cefataximide. PB1, PB3 shows the good resistance activity to the antibiotics. The curd produced by those strains has good quality and quantity of nutrients. Specially this study is for isolating, identifying, and characterizing the Probiotics. Isolated strains were identified by Gram staining, catalase assay, and 3 molecular identification methods; namely, (GTG) 5-PCR fingerprinting, This experiments shows the better anti-pathogen activity, and acceptable antibiotic susceptibility; it implies we can use these Probiotics in different purpose in food industry for modern food synthesis, for antimicrobial susceptibility test we are taking ampicin, karamycin, tetracycline, tripsin by using of the antibiotics concluded that various inhibition zones of given sample of probiotics.

Keywords- Probiotics, antimicrobial activity, curd, fermented rice, pickle, biochemical characterization

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INTRODUCTION

In India the traditional foods having some health benefits like rice which is the staple food of India have some beneficial health nutrients and also some beneficial microorganism after fermentation¹. The dairy products are rich with ample amount of health-enhancing factor such as enriched nutrients. Most traditional Indian food pickle is the best source of some beneficial microorganisms as the source of probiotics. Probiotics are microorganism's active factors,

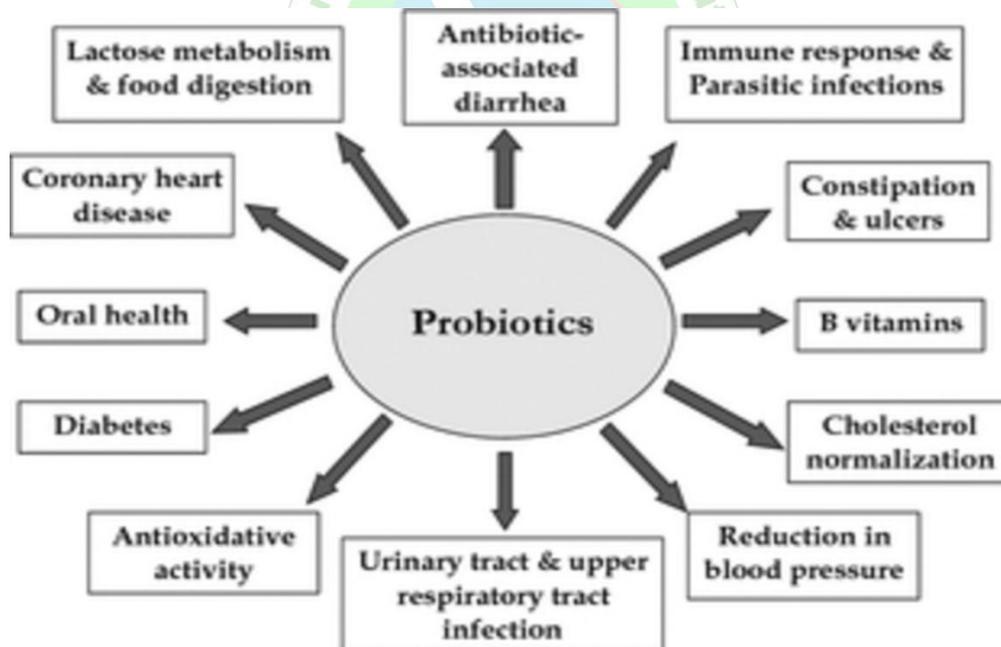
Which plays the main beneficial role on the host health². Probiotics have many benefits like it helps in absorption of nutrients, have antitoxin activity and antibiotics activity which significantly affect human body to maintain total physiology and metabolism¹.

Most of Probiotics are isolated from safe sources, such as fermented dairy products, fermented rice and the food products. Most of the probiotic bacteria are from LAB (lattice acid bacteria) so these bacteria could not be identified and differentiated by traditional biochemical methods, so we need advanced type of biochemical method of characterization of this bacteria for clear results. In human body the Probiotics plays the main role in GI tract for proper absorption of food especially the protein^{3,4}. The name Probiotics means uncountable bacteria. This bacteria have very long term growth it helps to increase and balance and reestablish the microbial population structure, with specific target Probiotics product affects the human alimentary tract as well as it reduce the risk of healing of human disease already known. Consumption of probiotic cells through food products is actually the most popular approach. In regular

basis the consumption of Probiotics is very much necessary but it must be in proper fermentation level. They have many multipurpose capacities because they have very long time survival. Probiotic bacteria can sustain in very harsh environment they can adapt themselves in every pattern that's why they can easily survive in GI tracts and easily viable in it. As Probiotics have very much good effect in host body, it can be taken normally in the form of Probiotics capsule, syrup and food. It needs to stable itself in GIT at least cell counts become 10^6 CFU g⁻¹. The main source of Probiotics is from dietary products. As they are countless bacteria as compare to other microorganisms, it can be used in specific medical advantage^{4,5}.

