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**ORIGINAL ARTICLE** **OPEN ACCESS**

# DETERMINATION OF ANTIBACTERIAL ACTIVITY OF BIFIDO BACTERIA ISOLATED FROM FERMENTED FOOD SAMPLES

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**ABSTRACT**
*The present study is aimed at determination of antimicrobial activity of Bifidobacteria (Probiotic) spp isolated from fermented food samples against enteric microorganism like E.coli. The homemade samples of Idali batter curd bakery samples of cheese and dhokla batter, Neera were collected and used for study. For enrichment of Bifidobacteria loopful of each sample was inoculated separately in 10 mL sterile MRS broth (de-Man Rogosa Sharpe) and were incubated at 28 to 30°Canaerobically for 48-h. After incubation a loopful of enriched sample was streak inoculated on sterile MRS agar plates for isolation of Bifidobacteria and incubated anaerobically at 28 to 30°C for 48-h and observed for typical well isolated colonies. The isolated colonies were purified and labeled as S1 to S8 and further used for their morphological and biochemical characterization. Subsequently, isolates were primarily identified as Bifidobacteria with reference to biochemical characteristics studies as perHolt et al 1986.Further, the selected promising isolates of S2, S5, S8 were inoculated in 50 mL of sterile MRS broth and incubated at 28 to 30°C for 48-h anaerobically to study ability of organisms to produce bacteriocin. After incubation, cultured broth was centrifuged at 10000 rpm for 15 min and supernatant obtained from each flask was used to study antibacterial activity against pathogenic E. coli using agar well diffusion method. Result showed that bacteriocin producing Bifidobacteria isolated from food samples have potential to inhibit (antagonistic activity)enteric pathogen i. e. E.coli.*
***Keywords:*** *Bacteriocin, Bifidobacteria , antagonistic activity, food pathogens, probiotic*

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## INTRODUCTION

The probiotic is derived from the Greek meaning 'for life' and has had several different meanings over the years (3). The term probiotic was first introduced by Kollath in 1953. Contrasting antibiotics, probiotics were defined as microbially derived factors that stimulate the growth of other microorganisms. In1989 Roy fuller suggested a definition of probiotics which has been widely used: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." Fuller's definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host (1). Lactic acid bacteria and *Bifidobacteria* are the most common types of microbes used as probiotics; but some yeasts and Bacilli may also be helpful. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures such as in milk yogurt, soy yogurt or as dietary supplement. The importance of the *Bifidobacteria* and the Lactic acid bacteria is that these groups of bacteria have several beneficial effects on the host, especially in terms of improving digestion and the effectiveness and intrinsic strength of the immune system. A product that stimulates *Bifidobacteria* is considered a bifidogenic factor (2).

## MATERIAL AND METHODS

### Sample collection

Samples such as Idli batter and curd were collected from home. Cheese, Dhokala batter were collected from nearby bakery situated in Karad and Neera was collected from nearby area in Karad.

**Enrichment and isolation of Bifidobacteria (12)** For enrichment of anaerobic flora of interest (*Bifidobcteria*), a loopful of each sample was inoculated separately in tube containing 10 mL sterile MRS

(deMan Rogosa and Sharp medium). These tubes were incubated at 28- $30^0$ C anaerobically in anaerobic jar for 48-h.

For isolation of *Bifidobacteria*, a loopful of enriched sample was streak inoculated on sterile MRS agar plates by four quadrant streak plate method. These plates were incubated at 28 to $30^0$C for 48-h anaerobically. And after incubation plates were observed for typical well isolated colonies. The cultures were purified by repeated sub culturing on MRS agar plates and incubated at $30^0$ C for 48-h.

The purified isolates were examined by Gram staining and motility and isolates showing motility were rejected while those found to be nonmotile were further studied for their biochemical tests for identification to genus level as *Bifidobacteria.*

**Study of biochemical characteristics of isolates-**

**Enzymatic characters**

**Catalase test**

The production of catalase enzyme and its activity was studied by using standard material and method.

A loopful of growth of each isolate was dipped with the help of glass rod in 30% v/v hydrogen peroxide ($H_2O_2$) and observed for evolution of gas bubbles as positive catalase test.

**Starch hydrolysis test**

All the samples were spot inoculated onto separate starch agar plates and incubated for 24-h anaerobically in anaerobic jar. Incubated plates were flooded with iodine solution and were observed for zone of hydrolysis around the colonies as indication of starch hydrolysis.

**Sugar fermentation test**

Tubes containing respective sugar pluspeptone water were inoculated with loopful of suspension of bacterial isolates and incubated at 28 to $30^0$C for 48-h. anaerobically in anaerobic jar. After incubation tubes were observed for acid formation. Various sugar tested were 1 % sorbitol, arabinose, ribose, lactose, maltose, sucrose, mannitol and cellobiose.

**Study of antibacterial effects of isolates**

To check the bacteriocin producing ability of isolates S2, S5, S8 were inoculated in 50 mL of sterile MRS broth and incubated at 28 to $30^0$C for 48-h anaerobically. After incubation, cultured broth was centrifuged at 10000 rpm for 15 min and supernatant obtained from each broth was used to study antibacterial activity against food pathogenic *E. coli* using agar well diffusion method.

