



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

IJRSET

International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 10, Issue 11, November 2021

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA

Impact Factor: 7.569

9940 572 462

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Isolation and Characterization of Probiotics from Fruits and Vegetables

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ABSTRACT: The increased consumer awareness of the relationship between diet and health corresponds to a greater demand for food promoters of a general state of well-being, such as the functional foods. Among the functional foods, foods with probiotics occupy a large market share. The probiotics are generally added to milk products (yogurt, fermented milks and cheeses), but the consumption of these products is excluded for individuals lactose intolerant, vegans and for low-cholesterol diets. The development of non-dairy products includes the use of fruits and vegetables as matrices to which add beneficial microorganisms. Fruits and vegetables are also a natural source of prebiotics. In this research work, the presence of probiotics in fruit and vegetables were analyzed, with a focus on the effect of the addition of these microorganisms on the technological and sensory aspects. In the present research 4 lactobacillus strains were isolated from potato, carrot, grapes and banana. The strains were named as strain1, strain 2, strain3 and strain4 using standard methods. These isolates were identified by colony morphology, Gram staining, catalase activity, acid tolerance, bile tolerance, Antibiotic susceptibility and Antimicrobial activity. It is believed that human intestine bile concentration is about 0.3% hence, isolated strains were tested for their resistance property. Cholesterol assimilation by the isolates were also tested in the presence and absence of the bile salts.

KEYWORDS: Fruits, Vegetables, Probiotics.

I.INTRODUCTION

The most common probiotic microorganisms used and marketed in food world wide belong to the lactobacillus and Bifidobacterium by Champagne et al.[1]. The beverages such as fruits and vegetable juices may be the next category of foodmatrices to serve as carriers of probiotic bacteria by Gibson et al [2]. Besides, probiotics foods containing fruit ingredients' are increasingly preferred by consumers and in addition to fruits, raw and fermented vegetables and cereals have been used as substrates for probiotic bacteria, once these food contain nutrients easily assimilated by probiotics by Hernández et al.[3].

II.METHODOLOGY

Collection of sample:

The sample (Fruits and vegetables) were collected from the chinnalampatti market, Dindigul district, Tamilnadu,India.

Isolation of probiotics from potato, carrot, grapes and banana:

The sample was finely chopped using sterile knife. Then it was serially diluted from 10^{-1} to 10^{-9} using sterile peptone water.1ml of aliquots from 10^{-3} from each of the four samples were plated into MRS(de man rogosa and Sharpe)agar. Pour plate technique was used to isolate the organisms, the plates were incubated at 37°C for 3 days under anaerobic conditions. After incubation the individual colonies were selected with streak plate technique.

**Physiological and biochemical characterization:****Antimicrobial activity:**

The MRS plates were prepared and 2mm well was made into agar plates. The 18 hours incubated indicator organism like *E.coli* were spread with sterile swab 20µg/ml of isolated strain culture was filled over the well, the plates were incubated at 37°C for 24hours under aerobically or anaerobically based on the indicator organism. After incubation the diameter of zones around the colonies were measured. The diameter of zone indicates the ability of antimicrobial activity of the culture. The isolates, which gave an inhibition zone bigger than 1mm, were determined to have antimicrobial activity.

Cholesterol reduction by probiotics:

Freshly prepared MRS broth was supplemented with 0.30% oxgall as a bile salt. Water soluble cholesterol was filter sterilized and added to the broth at a final concentration of 50-200µg/ml, inoculated with each strain at 1% level and incubated aerobically at 37°C for 24 hours. After the incubation period, the cells were centrifuged and supernatant was collected. The cholesterol concentration in the broth was determined using a modified colorimetric method. 1.5ml of FeCl₃ working solution was added to the tube containing sample, after through mixing the solution was allowed to stand for 10 minutes. Then 1ml of concentrated sulphuric acid was added. The solution was placed in the dark. After 45 minutes the absorbance was read at 560nm at different incubation periods. Cholesterol reducing activity was calculated as percentage by the treatment compared with control.

III. EXPERIMENTAL RESULTS AND DISCUSSION**Isolation and purification of bacterial strains**

Samples from fruits and vegetables (potato, carrot, grapes, banana) were taken from Chinnalampatti market, Dindigul. Lactic acid bacteria were isolated from various mediums at 37°C under anaerobic conditions. All the isolated strains were catalase positive. Strain 1, strain 2 and strain 4 were Gram positive. They are all non-motile, the isolated strains were confirmed as *Lactobacillus* sp. using various morphological, physiological and biochemical characteristics described in the 8th edition of "Bergeys Manual of Determinative Bacteriology".

Antimicrobial activity

The selected strains were examined according to their antimicrobial activity. For this purpose, strains were detected against the indicator microorganism such as *E.coli*. The diameter of inhibition zones showed that all of the isolates have antibacterial effect on the indicator microorganisms given in table 1.

