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*Research Paper*

# SCREENING FOR ANTIMICROBIAL EFFECT OF PROBIOTIC ORGANISMS AGAINST THE CHOSEN CLINICAL ISOLATES OF LEPROSY PATIENTS

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**Abstract**

Probiotic is a live microbial supplement which affects host's health positively by improving its intestinal microbial balancethere by support the host health by interfering the immune system in different ways such as; producing cytokines, stimulating macrophages, increasing secretory IgA concentrations [1, 2, 3]. Antimicrobial activity is one of the most important selection criteria for probiotics. Antimicrobial activity targets the enteric pathogens. The present research aims to evaluate the antimicrobial effect of probiotic organism and to compare the safety therapeutic index over the commercially available antibiotics against the chosen clinical isolates. Total of 2 probiotic sources of fresh dairy (Curd) and non-dairy food (cucumber) products were collected from the market. Isolation and purification of lactic acid bacteria from dairy and non-dairy sample using De Man, Rogosa and Sharpe Agar (MRSA). Evaluation of probiotic potentials such as temperature sensitivity, lactose utilization, NaCl tolerance were also evaluated. Antimicrobial activity tested against the chosen clinical isolates such as *Escherichia coli, Proteus sp, Klebsiella sp, Staphylococcus epidermidis, and Staphylococcus aureus.* The present study focusing on characterizing the potential benefits of probiotic organism of dairy and non-dairy source. In the present study, all the selected LAB isolates were able to survive at temperature 25°, 30°, 37° and 40°. It was observed that the lactic acid bacterial isolates were able to tolerate 1-6.5% NaCl concentration. Antibiotic susceptibility pattern against selected clinical isolates was observed by using Kirby-Bauer disc diffusion method which showed significant antimicrobial activity of probiotic over the commercial available antibiotics. Study Concludes by stating that probiotic as a prophylactic and therapeutic choice of a alternative medicine.

Key words: Probiotic, Bacteriocin, Anti-Microbial activity.

## INTRODUCTION

The word 'probiotic' comes from Greek language 'pro bios' which means 'for life' opposed to 'antibiotics' which means 'against life'. The history of probiotics began with the history of man by consuming fermented foods that is well known Greek and Romans consume very much [4, 5]. In 1908 a Russian researcher Ellie Metchnikoff, who has a Nobel Prize, firstly proposed the beneficial effects of probiotic microorganisms on human health. Metchnikoff hypothesized that Bulgarians are healthy and long lived people because of the consumption of fermented milk products which consists of rod shaped bacteria (*Lactobacillus spp.*). Therefore, these bacteria affect the gut microflora positively and decrease the microbial toxic activity [1, 4, 6]. The term 'probiotic' firstly used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms. After this year the word 'probiotic' was used in different meaning according to its mechanism and the effects on human health. The meaning was improved to the closest one we use today by Parker in 1974. Parker defined 'probiotic' as 'substances and organisms which contribute to intestinal microbial balance'. And provides health related benefits such as [1, 3, 7, 8]. - Managing lactose intolerance. - Improving immune system. - Prevention of colon cancer. - Reduction of cholesterol and triacylglycerol plasma concentrations (weak evidence). - Lowering blood pressure. - Reducing inflammation. - Reduction of allergic symptoms. - Beneficial effects on mineral metabolism, particularly bone density and stability. - Reduction of *Helicobacter pylori* infection. - Suppression of pathogenic microorganisms (antimicrobial effect). Effect of Immune system are promising such as producing cytokines, stimulating macrophages, increasing secretory IgA concentrations [1, 2, 3]. Some of these effects are related to adhesion while some of them are not [9]. Probiotic organism exhibit following mechanism of actions as follow [1, 10, 11, 12]. Production of inhibitory substances: Production of some organic acids, hydrogen peroxide and bacteriocins which are inhibitory to both gram-positive and gram-negative bacteria. 2. Blocking of adhesion sites: Probiotics and pathogenic bacteria are in a competition. Probiotics inhibit the pathogens by adhering to the intestinal epithelial surfaces by blocking the adhesion sites. 3. Competition for nutrients: Despite of the lack of studies in vivo, probiotics inhibit the pathogens by consuming the nutrients which pathogens need. 4. Stimulating of immunity: Stimulating of specific and nonspecific immunity may be one possible mechanism of probiotics to protect the host from intestinal disease. This mechanism is not well documented, but it is thought that specific cell wall components or cell layers may act as adjuvants and increase humoral immune response. 5. Degradation of toxin receptor. Antimicrobial activity is one of the most important selection criteria for probiotics and also will address the issue related to drug resistance and ensure the safety. The present research aims to evaluate the antimicrobial effect of probiotic organism and to compare the safety therapeutic index over the commercially available antibiotics.

