Studies on lowering of Cholesterol using an isolate of Lactic acid Bacteria

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***Abstract**: -*

Lactobacillales are an order of Gram-positive, low-GC, acid-tolerant, generally nonsporulating, either rod-shaped (bacilli) or spherical (cocci) bacteria that share common metabolic and physiological characteristics. These bacteria, produce lactic acid as the major metabolic end product of carbohydrate fermentation, giving them the common name Lactic Acid Bacteria (LAB). Along with their nutritional benefits the LAB play an important role in reducing cholesterol absorption and increasing cholesterol catabolism. They can bind with cholesterol in the intestine to stop it from being absorbed. They also help produce certain bile acids, which help metabolize fat and cholesterol in the body. Previous studies reported that lactobacilli can remove cholesterol in vitro via various mechanisms, such as assimilation, binding to the surface of cells, incorporation into the cellular membrane and co-precipitation with deconjugated bile.

The present study investigates the effect of Lactic Acid Bacteria on Cholesterol. The Lactic Acid Bacteria (LAB) used in this study was isolated from homemade curd. The isolated Lactic Acid Bacteria (LAB) was characterised and identified as Lactococci which was further studied for its activity on different cholesterol concentrations in MRS Broth. As Cholesterol-lowering activity is one of the most promising properties of lactic acid bacteria with probiotic characteristics. The isolated culture of lactic acid bacteria showed ability to assimilate cholesterol for different concentrations of cholesterol at the level almost 39% to 65%.

1.Introduction

Lactobacillales are an order of gram-positive, low-GC, acid-tolerant, generally nonsporulating, non-respiring, either rod-shaped (bacilli) or spherical (cocci) bacteria that share common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end product of carbohydrate fermentation, giving them the common name lactic acid bacteria (LAB). Lactic acid bacteria (LAB) constitute a ubiquitous bacterial group that is widespread in nature in niches of dairy (fermented), meat and vegetable origin, the gastrointestinal and urogenital tracts of humans and animals, and soil and water [1]. These microorganisms are well known for their ability to produce lactic acid as the main end-product of their anaerobic metabolism and for synthesizing a wide range of metabolites that beneficially affect the nutritional, sensorial, and technological properties of fermented food products. Their importance is associated mainly with their safe metabolic activity while growing in foods utilising available sugar for the production of organic acids and other metabolites. In addition, LAB, as part of gut microbiota

ferment various substrates such as biogenic amines and allergenic compounds into short-chain fatty acids and other organic acids and gases[2].

Cholesterol comes under class of lipid molecules. It is a sterol [3], a type of lipid. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. When chemically isolated, it is a yellowish crystalline solid. Cholesterol is a fat-like, waxy substance that helps the body make cell membranes, many hormones, and vitamin D.

Body needs some cholesterol to make hormones, vitamin D, and substances that help to digest foods. The body makes all the cholesterol it needs. Cholesterol is also found in foods from animal sources, such as egg yolks, meat, and cheese.

If the body has too much cholesterol in the blood, it can combine with other substances in the blood to form plaque. Plaque sticks to the walls of arteries. This build-up of plaque is known as atherosclerosis [4]. It can lead to coronary artery disease, where your coronary arteries become narrow or even blocked. Cholesterol is an important basic building block for body tissues, elevated blood cholesterol is a well known major risk factor for coronary artery disease. [5].

HDL, LDL, and VLDL are lipoproteins. They are a combination of fat (lipid) and protein. The lipids need to be attached to the proteins so they can move through the blood. Different types of lipoproteins have different purposes:

HDL stands for high-density lipoprotein. It is sometimes called "good" cholesterol because it carries cholesterol from other parts of the body back to the liver. The liver then removes the cholesterol from the body.

LDL stands for low-density lipoprotein. It is sometimes called "bad" cholesterol because a high LDL level leads to the build-up of plaque in arteries [6].

VLDL stands for very low-density lipoprotein. Some people also call VLDL a "bad" cholesterol because it too contributes to the build-up of plaque in your arteries. But VLDL and LDL are different; VLDL mainly carries triglycerides and LDL mainly carries cholesterol.

The most common cause of high cholesterol is an unhealthy lifestyle. This can include, unhealthy eating habits, eating lots of bad fats such as one type of saturated fat which is found in some meat, dairy products, chocolate, baked goods, and deep-fried and processed foods. Another type, trans fat, is in some fried and processed foods. Eating these fats can raise your LDL (bad) cholesterol.