Probiotics are defined as a live microorganism which is adequate amount confer health benefits to the host, a live microbial fed supplement that beneficially affect the host animal by improving its intestinal microbial balance, Probiotics are generally used to improve the health of both animal and human through the modulation of the intestinal micro biota⁵. Several well characterized strains of lactobacillus and Bifid bacteria are available for human use to reduce the risk of gastrointestinal infection or treat such infection⁸. The primary clinical interest in the application of Probiotics has been used in prevention of and treatment of gastrointestinal infection and disease therefore the modulation of an unbalanced indigenous micro biota from the rational Probiotics therapy^{3,7}.

Benefits of Probiotics



It is now an essential fact that the indigenous microbial communities is host Specific, location specific, very complex in composition and has been beneficially properties to host.

Probiotics Health benefits and its mechanism

- Resistance to enteric pathogens – Antagonism activity adjuvant effect increasing antibody production systemic immune effect.
- Aid in lactose digestion- Bacterial lactase acts on lactose in the small intestine.

Probiotics are microorganism that active factors, which show the beneficially impacts on the host health, Probiotics significantly affect the bioavailability of nutrient in the human body by facilitating the absorption of magnesium and calcium from milk proteins, lactobacillus and enterococcus species are common lactic acid bacteria gram positive and non toxic bacteria, which is usually consumed as probiotics⁹.

Probiotics bacteria under similar condition thus, these bacteria could not be identified and differentiated by traditionally phenotyping and biochemical methods. Such as sugar fermentation at genus levels as these techniques do not provide clear classification of result rapidly⁵. It is most importance that the Probiotics strains survive the sites where it is presumed to be active for maximum activity that strains should be able to proliferate and colonize at this specific location, the Probiotics microorganism should be of human origin and must be able to survive and grow in the in vivo condition^{8,9}.

And it must be able to tolerate low Ph and high concentration of both conjugated and de conjugated bile acids, Probiotics used should also be technologically compatible with the food manufacturing process and bacteria should be maintain the characterize sensory attributes of food production¹².

- Small bowel bacterial overgrowth- lactobacilli influence the activity of overgrowth flora, decreasing toxic metabolic production.
- Immune system modulation- strengthening of nonspecific and antigen specific defense against infection and tumors adjuvant effect in the immune response.
- Anti colon cancer effect- Anti mutagenic activity detoxification of carcinogenic metabolites alternation in pro cancerous enzyme activity¹³.

Antimicrobial properties

The intestinal microbial community is a complex ecosystem and introducing new organism into this highly competitive environment is difficult, thus microorganism can produce a product that inhibits the growth of existing organism have a characteristics advantage. The ability of Probiotics to establish in GI tracts is enhanced by their ability to eliminate competitors¹⁵.

Table 1: Probiotics bacteria

Probiotics	Compound
Lactobacillus GG	Wide spectrum antibiotic
<i>L. acidophilus</i>	<i>Acidolin, Acidophilin</i>
<i>L. bulgaricus</i>	<i>Bulgarican</i>
<i>L. plantarum</i>	<i>Lactolin</i>
<i>L. brevis</i>	<i>Lactobacillin</i>
<i>L. reuteri</i>	<i>Reuterin</i>
<i>L. sake L45</i>	<i>Lactocin S.</i>
<i>L. johnsonii</i>	<i>Lactocin F</i>
<i>L. helveticus</i>	<i>Helveticin</i>
<i>L. cremoris</i>	<i>Diplococin</i>

Anti carcinogenic properties

Probiotics suggest that diet and antibiotics can lower generation of carcinogenic in colon and reduce chemically induced tumors, these effect appear induced to be mediated through the intestinal microbial communities. A possible mechanism for these anticancer effects relies on inhibiting intestinal bacterial enzymes that convert procarcinogenes to more proximal carcinogens¹⁴.

Probiotics have also been found by several researchers to decrease fecal concentration of enzyme and secondary bile salt and reduce the absorption of harmful mutagens that may contribute to colon carcinogen.