## MATERIAL AND METHODS

### Probiotic Source:

Total of 2 probiotic sources of fresh dairy and non – dairy food products were collected from the market. Samples consisted of two groups: group I, fresh dairy product of curd; group II, fresh non - dairy product of Cucumber. Sample group II were washed with sterile distilled water, chopped with sterile cutter in small pieces and weighed 10g and smashed to isolate the product, on other hand the group I sample were taken weighed 10g and 10ml (for curd).

**Isolation and purification of lactic acid bacteria from Dairy sample**

For isolation of LAB, serial dilution agar technique was used. Ten gram of each sample (except curd taken 10 ml) was dissolved into 90 ml of MRS broth. After dissolving into MRS broth they were shaken homogeneously and were incubated at 37°C for 24 hours in an aerobic condition. In the serial dilution agar plate technique, 10ml of a stock solution was suspended and agitated in 90 ml water blanks to form a microbial suspension. Serial dilution of $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ were made by pipetting 10ml into 90ml water blanks. 10 ml of each dilution was inoculated to MRS agar plates (prepared by pouring 15ml of sterile and cooled molten media in plates) and incubated at 37$^{o}$C for 24 hours for bacterial growth. The plates were observed for appearance of colonies and number of colonies produced on each plate of different dilution was recovered [13]. Pure culture was obtained. Microscopic and Biochemical confirmation was done and culture stored at 4$^{o}$C.

**Evaluation of probiotic potentials of isolated bacterial cultures**

Bacterial cultures with good antimicrobial activity were selected for further determination of probiotic potential as follows:

**Temperature sensitivity**

The selected bacterial cultures were grown at varying temperatures, i.e. 25$^{o}$, 30$^{o}$, 37$^{o}$ and 40$^{o}$C for 48-72 hours. Then 0.1ml inoculum was transferred to MRS plates by pour plate method and incubated at 37$^{o}$C for 48hours. The growth of LAB on MRS agar plates was used to designate isolates as temperature tolerant [16].

**Lactose utilization**

The acid production by selected bacterial cultures was detected by observing the change in color of the medium. Sterilized fermentation medium (10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0) was inoculated with different cultures and incubated at 35$^{o}$C for 24-48 hrs. Change in color from red to yellow indicates the production of acid [17].

**NaCl tolerance**

Salt tolerance of selected bacterial cultures was assessed after 3days of incubation at concentration of 1-6.5% NaCl in MRS broth [13,18].Antagonistic activities Disc diffusion method was used to detect the antimicrobial activity as described above.

**Test microorganisms**

A total of five human blood bacterial pathogenic strains obtained from leprosy out of which three Gram negative namely *Escherichia coli*, *Proteus sp, Klebsiella sp*and two Gram-positive *Staphylococcus epidermidis*and *Staphylococcus aureus* respectively.

**Standardization of test microorganisms**

The tested microorganisms were standardized by using 0.5 McFarland standard. McFarland Standard was used as reference to adjust the turbidity of microbial suspensions so that their number will be within a given range. 0.5 McFarland gives approximate cell density of 1.5 x $10^{8}$ CFU/ml, having absorbance of 0.132 at wavelength of 600 nm. The microbial suspensions were prepared in their respective sterile nutrient broth and are compared either visually or by measuring the absorbance with that of the standard [14].

**Production and evaluation of antimicrobial metabolite from lactic acid bacteria**

MRS broth was used for antimicrobial metabolite production from lactic acid bacteria, 500mL conical flasks each containing 100 ml MRS broth autoclaved at 121$^{o}$C for 15 minutes and inoculated with colony of a LAB isolate grown on MRS agar. The inoculated

flasks were incubated at 37°C for 2-3 days under stationary condition. Then centrifuged at 10,000 rpm for 10 min. Antimicrobial activity of the supernatant was evaluated.

**Screening of isolated bacterial cultures for antimicrobial activity by Kirby-Bauer Method**

For screening of isolated probiotic bacterial cultures were inoculated to MRS broth incubated at 37°C for 24-48 hours on shaker to carry out the fermentation process. After incubation, 2 ml of each fermented culture broth and supernatant was taken to test the antimicrobial activity by Disc diffusion method against the six test microorganisms. Each isolated culture was screened against every test microorganism. An overnight culture of pathogens grown in their respective medium at 37°C was diluted to a turbidity equivalent to that of a 0.5 McFarland standard [15]. Susceptibility was evaluated based on zone of inhibition.

