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Isolation and identification of bacteria from curd and its application in probiotic chocolate

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ABSTRACT

The isolated and identified bacteria from curd sample are of spp. *Lactobacillus*. The bacteria of this spp. are lactic acid producers. These spp. resembles to *Lactobacillus acidophilus*. A probiotic is a microorganism known to be friendly and beneficial to its host when consumed. In today's time, probiotics are a very popular subject of research among scientists and pharmaceutical companies. Due to the over consumption of antibiotics, the normal micro flora of body does not survive. The spores obtained from pharmaceuticals are not good in taste, so generally children avoid eating them, hence we have made the probiotic chocolate. It is better to eat chocolate than medicine. The chocolate containing lyophilized *Lactobacillus* spp. may help directly for enhancing resistance against intestinal pathogens and in the prevention of diseases. Various studies have demonstrated that probiotic bacteria can support a healthy digestive tract and even help eliminate gastrointestinal dysfunction. Also, an optimal floral balance in the intestines can help produce a decreased transit time.

Keywords: Probiotics, *Lactobacillus acidophilus*, lyophilization, Probiotic chocolate.

INTRODUCTION

Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term Lactic Acid Bacteria is conventionally reserved for genera in the order *Lactobacillales*, which includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, in addition to *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*. [6]

Probiotics are products designed to deliver potentially beneficial bacterial cells to the microbiotic ecosystem of humans and other animals. Strains of lactic acid bacteria are the most common microbes employed as probiotics, especially *Lactobacillus* and *Bifidobacterium* species, but lactococci, some enterococci and some streptococci are also included as probiotics. Curd is a very good source for *Lactobacillus* species. [6]

Lyophilization technique is used for freeze drying of bacteria i.e. in the powdery form so we can easily add them into chocolate mixture. The viability of *Lactobacillus* species is not harmed in lyophilization technique upto some extents. [8]

MATERIALS AND METHODS

Curd is the best source for *Lactobacillus* spp. Among the other dairy products such as milk, buttermilk etc. Curd is taken in sterilized flask. Under the aseptic conditions curd was serially diluted from 10^{-1} to 10^{-14} from this 14 dilutions 10^{-5} 10^{-7} 10^{-9} are selected. For this 3 tubes spread plate technique further with streak plate technique is done on MRS medium. They are incubated in incubator at 37°C which is optimum temperature for *Lactobacillus*

species for 24 hours. After the period of incubation 3 isolated colonies were grown. Colony characterization is done for this 3 colonies found to be lactobacillus species. 1 colony shows 100% resemblance with lactobacillus acidophilus. Further catalase test was done.[7]

The isolated colony formed on the MRS agar plates was identified using gram stain, biochemical tests, The identification was performed according to Bergey's manual of determinative of bacteriology. The culture was kept in MRS agar slant and stored at 4 °C. For long term storage.

Gram staining test

The isolated bacteria were examined using gram staining kit and was observed under compound microscope with a magnification of 10x, 45x and 100x.

Motility test

Hanging-drop wet method was performed. The slide was observed under a light microscope with 45x magnification to check the motility of the bacteria

Catalase test

To perform this test, a single isolated colony was streaked on a glass slide and one drop of 3 % hydrogen peroxide was added on to it. The effervescence of oxygen indicated the positive response of the bacteria to catalase test.[7]

Lyophilization

One colony is taken from the curd streak plated and inoculated in 1000ml of nutrient broth. Incubation at 37°C for 24 hrs. Broth after 24-48 hrs shown lactobacillus species growth and these bacteria were freeze dried (powdery form) using lyophilization technique.[8]

Preparation of Chocolate

200gm of milk powder and 2 Chocolate cubes (40gm) Heated upto it melts. Then addition of some volume of milk (for solidification)is done. When it reaches the normal temperature, addition of freeze dried bacteria is done. Mixed it well and stored in freeze at low temperature. When it was solidified then cut into pieces. Wrapped the chocolates in Aluminium foil.[1][2]

