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Probiotics: Potential Bio-Therapeutics for Lowering Cholesterol

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Abstract

Hypercholestramia, accumulation of high cholesterol in the blood vessels which create blocks and result in heart attack. 34% of deaths in India is due to accumulate of cholesterol. To overcome the problem, this research focus on to regulate and maintain the level of cholesterol. Isolation of *Lactobacillus* on MRS medium was carried out using dairy products as sample. Biochemical characterization of isolates belongs to *Lactobacillus Spp*. Probiotic properties like haemolytic activity, bile salt tolerance assay, cell surface hydrophobicity and anti-microbial susceptibility was tested. *Lactobacillus* were non-haemolytic indicating the non-virulence nature and application as probiotics. Cell surface hydrophobicity and bile salt hydrolase activity benefits the *Lactobacillus* by enhancing its resistance to conjugated bile salts and increasing its survival in the gastrointestinal tract. *Lactobacilli* strains sustain in gut indicates their ability to retain during the therapy. Isolates CS2, CS3, MS1 and MS5 were proved to possess probiotic characters. MS5 was found efficient to hydrolyze bile and inhibits the cholesterol formation at initial stages leading to low levels of cholesterol accumulation. MS5 was found to reduce cholesterol up to 90% and proved to be efficient as therapeutic agent.

Keywords
Hypercholesterolemia, Lactobacilli, bile salt hydrolase activity

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INTRODUCTION

Probiotics are the "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" mainly lactic acid bacteria used as probiotic organisms, but Lactobacillus are use more conveniently at industrial level to desired probiotic products. *Lactobacillus* is a genus of Gram-positive, facultative anaerobic and micro-aerophilic, rod-shaped, non-spore forming bacteria *Lactobacilli* are generally non-motile and can survive in both aerobic and anaerobic environments. Cholesterol is essential for the formation of bile acids, which allow our body to digest fats. Cholesterol is also utilized by the body to produce cell membranes. Everybody needs some cholesterol in order to be healthy but if accumulation of cholesterol is high then it leads to the problematic like hypercholesterolemia or stroke, normal range of cholesterol per day. Death rate because of

cardiovascular disease like hypercholesterolemia is 34% as per the 2016 survey.

The goal of the presented work is to investigate the efficiency of *Lactobacillus* to lower the cholesterol. *Lactobacillus* will act on bile and produce hydrolase enzyme and degrades the bile acid into cholic acid, as bile is a precursor of cholesterol. As a result, lowering the accumulation of cholesterol at precursor level.

MATERIALS AND METHODS

Isolation of probiotic strains

Two Dairy samples were collected such as raw milk, curd from local market. These samples were serially diluted and spreaded on sterile MRS medium and these plates were incubated under microaerophillic conditions for 24 hours. The obtained isolates were studied for morphology and biochemical characterization for identifying *Lactobacillus spp.*

Characterization of probiotic organisms

Biochemical characterization

Ability of isolates for carbohydrate fermentation and enzymes synthesized were characterized biochemically. Biochemical tests include sugar fermentation tests (glucose, lactose, sucrose, and maltose), lysine decarboxylase, nitrate reduction, triple sugar iron, catalase, oxidase, citrate utilization and blood haemolysin test were performed.

Microbial adhesion ability

Suspension of isolates were prepared in sterile saline obtained from dairy products; Optical density was adjusted between 0.8 at 530 nm. The suspension was centrifuged at 6000 rpm for 10 minutes and wash the pellet twice with saline and re-suspend the pellet in saline. 3 ml of suspension was added to 0.6 ml of hexadecane, vortex mix carefully and then incubated 37°C for 30 minutes. Optical density was measured, and percentage hydrophobicity was calculated.

Antibiotic susceptibility testing

The suspension of each isolate was prepared in sterile nutrient broth and incubate for two hours and use as culture suspension, The media used for this activity was test Muller Hinton agar plates Onto the sterile MH plates the pre-incubated suspension was swabbed, After that each antibiotic disc was placed carefully by using forcep Plates was incubated for 48 hours in microaerophillic condition.

Acid tolerance assay

The sterile nutrient broth was prepared, and the pH was adjusted 2, 2.5, 3, 4, 5 by using 1N HCL. All tubes were inoculated with saline suspension of isolates and incubated at 37°C at microaerophillic condition for 24-48 hours. Growth was measured as increased in turbidity and its absorption maxima at 530 nm.

Bile salt tolerance assay

The sterile MRS broth was prepared with different concentrations of bile salt *viz.,* 0.5, 1, 1.5, 2. All tubes were inoculated and incubated at 37°C at microaerophillic condition for 24-48 hours. Growth was measured as increased in turbidity, checking its absorption maxima at 530 nm.

Bile hydrolase activity

The suspension of each isolate was prepared, and the culture was adjusted as 0.1. For this, sterile MRS agar with bile salt 0.3 % (w/v) and $CaCl_2$ (0.375g/l) was prepared. Agar well diffusion method was performed for bile hydrolase activity. 100µl of each suspension was loaded into separate wells, then plates were pre-incubated in refrigerator for 15 minutes for the suspension to diffuse (pre-diffusion) into medium. The plates were incubated under microaerophillic condition for 48 hours, after incubation, the plates were observed for halo zones formed due to the hydrolysis of bile salt, thin layer chromatography was performed by taking scrap from obtained halo zones for detection of glycine.