## RESULTS

**Table No:1 Probiotic Source and Organism**

| S.No | Category | Probiotic Product | Organism |
|---|---|---|---|
| 1 | Dairy | Curd | *Lactobacillus sp.* |
| 2 | Non-Dairy | Cucumber | *Bacillus sp.* |

**Table No: 2Temperature tolerance Of Probiotic Isolation**

| S.No | Organisms | Temperature Range | | | |
|---|---|---|---|---|---|
| | | $25^0$ | $30^0$ | $37^0$ | $40^0$ |
| 1. | *Bacilussp* | + | + | + | + |
| 2. | *Lactobacillus sp* | + | + | + | + |

**Inference**:
+→Presence

**Table No: 3Lactic Acid Production of Probiotic Organism**

| S.No | Organisms | LacticAcid Production |
|---|---|---|
| 1. | *Bacillus sp* | + |
| 2. | *Lactobacillus sp* | + |

**Inference:**
+ →Indicate Colour change from red to yellow – Lactic acid production.

**Table No: 4Salt Tolerance of Probiotic Organism**

| S.No | Organisms | Nacl Concentration Range in % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1% | 2% | 3% | 4% | 5% | 6% | 6.5% | 7% |
| 1. | *Bacillus sp* | + | + | + | + | + | + | Weakly +ve | - |
| 2. | *Lactobacillus sp* | + | + | + | + | + | + | Weakly +ve | - |

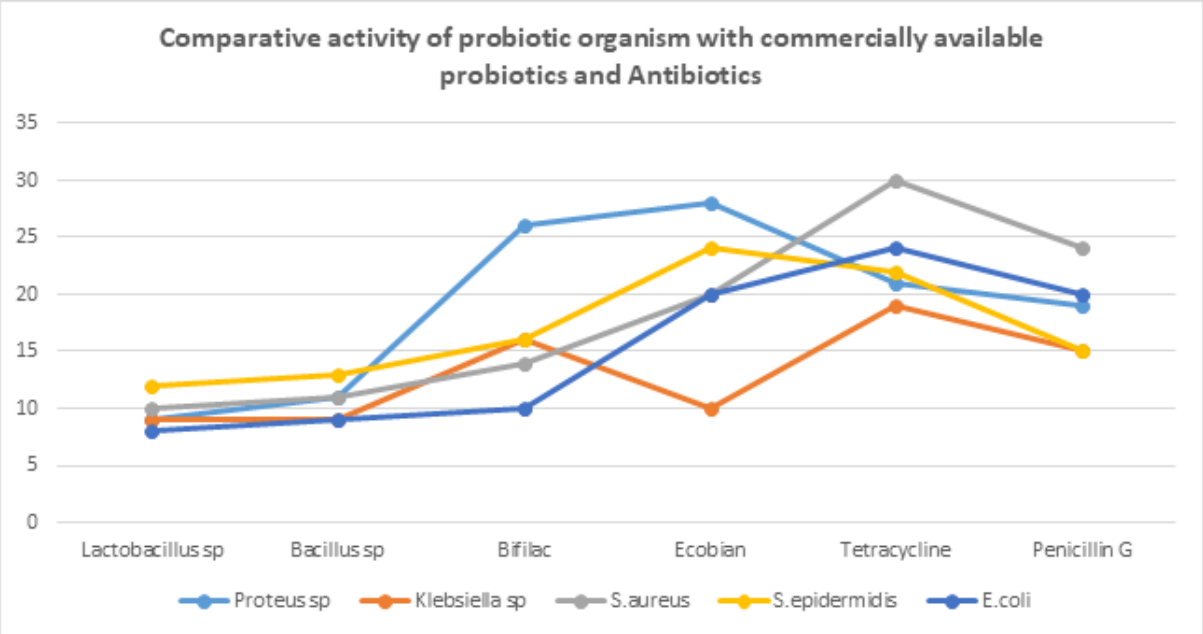
**Inference:**
**+→** Presence
**-→** Absence

**Table No: 5 Antagonistic Effect**

| S.No | Test organisms | Agar plate | Serial Dilution | |
|---|---|---|---|---|
| | | | $10^{-4}$ No. of Colonies | $10^{-6}$ No. of Colonies |
| 1. | *Proteus sp* | UTI Agar | 434 | 238 |
| 2. | *Lactobacillus sp* | MRS Agar | 224 | 113 |
| 3. | *Lactobacillus with Proteus* | UTI Agar | 137 | 73 |

