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Isolation, Identification and Screening of Cholesterol Degrading Probiotics

K. Vaishnavi, M. Krishma and P. Rajeswari*

The Standard Fireworks Rajaratnam College for Women, Sivakasi, Tamilnadu

*Corresponding Author E-mail: sholarrpl@gmail.com

ABSTRACT

In this study, Cholesterol degrading Lactobacillus was isolated from butter milk sample. The growth performance of Lactobacillus on MRS agar was identified by the colonies were formed due to the utilization of Lactose and by various biochemical tests. Lactobacilli are resistant to various antibiotics and bile salt. Growth performance of selected probiotic in various pH (such as 4, 7 & 9), Temperature (such as 4^0C, 37^0C, 50^0C), and various concentration of Bile salt (such as 0.1g, 0.2g, 0.3g,0.4g and 0.5g) was detected. In vitro study of cholesterol degradation by probiotic was carried out and it was based on the growth performance and percentage of cholesterol removed from liquid cholesterol. The result of in vitro study reveals Lactobacillus showed the better degradation (21.5%) of cholesterol degraded in liquid cholesterol (at 600 µg/ml).

Key words: *Cholesterol, Lactobacillus, Probiotics, Bile salt and MRS medium.*

INTRODUCTION

Cholesterol is an important basic block for body tissue; elevated blood cholesterol is well-known major risk factor for coronary heart disease[1]. Bile a water-soluble end product of cholesterol in liver is stored and concentrated in the gallbladder and released into the duodenum upon the ingestion of food[5]. Cholesterol being a precursor of bile acid converts its molecule to bile acids replaces those lost[4,5]. Cholesterol is used to synthesis new bile acid in a homeostatic response, resulting in lowering of cholesterol[2]. Many attempts have been made to elucidate the mechanism; one proposed mechanism is the assimilation of the cholesterol by cell wall during growth[3]. Another mechanism is deconjugation of bile salt by bacteria producing bile salt hydrolase.

Lactic acid bacteria capable of lowering blood cholesterol by sticking in the intestinal wall then multiply and producing the enzyme bile salt hydrolase (BSH), which resulted in increased acid conjugated bile that is not easily absorbed from the small intestine compared with bile acid conjugation. Conjugated bile acids by high hence decreasing intestinal absorption of fat and fatty deposits to be reduced[7].

MATERIALS AND METHODS

I. Sample Collection:

Butter milk was collected from the hostel in the Standard Fireworks Rajaratnam College for Women at Sivakasi to isolate the bacterium *Lactobacillus.*

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II. Isolation and Identification of *Lactobacillus*

Selective medium was used for the isolation of *Lactobacillus* was MRS medium. And it was identified by using microscopic observation and by various biochemical tests such as Indole test, Methyl red, Voges Proskauer, Citrate utilization test, Urease test, Oxidase test, Catalase activity and carbohydrate fermentation were carried out for the identification of *Lactobacillus.*

III. Screening and Characterization of Lactobacillus for Cholesterol Degradation[6]

The cholesterol degraders were screened based on the bile tolerance, pH resistance, temperature resistance and antibiotic resistance.

a) Growth of cholesterol degrading probiotics in various concentration of bile salts in MRS medium with different pH at different temperature

Prepare each glass vials with five concentration of bile in MRS media. Make into three set of pH such as 4, 7 & 9. Each vial was inoculated with 1% of broth culture of *Lactobacillus*. Incubate the vials at different temperature such as 4ºC, 37ºC, and 50°C for 24 hours.

b) Antibiotic resistance

Sterile disc was immersed with various antibiotics such as Amoxycillin, Ranitidine, Ampicillin, Erythromycin, Penicillin G, Tetracycline, and Streptomycin. The disc was placed in the Muller Hinton agar plates swabbed with *Lactobacillus* culture. The plates were incubated at 37°C for 24 hours.

IV. Cholesterol assimilation by using water-soluble cholesterol[6]

Day - 1

For cholesterol assimilation by Probiotics, 1% of *Lactobacillus* culture was inoculated into freshly prepared MRS broth, supplemented with bile salt and Water - Soluble cholesterol at various concentrations such as 200µg/ml, 400µg/ml and 600µg/ml respectively. Then the glass vials were inoculated with *Lactobacillus* culture and anaerobically incubated at 37°C for 24 hours.