Probiotics in Diabetes and Obesity

The role of gut flora in the pathology of insulin resistance and obesity has been well documented, animal and human studied have been suggested that gut flora enhance the body weight gain and increase the insulin resistance and these phenotype are transmissible with gut flora during the implantation studies of gut micro flora^{1,5}.

Mechanism action of Probiotics

Probiotics bacteria have multiple and diverse influence on the host. Different organism can influence luminal environment, epithelial and mucosal barrier function and the mucosal immune system, the numerous cell type affected by Probiotics involve epithelial cell, dendritic cell, monocytes, and microphages^{2,6}.

MATERIAL AND METHODS

Sample collection

Curd, pickle and fermented rice sample were collected from various market and fermented rice sample were

collected from home making, two sample curd and pickle were chosen from different area Bangalore and store 37⁰c in incubator.



Figure 1: Serial Dilution

Serial dilution

To isolated antibiotics production microbes from sample, to isolate degrade microbes to remove contamination and to keep and clean the environments that are present in different seasons summer, winter, autumn.

Methods

- Serial dilution methods is one of the most old and usable methods which is use for the isolation of bacterial colony.
- In this method we take concern sample soil, water, milk, food make dilution in test tube.
- Inoculated this sample from the diluted test tubes in the prepared nutrient media plates by using pure plate methods and then incubated the cultured plates at 37⁰c for 24 hours.
- After 24 hours we absorb the cultured plates, the growth will appear on each plates.

A serial dilution is a the stepwise dilution of a substance in solution, the dilution factors at each step is constant, resulting in geometric progress of the concentration in log fashion, a tenfold serial dilution could be 1M, 0.01,0.02,0.03,0.04,0.05,0.06,0.07,0.080,0.09,0.1M respectively.



Figure 2: Serial Dilution

Identification of Probiotics bacteria

Gram staining

Prepare the smear in a clean slide make it heat fix, then add drops of crystal violet wait for 2min then with distil water then add gram iodine wait for 1 min then clean with alcohol after that add counter strain saffrin and observe under microscope.

Biochemical characterization of bacteria

Indole test

Peptone broth was prepared and sterilized at 121°C for 15 min and inoculated with test organism, incubated the medium at 37°C for 24 hours, Added 1 ml of Kovac reagent to tubes including control. Shook and observed the tubes for presence of rings.

Methyl Red test

Prepared MR-VP broth in two flasks, inoculate the broth with the test organism and incubated for 24 hours at 37°C, after 24 hours of incubation transferred 5 ml of broth into two test tubes. To the each broth culture added 5 drops MR indicator the tubes and shake them. Examine the colors of the each culture.

Voges – proskauer test

Prepared MR-VP broth in two flask, inoculate the broth with the test organism and incubated for 24 hours, prepared BARRITT reagent A and B. after 24 hours of incubation 0.5 ml of reagent A and 0.2 ml of reagent B was added to the broth and observe for color change.

Citrate utilization test

Prepared citrate agar slant and inoculated each of the test organism into appropriately labeled tubes by means of a loop, the slant was left UN inoculated that serve as control, incubated for 24 hours at 37°C. After 24 hours all agar slant were examined for the presence of growth and coloration of the medium.

Catalase test

Transferred small quantity of culture from the plates on glass slide, add 1 drop of 3 % H₂O₂ observe bubbles formation.

Oxidase test

Taken oxidase disc in clean microscopic slide, pasted the culture on the oxidase disc and observed for color changes.

Nitrate test

Prepared nitrate broth and inoculated each of the test organisms into its appropriately labeled tubes means of a loop. The last slant was left un inoculated that serve as control, incubated all culture for 24 hours at 37°C, after 24

hours add one dropper full of sulfanilic acid and one dropper full of α naphthylamine to each broth. Broth were examined for the change in coloration of the medium, a color change to red indicates a positive nitrate reduction test.

Starch test

Prepared starch agar and inoculated each of the test organism into its appropriately labeled tubes by means of a loop, the last plates was left un inoculated that serve as control, incubated all culture for 24 hours at 37°C. after 24 hours all agar slants were examined for the presence of growth and zone formation on the medium, add iodine solution to see the zone formed more vividly.