**Table No:6 Comparative activity of probiotic organism with commercially available probiotics and Antibiotics**

| S.no | Test Organisms | Isolated Probiotics | | Commercially available Probiotics | | Antibiotics | |
|---|---|---|---|---|---|---|---|
| | | ***Lactobacillus sp*** | ***Bacillus sp*** | **Bifilac** | **Ecobion** | **Tetracycline** | **Penicillin G** |
| 1. | *Proteussp* | 9mm | 11mm | 26mm | 28mm | 21mm | 19mm |
| 2. | *Klebsiellasp* | 9mm | 9mm | 10mm | 10mm | 19mm | 15mm |
| 3. | *Staphylococcus aureus* | 10mm | 11mm | 14mm | 20mm | 30mm | 24mm |
| 4. | *Staphylococcus epidermidis* | 12mm | 13mm | 16mm | 24mm | 22mm | 15mm |
| 5. | *E-coli* | 8mm | 9mm | 10mm | 20mm | 24mm | 20mm |




**DISCUSSION:**

The medical world has long been interested in finding out an alternative choice of medicine with minimal side effects ad for better hormones human health. Probiotic organisms found to be an alternative choice with demonstratable health benefits and

have generally regarded as safe status with the proven low risk of inducing or being associate with etiology of diseases.

The present study focusing on characterizing the potential benefits of probiotic organism of dairy and non-dairy sources. Dairy Sources: Curd is a regular and common food component of India with special reference to south India. Curd has a part of milk that coagulates when the milk sours or treated with enzymes. The curd bacteria especially lactic acid bacteria have been well accepted as GRAS (Generally Recognized As Safe) for human [19]Apart from dairy source it has been documented that non-dairy product which include beet root, cucumber, cabbage possess a good probiotics niche. In this study we have selected curd (Dairy product), cucumber (Non-Dairy product) as probiotic source.

Probiotics products consists of different enzymes, vitamins, capsules and some fermented food contains microorganism which have beneficial effects on the health of host. They can contain one or several species of probiotic bacteria. Most of the product which destine human consumption are produced in fermented milk or given in powder or tablet. These capsules and tablets do not used for medical application. They are just used as health supporting products. The oral consumption of probiotic microorganism produces a protective effect on the GUT flora. Lots of studies suggest that probiotic have beneficial effect on microbial disorder of the GUT, but it is really difficult to show the clinical effects of such product. The probiotic preparation used for traveller's diarrhea, Antibiotic associated diarrhea and acute diarrhea which shown that they have positive therapeutic effect. [1, 4, 9].

In present study the lactic acid bacterial isolates were able to tolerate 1-6.5% NaCl. NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria. If the lactic acid bacteria was sensitive to NaCl then it would not be able to show it's activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacterial isolates, whereas Hoque et al. (2010) observed the NaCl (1-9%) tolerance of their *Lactobacillus sp.* isolated from yoghurts. The present experimental results were similar to the work done by Adebayo-tayo and Onilude (2008).

Antibiotic susceptibility pattern against selected clinical isolates was observed by using Kirby-Bauer disc diffusion method, The test organisms was sensitive to drugs such as Penicillin G (*Proteus* - 19mm, *Klebsiella* – 15mm, *Staphylococcus aureus* – 24mm, *Staphylococcus epidermidis*– 15mm, *E. coli* – 20mm), Tetracycline(*Proteus* - 21mm, *Klebsiella* – 19mm, *Staphylococcus aureus* – 30mm, *Staphylococcus epidermidis*– 22mm, *E. coli* – 24mm), Study document the existence of sensitivity but there is a chance of developing multi drug resistance over the period of time with adverse side effects. The issue of multi drug resistant and side effects could be addressed with the help of probiotic organism which act as an alternative choice of the therapeutic causes with minimized side effects

Probiotic activity tested against the chosen bacterial strains evidenced that gram positive bacteria was more sensitive than gram negative bacteria to lactic acid bacterial isolates. Effective probiotic activity was observed in dairy sample compared to non-dairy sample. The antibacterial activity was may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compounds. Antagonistic effect between *lactobacillus* and *Proteus* was well documented.

Antimicrobial activity is one of the most important selection criteria for probiotics. Antimicrobial activity targets the enteric undesirables and pathogens [20]. Antimicrobial effects of lactic acid bacteria are formed by producing some substances

such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins[1].

## CONCLUSION

Probiotics activityof dairy source against chosen clinical isolates stated a promising alternative therapeutic target which could be achieved with the help of probiotics with minimized side effects over commercially available antibiotics.

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