Day - 2

The cells were harvested after the incubation period by centrifugation at 10,000 rpm at 4°C for 10 minutes. The cell pellet was washed twice with sterilized distilled water. The cell pellet was suspended in MRS broth containing 0.1 gm of bile salt and various concentrations of water-soluble cholesterol (200µg/ml, 400µg/ml and 600µg/ml) in three vials. This setup was anaerobically incubated at 37ºC for 24 hours.

Day - 3

After the incubation period, cholesterol assimilation ability of *Lactobacillus* to remove the cholesterol; from the media was calculated as percentage from the following equation.

$$\textbf{Cholesterol assimilation (A) = 100 – (B/C)*100}$$

Where, A= % of cholesterol removed, B=absorbance of the sample containing cells and C= absorbance of the sample without cells.

RESULTS AND DISCUSSION

Isolation and Identification

Cholesterol degrading probiotics was isolated and identified based on biochemical characteristics. By microscopic observation the *Lactobacillus* was observed as gram positive, rod shaped and non-motile. The result of various biochemical test such as Indole test (-ve), Methyl red (-ve), Voges Proskauer (-ve), Citrate utilization test (-ve), Urease test (-ve), Oxidase test (-ve), Catalase activity(-ve) and carbohydrate fermentation (+ve to Lactose, Sucrose & Glucose) were observed.

Screening and Characterization

The growth rate of *Lactobacillus* in various concentration of bile salt (0.1g, 0.2g, 0.3g, 0.4g & 0.5g) and various pH (4, 7 & 9) conditions at different temperature (4°C, 37°C & 50°C) were observed and monitored at 620nm (Table-1).

Table – 1: Growth rate of *Lactobacillus* at Various Bile salt concentration, pH and Temperature

pH	Concentration of bile salt	Optical Density (4°C)	Optical Density (37°C)	Optical Density (50°C)
4	0.1g	0.102	0.192	0.240
	0.2g	0.158	0.412	0.318
	0.3g	0.09	0.130	0.084
	0.4g	0.242	1.184	0.345
	0.5g	0.019	0.030	0.003
7	0.1g	0.09	0.223	0.074
	0.2g	0.134	0.320	0.067
	0.3g	0.139	0.160	0.030
	0.4g	0.154	0.344	0.293
	0.5g	0.48	0.323	0.046
9	0.1g	0.193	0.421	0.224
	0.2g	0.001	0.462	0.400
	0.3g	0.018	0.360	0.053
	0.4g	0.056	0.426	0.020
	0.5g	0.029	0.354	0.020

Antibiotic resistance

The result of antibiotic resistant of cholesterol degrading Probiotic organism was observed and tabulated (Table-2).

Table-2: Antibiotic resistance of *Lactobacillus* against different antibiotics

Antibiotic / Organisms	*Lactobacillus* (Zone formation. dm in cm)
Streptomycin	2.3
Penicillin G	1.5
Ranitidine	-
Tetracycline	2.5
Ampicillin	0.9
Amoxyline	1.5
Erythromycin	-

Cholesterol Removal Method:

Assimilation of Cholesterol:

The growth performance of *Lactobacillus* in the medium containing water-soluble cholesterol at various concentrations (200µg/ml, 400µg/ml & 600µg/ml) was observed spectroscopically at 620nm. Medium containing 600 µg/ml of cholesterol showed the maximum growth and maximum percentage (21.5%) of cholesterol degradation by *Lactobacillus* at 3 days (Table-3).

Table-3: Effects of *Lactobacillus* on cholesterol degradation at 3 days

Concentration of Water-Soluble Cholesterol (µg/ml)	Optical Density	Percentage of Cholesterol Degradation (%)
200µg/ml	0.122	17.3
400µg/ml	0.142	20.1
600µg/ml	0.151	21.5

CONCLUSION

The *Lactobacillus* was isolated from butter milk sample and it was identified based on various biochemical tests. Then the culture was screened and characterized by pH resistance, Bile salt resistance, temperature resistance and Antibiotic resistance. From this present study, it was concluded that the

Lactobacillus showed the better degradation (21.5%) of cholesterol in the medium containing 600µg/ml of water-soluble cholesterol.

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