## RESULTS AND DISCUSSION

**Isolation of *Bifidobacteria f*rom collected samples.**

A total of eight isolates were obtained from the different samples collected. Details about the isolates are recorded in Table-1

**Table-1 List of isolates obtained from different sources.**

| Source | Isolates |
|---|---|
| Curd | S1 |
| | S2 |
| Idali | S3 |
| | S4 |
| Cheese | S5 |
| | S6 |
| | S7 |
| Neera | S8 |

**Table-2. Details of colony characteristics, Gram nature and motility of *Bifidobacteria* isolated from fermented food samples**

| Codes of isolates | Size | Shape | Colour | Margin | Elevation | Opacity | Consistency | Gram nature And morphology | Motility |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 2mm | Circular | White | Regular | Convex | Opaque | Moist | Gram positive rod | Motile |
| S2 | 1mm | Circular | White | Regular | Convex | Opaque | Moist | Gram positive rod With bifido morphology | Non motile |
| S3 | 2mm | Circular | Off White | Entire | Raised | Opaque | Mucoid | Gram positive rod | Motile |
| S4 | 2mm | Circular | Off White | Entire | Raised | Opaque | Moist | Gram positive rod | Motile |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S5 | 3mm | Round | Off White | Regular | Convex | Opaque | Mucoid | Gram-positive rod with Bifido morphology | Non motile |
| S6 | 1mm | Circular | White | Entire | Flat | Opaque | Moist | Gram positive rod | Motile |
| S7 | 1mm | Circular | Pale yellow | Regular | Raised | Opaque | Moist | Gram positive rod | Motile |
| S8 | 2mm | Round | Off white | Entire | Convex | Opaque | Mucoid | Gram positive rod With Bifidomorphology | Non motile |

**Biochemical Properties**

Those isolates which were found to be nonmotile with Bifido morphology were further studied for their biochemical characterization.

**Enzymatic characteristics of the isolates**

**Table-3. Enzymatic characteristics of the isolates**

| Sr. No. | Test | Isolates | | |
|---|---|---|---|---|
| | | S2 | S5 | S8 |
| 1 | Catalase Production | - | - | - |
| 2 | Starch hydrolysis test | + | + | + |

From Table-3 it can be seen that, all three isolates have shown positive results for starch hydrolysis, while all the three were catalase negative.

**Table-4. Sugar fermentation test of isolates**

| Sr. No. | Test | Isolates | | |
|---|---|---|---|---|
| | | S2 | S5 | S8 |
| 1 | Lactose | + | - | + |
| 2 | Arabinose | + | - | + |
| 3 | Maltose | + | - | - |
| 4 | Ribose | + | + | + |
| 5 | Sorbitol | + | + | + |
| 6 | Sucrose | + | + | + |
| 7 | Mannitol | - | - | - |
| 8 | Cellobiose | - | - | + |

Isolates vary in their sugar utilization ability, results of which are shown in table -4. IsolateS2 was found to be positive for most of the test sugars except mannitol and cellobiose. Isolates S5 showed positive results for ribose, sorbitol & sucrose while for remaining sugar it showed negative results. IsolateS8 showed sugar utilizing ability for lactose, arabinose, ribose, sorbitol, sucrose whereas remaining two sugars i.e maltose andmannitol were not fermented by this isolate. It showed that three isolates were metabolically active.

**Table- 5 Tentative identification of Isolates.**

| Isolate | Tentative identified species |
|---|---|
| S2 | *Bifidobacterium angulatum* |
| S5 | *Bifidobacterium subtile* |
| S8 | *Bifidobacterium adolescentis* |

From Gram property, morphological, biochemical and cultural characteristics of the isolates and with reference to Holt et al 1994,(12) the three isolates were confirmed to be members of genus *Bifidobacteria*. Tentative identified species are shown in Table-5.

**Table -6 Inhibitory activity of centrifuged fermented broth against test organism**

| Supernatant obtained from isolate | Diameter of the inhibitory zone ($mm^3$) including well diameter against food pathogenic *E. coli* |
|---|---|
| S2 | 19 |
| S5 | 24 |
| S8 | 16 |

Each isolate was evaluated for its probiotic potential i.e. inhibitory activity against a common intestinal food pathogenic organism *E. coli.* The results are present in Table-6. All the isolates were able to show inhibitory activity against test organism. Supernatant obtained from isolate S5 showed largest zone of inhibition of 24 mm against *E.coli.* This was followed by S2 and S8.

## CONCLUSION

During the course of investigation, it was found that, many Indian fermented food samples like curd, dhokla, batter etc. are rich in *Bifidobacteria* and relevant *Lactobacilli* species. Total eight bacterial isolates were obtained. Three isolates were belonging to genus Bifidobacterium. Tentative identification suggests that the isolates S2, S5, S8 are *Bifidobacterium angulatum, Bifidobacterium subtile and Bifidobacterium adolescentis* respectively. All these three isolates are having probiotic potential which was tested in vitro against *E. coli*. Prominent inhibitory activity was shown by isolate S5 i. e. *Bifidobacterium subtile* followed by S2 and S8. Further work regarding the final geneticidentification of these isolates is needed. Also, evaluation of these cultures for their probiotic potential should be tested *in vivo.* The pure bacteriocins of three isolates have potential to control food pathogenic enterotoxic *E. coli* and it can be further explored.

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