Gelatin test

Prepared gelatin slant and inoculated each of the test organism into its appropriately labeled tubes by means of a loop. The slant was left un inoculated that serve as control, incubated all culture at the bacterium optimal growth temperature for up to 1 week and checked every day for gelatin liquefaction. Gelatin normally liquefies at 28°C and above, so to confirm that liquefaction was due to gelatinase activity. The tubes are immersed in an ice bath for 15 to 30 min, Afterwards tubes are tilted to observe if gelatin has been hydrolyzed, hydrolyzed gelatin will results in liquid medium even after exposure to cold temperature (ICEBATH), and while the UN inoculated control medium will remain solid.

Antimicrobial susceptibility

The performance of antimicrobial susceptibility testing by clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents.

The disk diffusion susceptibility methods is simple and practical and has been well standardized, the test performed by applying bacterial inoculums of approximately $1-2 \times 10^8$ cfu/ml to the surface of large Mueller Hinton Agar plates, up to 12 commercially prepared fixed concentration paper antibiotics disk are placed on the inoculated agar surface. Plates are incubated for 16 – 24 hours at 37°C prior to determination of results; the zones of growth inhibition around each of the antibiotics are measured to the nearest millimeter. The diameter of the zones is related to the susceptibility of the isolate and to the diffusion rate of the drug through agar medium.

RESULTS AND DISCUSSIONS

Table 2: Biochemical Test

Biochemical test	Fermented Rice	Pickle	Curd
Indole	+	+	--
Methyl Red	--	--	+
Voges- Proskauer	+	+	--
Citrate utilization	--	--	+
Catalase	+	+	+
Oxidase	--	--	+
Nitrate	+	+	--
Starch	--	+	+
Gelatine	+	--	+
Gram staining	--	+	+