Cholesterol lowering ability of *Lactobacillus*

The sterile MRS was supplemented with 0.02 gm% cholesterol which was used as standard for colorimetric assay. Tube containing 10 ml of nutrient broth and 0.02 gm % cholesterol was inoculated with 0.1 ml of suspension of isolated organisms. After inoculation the broth was incubated at microaerophillic condition for 48 hours. The left-over cholesterol was estimated by $FeCl_3$-H_2SO_4 and the absorbance was determined at 530 nm. Cholesterol reacts with $FeCl_3$-H_2SO_4 and forms brown colour complex.

RESULTS

Isolation of probiotic organisms

Samples were serially diluted and plated on sterile MRS medium. As the dairy products is considered as a rich source of microbes and its ability that produce various probiotics. A total of 15 isolates were obtained and were selected on the basis of morphology. These isolates were designated as MS1 to MS10 for raw milk sample and CS1 to CS5 for curd sample. Further identification was carried out by using Gram staining, all strains showed Gram positive nature.

Characterization of probiotic organisms

Biochemical characterization

Enzyme production and carbohydrate utilization by isolates was carried by biochemical characterization. Isolates MS5, MS6, CS2, CS3 showed catalase positive, nitrate reduction, citrate utilization, carbohydrate utilization by producing acid, non-haemolytic

Microbial adhesion to hydrocarbon by using n-hexadecane.

The hydrophobicity percentage for the isolates ranged between 0.11% and 85%. Strains with hydrophobicity more than 40% were considered hydrophobic. The hydrophobicity of strain MS5 showed greater affinity towards the solvent used for the study. The strain MS5 showed 85% affinity towards n-hexadecane. Maximum strains show more than 40% hydrophobicity. Percentage hydrophobicity suggests the adhesion of probiotic strains to intestinal epithelial cells is an important prerequisite for colonization in the gastrointestinal tract.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of obtained isolates was carried out using eight discs such as Gentamycin, Penicillin G, Erythromycin, Ampicillin, Streptomycin, Chloramphenicol, Vancomycin and Kanamycin.

All observation was made on the basis of Kirby Bauer manual, the *lactobacillus* was resistant to antibiotics. Resistance to antibiotics help the individual to retain the gut flora during the therapy.

Acid tolerance assay for *Lactobacillus*

Acid tolerance assay was carried using different pH ranges of MRS broth adjusted at 2, 2.5, 3, 4, and 5. Growth was observed at low pH ranges indicate the acid tolerance of *Lactobacillus*. This peculiar property of proved retention ability and survival in the gut.

Bile salt tolerance assay for *lactobacillus*

Survival of *Lactobacillus* in the presence of bile salt was studied. Varying concentrations of bile salt was adjusted. A significant absorbance was observed in all tubes indicates the tolerance of bile in the gut.

1. Bile hydrolase activity

Visible halos around the wells indicate the positive BSH activity of the strains when supplemented with 0.3%(w/v) bile salt and another is $CaCl_2$ (0.375g/l). Results were then assessed by measuring the diameters of halos. Four isolates out of fifteen namely MS5, MS6, CS2, CS3 from dairy products were efficient to hydrolyse the bile and cholesterol lowering effects have been linked to bacterial bile salt hydrolase (BSH) activity. Organisms produce bile hydrolase enzyme which degrades the bile into cholic acid and the $CaCl_2$ helps to precipitate the hydrolase enzyme, which is then confirmed by thin layer chromatography were the test and control shows same retardation factor, the standard glycine was used as a control.

Bile Hydrolase activity

2. To evaluate cholesterol lowering ability of *Lactobacillus.*

Hypercholestramia is the presence of excess cholesterol in blood stream that blocks the normal flow of blood leads to heart attacks, In India death rate because of Hypercholesterolemia is 34% as per 2016 survey. Cholesterol reduction ability was evaluated and found that up to 90% of cholesterol level reduced by the *Lactobacillus* strain MS5. Hence isolated *Lactobacillus* strain are useful for lowering cholesterol and help in resolving the problem of hypercholestramia.

DISCUSSION

The WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World. It was reported that hypercholesterolemia contributed to 45% of heart attacks in Central and Eastern Europe from 1999 to 2003.The risk of heart attack is three times higher in those with hypercholesterolemia, compared to those who have normal blood lipid profiles. The WHO delineated that unhealthy diets such as those high in fat, salt and free sugar, and low in complex carbohydrates, fruits and vegetables, lead to increased risk of cardiovascular diseases.

To overcome this consequence in the locality we made efficient health drink to lowers the rate of cholesterol accumulation in our system by using *Lactobacillus.* The cholesterol-lowering potential *Lactobacillus* of has been discussed in studies for years. In this study, several *in vitro* experiments were performed to evaluate the ability of *Lactobacillus* to reduce cholesterol levels. Microbial BSH activity in the host results in the reduction of cholesterol levels, deconjugated bile acids are less soluble and are less likely to be absorbed from the intestinal lumen than

are conjugated bile salts, free bile is more likely to be excreted through the intestinal tract. Therefore, with the help of BSH, deconjugation of bile salts could lead to a reduction of serum cholesterol by reducing cholesterol absorption through the intestinal lumen. This increases the demand for cholesterol for *de novo* synthesis of bile acids to replace their loss through faeces. In our study, the cholesterol reduction was studied by using ferric chloride method and it was found to be 90%. Among all the experiments and study, the isolate MS5 has proved as an efficient probiotic organism because no haemolysis was observed on SIBA, 85% hydrophobic to the n-hexadecane, grows at low pH, survive in different concentration of bile salts.

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