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A Study on Cholesterol Degradation by *Lactobacillus*

KEYWORDS: Cholesterol, *Lactobacillus* and MRS medium.

K. VAISHNAVI	M.KRISHMA	P.RAJESWARI
Department of Microbiology, The Standard Fireworks Rajaratnam College for Women, Sivakasi. Virudhunagar (Dst),Tamil Nadu, India.	Department of Microbiology, The Standard Fireworks Rajaratnam College for Women, Sivakasi. Virudhunagar (Dst),Tamil Nadu, India.	Department of Microbiology, The Standard Fireworks Rajaratnam College for Women, Sivakasi. Virudhunagar (Dst),Tamil Nadu, India.

ABSTRACT *The aim of this work was made to isolate, screen and characterize probiotic organisms for Cholesterol degradation. Cholesterol degrading probiotic Lactobacillus was isolated form butter milk sample using MRS medium and identified based on the morphological and biochemical characteristics. Then the strain was further subjected for cholesterol degradation. Lactobacillus isolate showed better results (27.8% of degradation) seen in 3days.*

Introduction

Cholesterol is a compound belonging to steroid family of molecules (Steinberg, D .2006). Cholesterol plays major role in human health. Normally, it is need in the body to insulate nerves makes cell membranes and produce some hormones, vitamin D and substance aid for digestion and also need to build healthy cells (HongbaoMa.2004). Excess cholesterol in the blood stream can form plaque in arterial wall (Steinberg, D and Witztum JL. 2002). The cholesterol and plaque build-up causes the arteries to become thicker, harder and sometime blocking the blood flow to the heart and other vital organs. When too much low-density lipoprotein (LDL) deposits inside the arterial walls where if it is oxidized, it can build-up as hard deposits and cause atherosclerosis, the disease process that under lies heart attack. Thus the synthesis and utilization of cholesterol must be tightly regulated in order to prevent over accumulation and abnormal deposition with in the body (Fernandez de las Heras, L. 2011). Probiotics strains especially lactic acid bacteria have major role to play in the cholesterol level reducing mechanism (Fuller.R .1989).

Lactobacillus bacteria and *Bifidobacteria* in the host decreased blood cholesterol levels by either decreased total intestinal absorption or removal through solid excretion or by interrupting the entero hepatic cycle of bile acids. *Lactobacillus* bacteria suppressed the re-adsorption of bile acids carrying cholesterol and enhance the removal of cholesterol from blood through faeces (Hosono, A, 2000). These beneficial organisms are able to deconjugate with bile acids such as taturocholic or glycocholic acid. Deconjugation of bile acid may helps to decrease the serum cholesterol in humans, the synthesis of bile acids from cholesterol concentration can reduce the total cholesterol in the body (Buck. M and S.E Gilliland.1994).

Materials and Methods:

Sample Collection:

1. Butter milk was collected from the hostel in the Standard Fireworks Rajaratnam College for Women, Sivakasi to isolate the bacterium *Lactobacillus.*
2. Serum sample of hypercholesterolemia and hypocholesterolemia patients were collected from Clinical Laboratory, Sivakasi.

Isolation of *Lactobacillus:* (Raghavan *et al.*, 2011)

Selective medium was used for the isolation of *Lactobacillus* was MRS medium with the following composition: Peptone from casein- 10gm, Yeast extract- 8 gm, D (-) glucose- 20gm, Di-potassium hydrogen phosphate- 2 gm, Tween 80- 1 ml, Di –ammonium hydrogen citrate- 2gm, Sodium acetate- 5 gm, Magnesium sulphate- 0.2 gm, Manganese sulphate- 0.04 gm, Distilled water- 1000 ml, pH- 5.7±0.2.

Identification of *Lactobacillus*

Microscopic observations of the bacterial isolates were studied using Gram Staining and motility tests (Hanging drop Technique). 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*.

Cholesterol removal method: (Raghavan *et al.*, 2011)

Cholesterol assimilation by using Blood serum cholesterol:

Day - 1

For cholesterol assimilation by Probiotics, 1% of *Lactobacillus* culture was inoculated into freshly prepared MRS broth, supplemented with bile salt and hyper and hypocholesterolemic patient's serum having cholesterol at various concentrations such as 180µg/ml, 200µg/ml, 220µg/ml and 240µg/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 patient's serum having cholesterol at various concentrations (180µg/ml, 200µg/ml, 220µg/ml and 240µg/ml) in four vials. This setup was anaerobically incubated at 37°C 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 equations.

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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
The aim of this work was to isolate, screen and characterize probiotic organisms for cholesterol degradation. The result of various morphological analysis and biochemical test such as Indole test, Methyl red, Voges Proskauer, Citrate utilization test, Urease test, Oxidase test, Catalase activity and Carbohydrate fermentation were tabulated (Table.1).

Table -1: Biochemical Characterization

Biochemical test	Results
Gram Staining	Gram Positive- rod
Motility test	Non-Motile
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
C Citrate utilization test	Negative
U Urease test	Negative
Oxidase test	Negative
Catalase test	Negative
C Carbohydrate Fermentation test	Positive to Lactose, Sucrose and Glucose

Cholesterol Removal Method:
Assimilation of Cholesterol:
The growth performance of *Lactobacillus* in the medium containing patient's serum having cholesterol at various concentrations (180µg/ml, 200µg/ml, 220µg/ml and 240µg/ml) was observed spectroscopically at 620nm. Medium containing 240 µg/ml of blood serum cholesterol showed the maximum growth and maximum percentage (27.8%) of cholesterol degradation by *Lactobacillus* at 3 days (Table-2).

Table-2: Effects of *Lactobacillus* on medium containing cholesterol (from patient's serum) degradation at 3 days.

Serum cholesterol (mg/dl)	Optical Density	Percentage 0f Cholesterol Degradation (%)
180 mg/dl	0.070	9.2
200 mg/dl	0.154	18.1
220 mg/dl	0.212	23.3
240 mg/dl	0.268	27.8

Conclusion
The *Lactobacillus* was used as a selected probiotic to potentially reduce the cholesterol. From this present study, it was concluded that the *Lactobacillus* showed the better degradation (27.8%) of cholesterol in the medium containing cholesterol (240 µg/ml) from blood serum. Hence it was suggested that these *Lactobacillus* can be a better option for *in vivo* treatment of patient with hypercholesterolemia.

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