Lack of physical activity, with lots of sitting and little exercise. This lowers your HDL (good) cholesterol. Smoking, which lowers HDL cholesterol, especially in women. It also raises LDL cholesterol.

Genetics may also cause people to have high cholesterol. For example, familial hypercholesterolemia (FH) is an inherited form of high cholesterol [7]. Other medical conditions and certain medicines may also cause high cholesterol.

2.Materials and Methods

2.1 Collection of sample: -

The bacterial strain was isolated from home-made curd in order to get wider diversity of LAB. For selection of LAB, MRS broth was used in which 1 ml of home-made curd sample was processed under sterile conditions by suspending it in 9mL of normal saline and was vortexed for proper mixing and then the broth was incubated at room temperature for 48hrs. After 48hrs the overnight aerobically enriched sample was then diluted serially by standard serial dilution procedure and then from the last three dilution a loop-full suspension from

MRS broth was streaked on MRS-agar plates and incubated at 37°C for 48hrs and observed for the growth of colonies. [8].

2.2 Identification: -

For identification and characterization, the well isolated colonies were further tested for their physiological and biochemical characters used routinely in microbial taxonomy. [9]

2.3 Gram staining: -

Gram staining was performed for all isolated strains according to the standard procedure.

2.4 Morphological characterization: -

This was done with respect to size, shape, margin, motility, opacity, elevation, gram character, colour.

2.5 Physiological Characterization of Isolates: -

After confirming the purity of culture, the isolates were further identified by relevant biochemical tests.

2.6 Citrate Utilization Test: -

The isolate was streaked on Simmons citrate agar and incubated at 37°C for 48hrs. After incubation, the appearance of blue coloration indicated the positive test for citrate utilization and was recorded accordingly for the isolate tested.

2.7 Catalase test:-

A drop of 3% hydrogen peroxide was added to a fresh culture on a sterile glass slide and mixed well. Observation of bubbles or froth, indicated catalase-positive and no bubble or froth indicated catalase negative.

2.8 Urease test:-

Urease agar slant was prepared and well isolated colony was streaked on slant and incubated for 48 hrs at 37^0c. Positive test shows colour change from yellow to pink.

2.9 Indole test :-

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium which gives positive results such as the solution in the test tube produces cherry red or brown oily layer at the top of the broth**.**

2.10 Sugar fermentation: -

Sugar fermentation test was performed using 1% (w/v) sugar in MRS broth. Dextrose, fructose, sucrose, maltose and lactose were used in this test. Phenol red solution was used as indicator. 10 ml media was prepared and an inverted Durham's tube was inserted in each of the test tubes. Fresh culture was inoculated and incubated at 37°C for 48 hrs. Media without sugar was used as negative control. Results were observed by change of colour and gas formation.

2.11 Assimilation of Cholesterol: -

Standard Serum Cholesterol solution manufactured by coral clinical systems was used. Three different concentrations of Cholesterol of 100,200 and 300 microlitres were added in 1 ml of

bacterial suspension from MRS Broth. After adding the cholesterol, absorbance was measured immediately at 540nm.The tubes were then incubated for 48hrs. The suspensions were centrifuged in ultra-speed centrifuge for 10mins in order to separate cell biomass and to obtain clear MRS Broth supernatant. Absorbance was measured at 540nm using the supernatant after incubating it for 48hrs. [10].

3.Results

Turbidity was observed in MRS broth and colonies were observed on agar plate. The isolate obtained was considered as Lactic Acid Bacteria (LAB) by their characterization.

3.1Morphological characterization:-

Size	0.5-2.0 μm in diameter.
Shape	Spherical cocci
Colour	Milky white
Margin	Smooth
Elevation	Convex
Opacity	Transluscent
Motility	Non motile
Gram Character	Positive

3.2 Biochemical tests

Catalase Test: -

No bubbles were observed after addition of H_2O_2.Test is negative

Citrate utilization test: -

Positive test was observed as the colour changed from green to blue.

Urease test: -

Positive test was observed as the colour changed from yellow to pink .

Indole test: -

Positive result was observed as the solution in the test tube produced cherry red or brown oily layer at the top of the broth.