Table 1 : Antimicrobial activity of strains against test pathogen

Bacterial isolates	<i>E.coli</i> (inhibition zone mm)
Strain1	8.6±2.05
Strain2	9.83±0.04
Strain3	7.19±0.40
Strain4	7.61±0.16

Results were shown in Mean ± Standard deviation

Cholesterol reduction by probiotics

All the isolates were studied for cholesterol reducing capability in the cell free broth at a concentration ranges from 50µg/ml to 200µg/ml. The cholesterol reduction test was performed by addition of 0.3% bile salt and without addition of bile salts at 24 and 48 hours given in figure 1, 2, 3 and 4.



Figure 1 : Cholesterol reduction by *Lactobacillus* sp. without bile salts after 24 hours

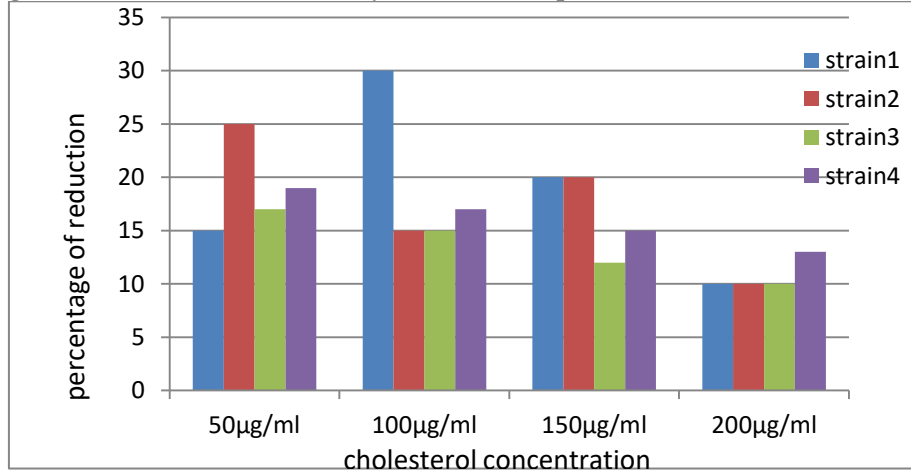


Figure: 2 Cholesterol reduction by *Lactobacillus* sp. without bile salts after 48 hours

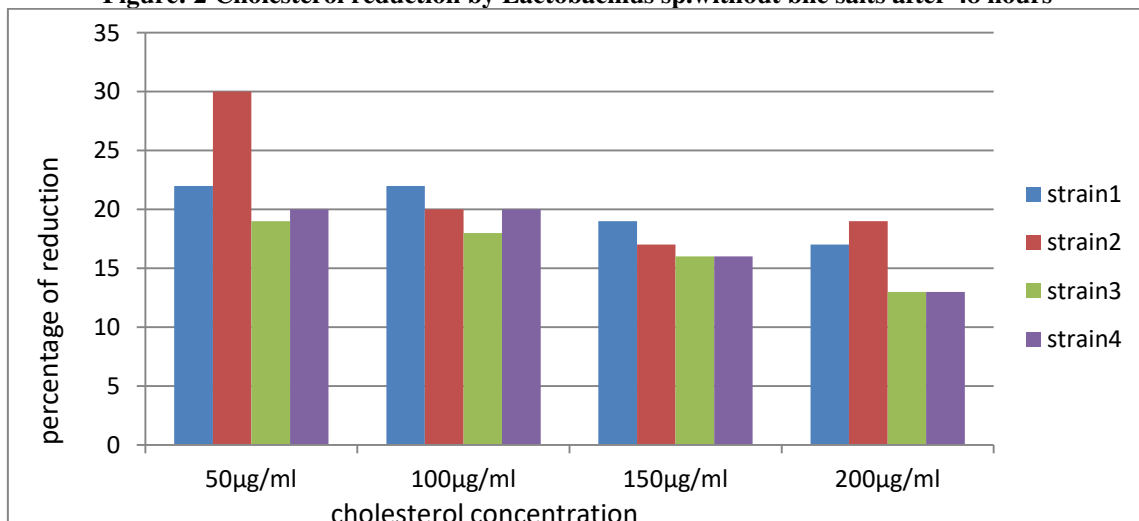


Figure: 3 Cholesterol reduction by *Lactobacillus* sp. with bile salts after 24 hours