RESULTS AND CONCLUSION

Identification of *Lactobacillus spp.*

The isolated bacteria were observed by compound microscope. It is clear that the bacteria was gram positive, rod shaped coccobacilli, occurring singly or in chains. The gram staining results indicated that the isolated bacteria could be identified as lactobacillus acidophilus. Hanging-drop wet method showed that the isolated bacteria were nonmotile. The nonmotile behavior is a characteristic of *L. acidophilus*. Therefore The curd sample bacterium resembles characters similar to lactobacillus acidophilus. The catalase test is one of the most useful diagnostic tests for the recognition of bacteria due to their simplicity. In performing catalase test, no bubble was observed indicating that the isolated bacterium is catalase negative and could not mediate the decomposition of H₂O₂ to produce O₂. It is well known that *Lactobacillus* is catalase negative. Thus, the results obtained coincided with *L. acidophilus* strain characteristics.[5]

Lyophilization and preparation of chocolate-

By using lyophilization technique the lactobacillus species were freeze dried. The freeze dried lactobacillus species were in powdery form. This powder was added at the last step of preparation of chocolate. The probiotic chocolate is prepared. The chocolate is easily assimilated and it is better to eat a chocolate than medicinal tablets. [8]

Cell count:

$$\begin{aligned}
 &= \frac{\text{no.of cells in culture} \times 10^6}{\text{dilution factor}} \\
 &= \text{for 1 ml-}146 \times 10^6 \\
 &= \text{for 1000 ml-}146 \times 10^9
 \end{aligned}$$

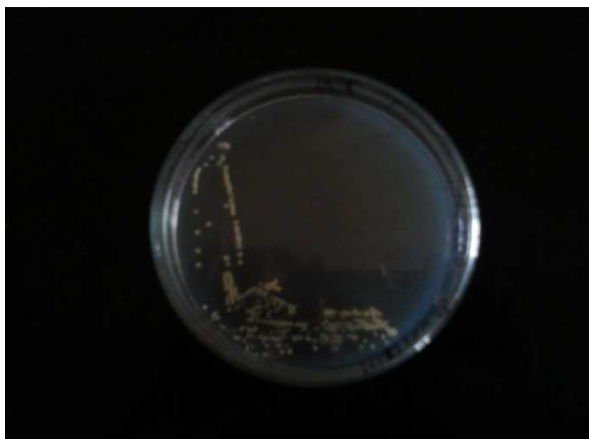


Fig 1. *Lactobacillus* spp. Grown on Nutrient Agar Plate

Sample	Size	Shape	Color	Consistency	Margin	Elevation	Opacity	Gram Character	Motility	Endospore	Capsule
Curd	0.5	Rod	White	Mucoid	Irregular	Flat	Opaque	Gram +Ve	Non Motile	Absent	Absent
Curd	0.3	Cocci	Yellowish White	Moist	regular	Flat	Opaque	Gram +ve	Non Motile	Absent	Absent
Curd	0.4	Rod	White	Mucoid	Irregular	Raised	Opaque	Gram +ve	Non motile	Absent	Absent

Table 1. Morphological Characteristics of curd sample

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REFERENCES

- [1] Barry Callebaut, *First probiotic chocolate on industrial scale in partnership with Lal'food*, Nov 28, **2007**
- [2] S.Possemiers, M Marzorati, W Vrstraete, T Van De; *Wiele International journal of food microbiology* **2010** Volume:141,Issue:1-2,Publisher:Elsevier B.V.,Pages;97-103.Pubmed:20452073
- [3] M Ratna Sudha, Prashant Chauhan, Kalpana Dixit, Sekhar Babu, Kaiser Jamil, *Genetic Engineering and Biotechnology Journal*, Volume 2010.
- [4] Lactospore, *A lactic acid bacillus preparation*, Sabinsa Corporation,
- [5] Bergey's Manual of Systematic Bacteriology, first edition, vol. 2, The Williams & Wilkins Co., Baltimore, 1986
- [6] Todar's online textbook of bacteriology, Keeneth todar, PhD
- [7] Hassan Par and K.K Peh, *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol 6, Issue 1, **2014**
- [8] G R. Nireesha, L. Divya, C. Sowmya, N. Venkateshan, M. Niranjana Babu and V. Lavakumar, *International Journal Of Novel Trends In Pharmaceutical Sciences*, Volume 3 ,Number 4, Oct, **2013**.