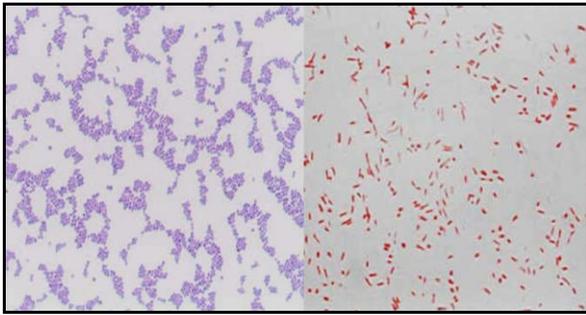


Figure 3: Gram Staining

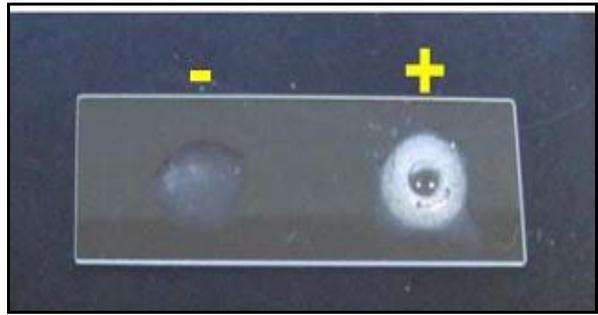


Figure 4: Catalase Test



Figure 5: Citrate test

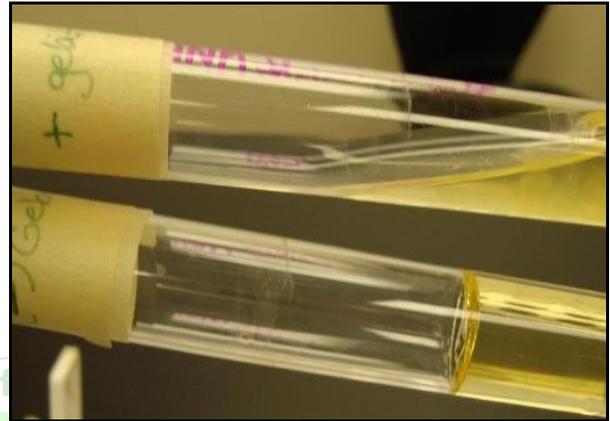


Figure 6: Gelatin test

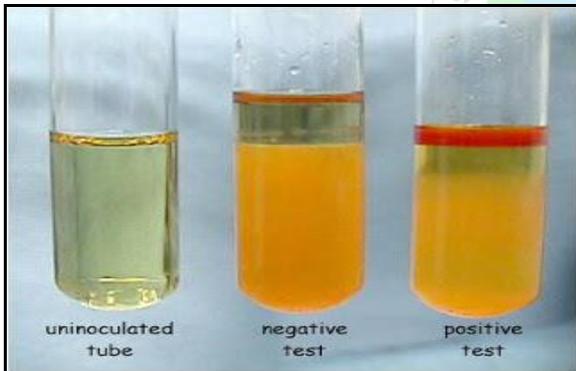


Figure 7: VP Test



Figure 8: Methyl Red



Figure 9: Starch Negative



Figure 10: Starch Positive



Figure 11: Urease Test

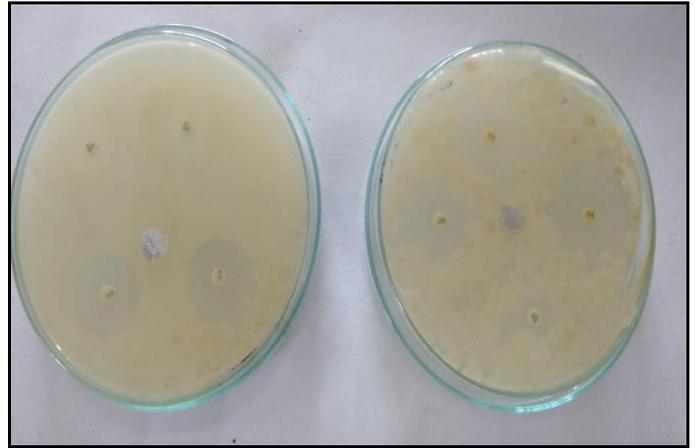


Figure 12: Antimicrobial susceptibility Test

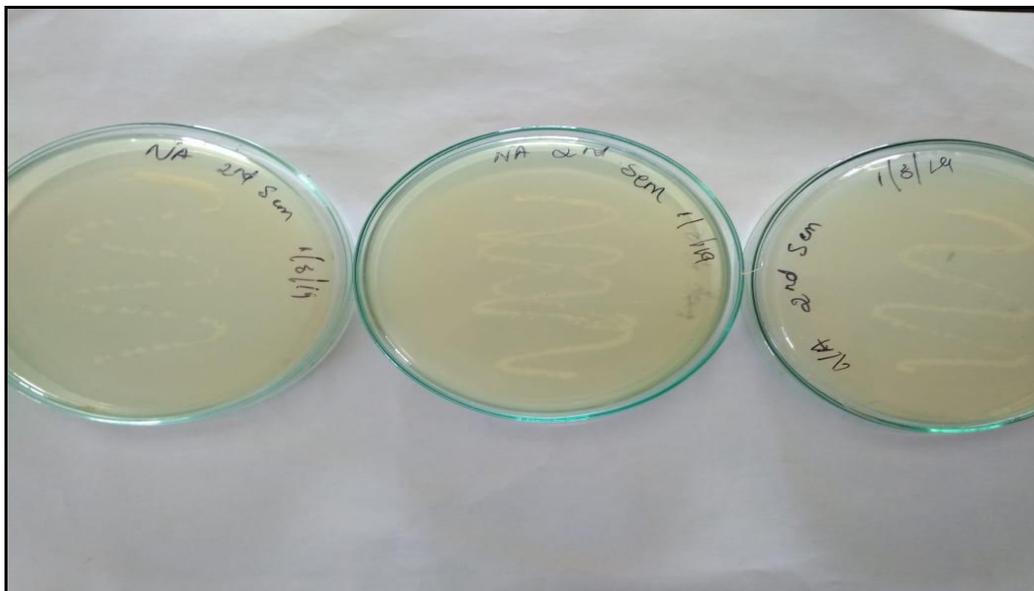


Figure 13: Screening of Probiotics Bacteria

CONCLUSION

Probiotic bacteria in fermented rice, curd, pickle could be a very good choice for value added products, the probiotic bacterial strain particularly lactobacillus casei and acidophilus with valuable count have the ability to produce good quality of curd, pickle and fermented rice and it can inhibit the pathogenic bacteria mass. It has been observed throughout the present research work, the curd produce by the identified strains showed good nutritional values, hence the study of characterization of probiotic bacteria and biochemical characterization is strongly supports the knowledge about the selection of inoculation for curd, fermented rice and pickle preparation especially on probiotic properties to promote the which bacteria of strain is present for which products and how its promote people health.

It would be possible to provide the basic information for the various production of probiotic feed products for various sources, it is also anticipated that deliverables of research work would be promote establishment of various society based environmentally suitable products industries by wider participation of vulnerable poor and destitute for society.

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