



PRELIMINARY STUDIES ON PROBIOTIC POTENTIAL OF SELECTED LACTOBACILLUS VIT SSV STRAINS SCREENED FROM CURD SAMPLES OF VELLORE, BIHAR, HARYANA AND VARANASI

SANJEEB KUMAR MANDAL* AND SUNEETHA VUPPU

School of Bio Sciences and Technology, VIT University, Vellore-632 014, Tamilnadu, India

ABSTRACT

Curd, Dahi or perugu is a potential source of probiotic *Lactobacilli*. In the present study, four different strains of *Lactobacillus* spp. were isolated from curd in different regions like Vellore, Varanasi, Haryana, Bihar which were subjected to preliminary screening and characterization and further examined for the presence of probiotic properties and differentiated on the basis of biochemical testing and growth kinetics. Different *Lactobacillus* strain produces a different concentration of lactic acid which depends on strain and ecological diversity. By titration method we got the concentration of the lactic acid in different sample. Cultures were subjected to characterize its antioxidant activity. No scientific evaluation of the antioxidant activity of *Lactobacillus* from curd sample has been reported so far. Therefore, it was thought worthwhile to evaluate the antioxidant activity of *Lactobacillus*. Acid tolerance of the cultures with microbial and ecological diversity was studied by incubating the organisms in MRS broth. The NaCl tolerance and bile tolerance of the isolated microbial strains were evaluated which indicate its probiotic potential.

KEY WORDS: *Lactobacillus*, Probiotics, Growth kinetics, Lactic acid, Titration



SANJEEB KUMAR MANDAL

School of Bio Sciences and Technology, VIT University, Vellore-632 014, Tamilnadu, India

*Corresponding author

INTRODUCTION

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host^{1,2}. Lactic acid bacteria generally called as LAB and *Bifidobacteria* are the most common types of microbes used as probiotics. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements. The Microorganisms involved are usually lactic acid producers^{3,4,5}. The followings are the examples of reported microbes which are considered to be Probiotics for human^{4,5,6,7,8,9}

| Name of Micro Organisms |
|-------------------------|
|-------------------------|

| |
|--|
| <p><i>Lactobacillus species, Bifidobacterium species, Enterococcus faecium, Lactococcus lactis, Leuconstoc mesenteroides, Pediococcus acidilactici, Streptococcus thermophilus and some non-lactic acid bacteria: Bacillus subtilis, Escherichia coli strain nissle, Saccharomyces boulardii, Saccharomyces cerevisiae etc. Lactobacillus acidophilus, L. acidophilus DDS-1, Lactobacillus blugaricus, Lactobacillus rhamnosus GG, Lactobacillus plantarium, Lactobacillus reuteri, Lactobacillus salivarius, Lactobacillus casei, Lactobacillus johnsonii, and Lactobacillus gasseri.</i></p> |
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Lactobacillus has been used for treating and preventing a wide variety of diseases and conditions.

In this paper different strains of *Lactobacillus* spp. were isolated from different region's curd like Vellore, Varanasi, Haryana, Bihar which were subjected to preliminary screening and characterized and examined for the presence of probiotic properties and differentiated on the basis of biochemical testing and growth kinetics.

MATERIALS AND METHODS

Screening and Isolation of Bacteria

For screening of potential *Lactobacillus* we have collected curd samples from different regions (Vellore, Varanasi, Bihar and Haryana) aseptically in sterile vials (Cryo Vials from Abdos Labtech Private Limited) and stored it for our study. MRS media was prepared for the isolation of the culture. Then we have isolated the colony on Petri plates by streak plate method and identified it^{10,11}.

Morphological study by staining method

Simple stains and Gram staining are bacteriological laboratory techniques used to differentiate bacterial species into groups based on formed within the vegetative cells^{12,13}.

Growth kinetics studies for isolated strain: O.D at 590 nm value was measured for 48 hrs. at a time interval of 1 hr¹⁴. Different growth phases were determined. Graph was

plotted between numbers of cells (O.D value) Vs time at room temperature^{15,16}.

Biochemical test

Different types of biochemical tests like Indole test, Catalase test, Citrate utilization test, Starch hydrolysis test, Fermentation of carbohydrate, Mannitol Motility test and Methyl red test are performed.

Characterization and estimation test

Lactic acid estimation

The presence of lactic acid, produced during the lactic acid fermentation is responsible for the sour taste and for the improved microbiological stability and safety of the food^{17,18}.

Assay for tolerance (Bile salt tolerance, NaCl tolerance and Acid tolerance)

In order to exert a beneficial effect in the digestive tract, probiotic culture must survive passage through the stomach and be tolerant to

the bile salts concentrations in the small intestine¹⁹. For an effective probiotics; *Lactobacillus* must survive the condition of low pH. Since the pH of stomach is acidic around 3 so for the beneficial effect of *Lactobacillus* it must survive the condition of pH 3. Acid tolerance of the cultures was studied by incubating the organisms in MRS broth^{20,21}. Acid tolerance was determined by comparing the plate count with respect to control^{22,23}.