Sugar fermentation test: -

Dextrose	Gas production and colour change from red to yellow
Sucrose	Gas production and colour change from red to yellow
Lactose	Gas production and colour change from red to yellow
Fructose	Gas production and colour change from red to yellow
Maltose	Gas production and colour change from red to yellow

3.4 Assimilation of Cholesterol: -

Reading was taken immediately when bacteria and cholesterol were added in the broth. Controls were kept. After incubating it for 48 hrs it was centrifuged and supernatant was collected. The absorbance was taken at 540nm .

Table 1: Absorbance readings

Absorbance for 0 hr:-	Absorbance after 48 hrs:-
1. 100 μl = 0.286	**1. 100 μl = 0.099**
2. 200 μl = 0.412	**2. 200 μl = 0.209**
3. 300 μl = 0.528	**3. 300 μl = 0.322**

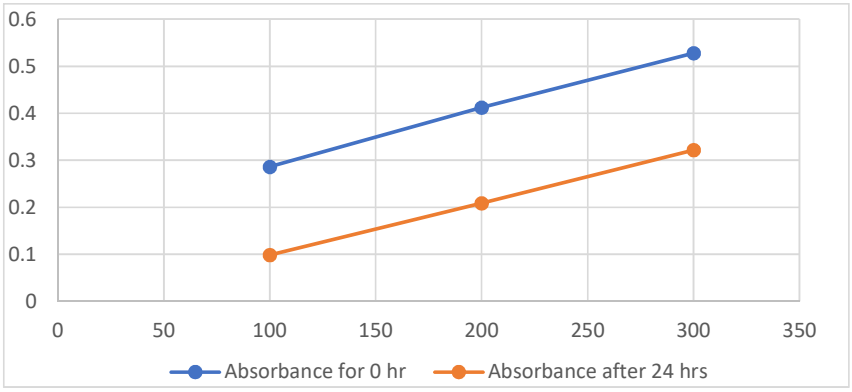

Fig1 : Comparison of 0 hour and 24 hours absorbance

Cholesterol Assimilation: -

Formula :- The cholesterol assimilated by probiotic LAB strains was determined as follows: - **cholesterol assimilated (μl in 3mL) = [cholesterol (μl in 3mL)]0 h − [cholesterol (μl in 3mL)]48 h.** [10]

Therefore,

1. For 100μl Cholesterol in 3mL = 0.286 – 0.099 = **0.187**
2. For 200 μl cholesterol in 3ml = 0.412 – 0.209 = **0.203**
3. For 300 μl cholesterol in 3ml = 0.528 – 0.322 = **0.206**

Cholesterol assimilated by LAB was also calculated in terms of percent cholesterol assimilation [10].

% cholesterol assimilated = [cholesterol assimilated (μl in 3mL) /cholesterol (μl in 3mL) 0h] × 100%.

1. For 100μl Cholesterol in 3mL = [0.187/0.286] x 100 = **65%**
2. For 200 μl cholesterol in 3ml = [0.203/0.412] x 100 = **49%**
3. For 300 μl cholesterol in 3ml = [0.206/0.528] x 100 = **39 %**

4. Discussion

The study shows that lactic acid bacteria has shown reduction in cholesterol level. The risk of developing Coronary Artery Disease (CAD), the leading cause of death, is directly associated with elevated cholesterol levels (Wei Ouyang et al., 2013). The hypocholesterolemic effects of probiotic bacteria have been linked to intrinsic bile salt hydrolase activity, cholesterol assimilation and incorporation in cellular membranes, and the production of compounds, such as Ferulic Acid (FA), that can inhibit the activity of enzymes, including 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase [10]. Cholesterol assimilation by probiotic bacteria in the gastrointestinal tract would allow for the reduction of cholesterol absorption by enterocytes and excretion of the cholesterol from the host. This would, in turn, lead to a decreased risk of developing Coronary Artery Disease (CAD). The goal of the present work was to investigate lactic acid bacteria for their ability to assimilate cholesterol from bacterial culture media. Screening for cholesterol-lowering properties, in vitro, has become an important criterion in the selection of bacterial strains for in vivo probiotic investigations. Initially, MRS bacterial culture media was supplemented with cholesterol and the lactic acid bacteria were added for 24 h of incubation. Lactic acid bacteria were shown to successfully assimilate cholesterol. The highlight of results obtained in this study is in the fact that in the vast majority of published studies show the ability of assimilation of cholesterol of about 12% to 57% of *Lb. acidophilus* [11] and our isolates of lactic acid bacteria showed the ability to assimilate cholesterol of about 39 % to 65%.

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