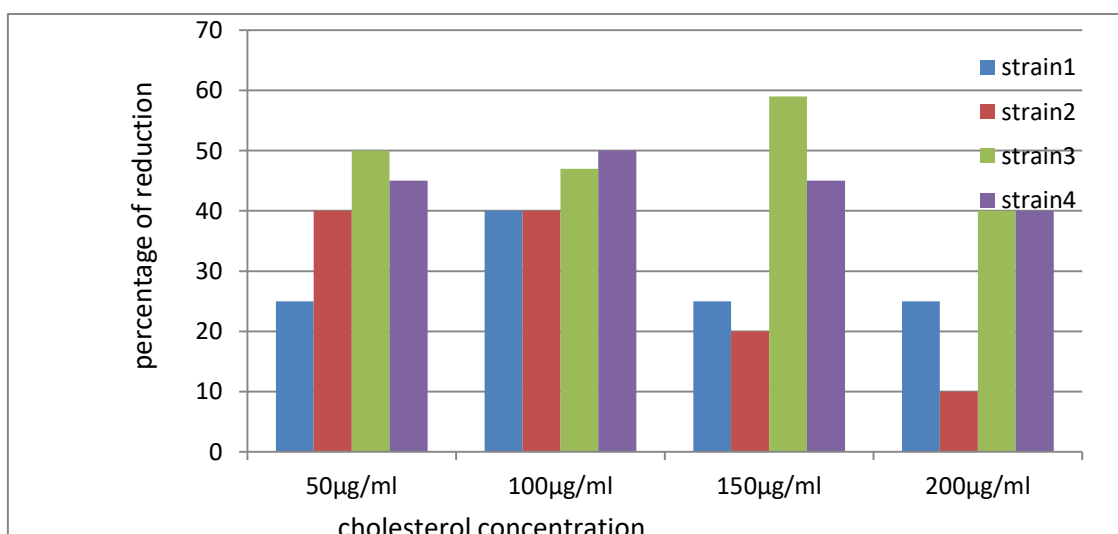
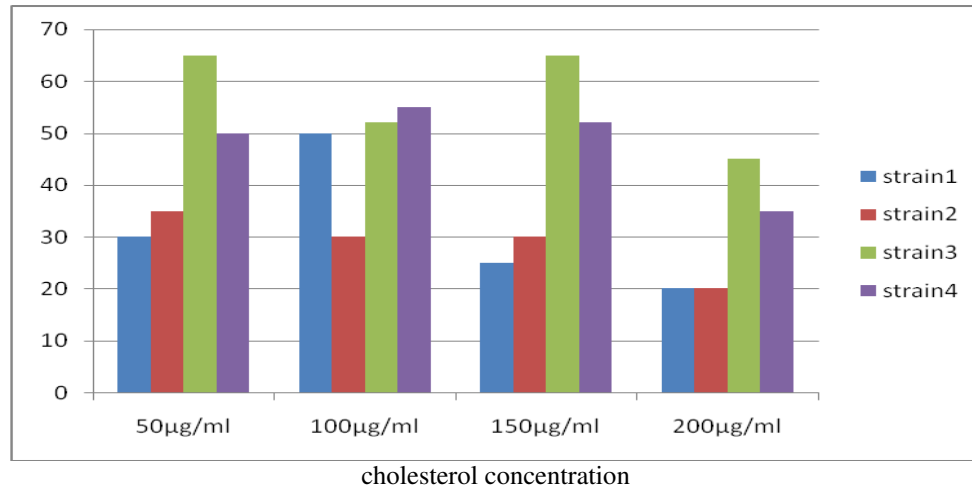


Figure: 4 Cholesterol reduction by *Lactobacillus* sp. with bile salts after 48 hours

Probiotics have the potential for contributing greatly to human and animal health via wide range of applications. Historically, probiotics have been used in food for humans and animals without any side effects, while providing for the balance of intestinal flora. The health promoting effects of probiotics have been widely explored and include stabilization of indigenous microbial population, boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from from various causes, alleviation lactose intolerance, increased nutritional value of foods, reduction of serum cholesterol levels, antimutagenicity and anticarcinogenicity, reduction of the risk of inflammatory bowel conditions, improvement of digestion of proteins and fats, synthesis of vitamins, and detoxification and protection from toxins.

In this study the first step was taken to use the isolates as cultures for probiotic products. The main criteria of being probiotic strains were determined and the selected isolates were identified by Alegre et al.[4]. LAB is one of the major inhabitants of the human, further gastrointestinal tract and is commonly used in fermentation of various kinds of food products there is mounting scientific evidence that LAB fermented food products positively influence the health of humans by Bevilacqua et al. [5]. NaCl is an inhibitory substance which antagonizes the growth of certain types of bacteria. All strains were able to grow at 4% NaCl concentrations. Strain 4 shows more growth at 6.5% NaCl concentration, rest of them show least growth compared to growth of strains in 4% NaCl concentration. Although, the bile concentration of the human gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3% and the staying time is suggested to be 4 hours. According to our finding, all of the study isolates are able to grow up in 0.05-0.3% bile salts. Strain 4 is considered as the best candidate for bile tolerance. In order to justify the varying cholesterol-lowering effect exhibited by various strains of probiotics, or types of prebiotics, researchers have endeavored to reveal the mechanisms of probiotics on hypocholesteremic effect through invitro and in vivo studies. Due to the production of organic acids and other low molar mass metabolites, the isolated strains shows strong antimicrobial activity by Gebbers [6].

IV.CONCLUSION

LAB is one of the major inhabitants of the human, further gastrointestinal tract and is commonly used in fermentation of various kinds of food products there is mounting scientific evidence that LAB fermented food products positively influence the health of humans

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