Cholesterol reduction assay

Cholesterol is an important and necessary for human health however high levels of cholesterol leads to damage of arteries and cardiovascular diseases^{24,25}. *Lactobacillus* has ability to decrease serum cholesterol level to some extent depending on the strains^{26, 27, 28}.

RESULTS AND DISCUSSION

Isolation of bacteria

Lactobacillus screened from different regions of India like Vellore, Varanasi, Bihar, and Haryana which we designated as VIT SSV I, VIT SSV II, VIT SSV III, VIT SSV IV. Bacterial colonies were isolated from the different regions curd samples by the pour plate technique and a pure culture was obtained by the streak plate technique.

Morphology of bacteria

By simple staining and the Gram staining method we got the characteristics of bacteria is rod shaped bacteria which is the characteristic of *Bacillus* species.

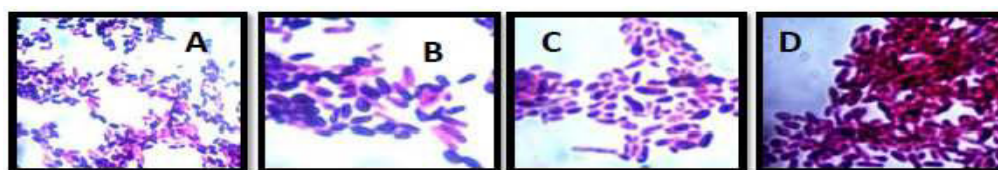
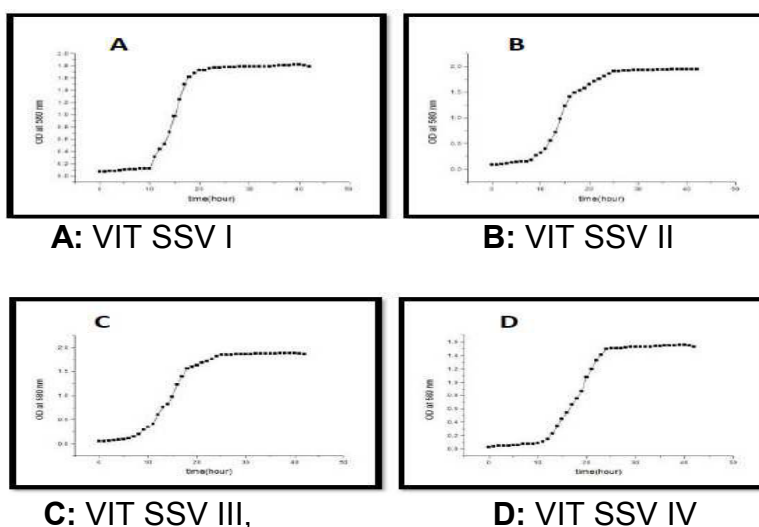


Figure1
Gram's Staining

(Note: A: VIT SSV I, B: VIT SSV II, C: VIT SSV III, D: VIT SSV IV)

Growth Kinetics



Graph 1
Growth kinetics of different strains

Table 1
Plate Count & CFU, Biochemical testing,
Carbohydrate Fermentation, Lactic Acid.

| Test | VIT SSV1 | VIT SSV2 | VIT SSV3 | VIT SSV4 | Table Name |
|-----------------------------------|-------------------|-------------------|-------------------|-------------------|-----------------------|
| Colony Number | 27 | 29 | 44 | 53 | (a) Plate Count & CFU |
| CFU(unit/ml) | 2.7×10^8 | 2.9×10^8 | 4.4×10^8 | 5.3×10^8 | |
| Indole Test | -ve | -ve | -ve | -ve | |
| Catalase Test | -ve | -ve | -ve | -ve | |
| Citrate Utilization Test | +ve | +ve | +ve | +ve | (b) Results of |
| Starch Hydrolysis Test | +ve | +ve | +ve | +ve | biochemical test |
| Fermentation of Carbohydrate Test | Glucose | +ve | +ve | +ve | |
| | Sucrose | +ve | +ve | +ve | |
| | Lactose | +ve | +ve | +ve | |
| Mannitol Test | -ve | -ve | -ve | -ve | |
| Methyl Red Test | +ve | +ve | +ve | +ve | |
| Glucose | Acid | +ve | +ve | +ve | (c) Result of |
| | Gas | -ve | -ve | -ve | |
| Sucrose | Acid | +ve | +ve | +ve | fermentation |
| | Gas | -ve | -ve | -ve | |
| Lactose | Acid | +ve | +ve | +ve | |
| | Gas | -ve | -ve | -ve | |
| NaOH vol(ml) | 23 | 22 | 11 | 13 | (d) Lactic acid |
| Lactic Concentration(mg/ml) | 2.04 | 1.98 | .99 | 1.17 | estimation |

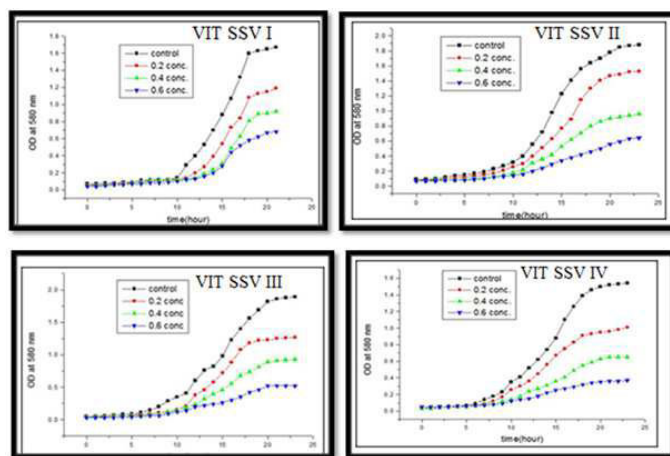
Characteristic and estimation test

Lactic acid estimation

In the lactic acid estimation we determined the concentration of lactic acid by the titration method.

Bile salt tolerance

Optical density (at 580 nm) of *lactobacillus* strain reached to about 1.88 (VIT SSV II) to 1.54 (VIT SSV IV) for the control. In addition of bile salts to broth, the growth of strains also varied considerably. VIT SSV I strains appeared to be the most resistant to bile salts while the growth rate of VIT SSV III and VIT SSV IV greatly affected by the bile salt addition at 0.6 % conc. The optical density of VIT SSV III reached up to 0.52 and for VIT SSV IV it reached up to only 0.37 which reveals the minimum growth of VIT SSV IV.



Graph 2
Bile Salt Tolerance Estimation

NaCl tolerance test

The isolated *Lactobacillus* from curd was able to tolerate 1-9 % NaCl. Maximum growth was indicated as double positive sign (++) and normal growth by single positive sign (+) and no growth by negative sign (-).

| Concentration of NaCl % | VIT SSV I | VIT SSV II | VIT SSV III | VIT SSV IV |
|-------------------------|-----------|------------|-------------|------------|
| 1 | ++ | ++ | ++ | ++ |
| 2 | ++ | ++ | ++ | ++ |
| 3 | + | ++ | + | ++ |
| 4 | + | + | + | + |
| 5 | + | + | + | + |
| 6 | + | + | + | + |
| 7 | + | + | + | + |
| 8 | + | + | + | + |
| 9 | + | - | - | - |
| 10 | - | - | - | - |

Table 2
Tolerance to NaCl of isolated Lactobacilli

Acid tolerance

Different strains show different growth at different pH. At pH 2 very few colonies formed. At pH 3 all strains survived but their viability decreased. Given bar chart shown the viability of all four strains.

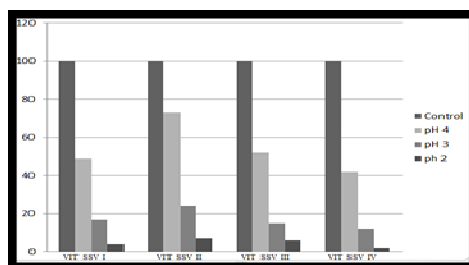


Figure 2
Viability of different strains at different pH

Cholesterol reduction assay

The percentage of cholesterol assimilated in 24 hour at 37°C in MRS broth revealed a wide variation among strains. The assimilation ranged from 20.3% to 41.9% resulted from 12.24µg/ml to 30.13µg/ml. The highest assimilation was shown by VIT SSV II.

Table 3
Cholesterol Assimilation in Test Samples

| Test Sample | O.D at 540 nm | % Assimilation |
|-------------|---------------|----------------|
| Control | 0.74 | - |
| VIT SSV I | 0.53 | 28.4 |
| VIT SSV II | 0.43 | 41.9 |
| VIT SSV III | 0.48 | 35.1 |
| VIT SSV IV | 0.59 | 20.3 |

CONCLUSION

Characterization and determination of probiotic properties of lactic acid bacteria isolated from the curd sample of different regions (Vellore, Varanasi, Bihar and Haryana) was the aim of this study. Isolated *Lactobacillus* was found to be rod shaped Gram positive and non-endospore forming bacteria in nature. Out of 10 different curd samples we have screened four potential strains VIT SSV I, VIT SSV II, VIT SSV III and VIT SSV IV and these strains possessed different types of growth kinetics which reveal the different time period of lag phase, log phase and stationary phase. It can be due to the ecological divergence. To determine the probiotic characteristic of these isolated strains, different Biochemical tests were performed such as tolerance to acid, bile and NaCl and these isolates were characterized for the estimation of lactic acid, and cholesterol reduction. According to Bergey's manual and with the results obtained from biochemical test this microorganism was tentatively identified as

Lactobacillus delbrueckii. All strains show different characteristics like cholesterol reduction, tolerance towards bile salt, acids. It was found that VIT SSV I has the highest tolerance for the NaCl and bile and VIT SSV II are the most viable for the acid. VIT SSV II has the maximum cholesterol assimilation power which means it reduces the maximum cholesterol.

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