



# Cholesterol Degradation Activity of Probiotic Isolates from Curd

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## Abstract

Cholesterol is a waxy substance present in all types of the body cells. The presence of higher concentration of low density lipoprotein (LDL) is characterized by abnormal cholesterol level and is associated with cardiovascular diseases which lead to the development of atheroma in arteries known as atherosclerosis. Probiotics mean live microorganisms that have beneficial effects on their host's health. The medical world has long been interested in nutrient properties of curd. Curd is commonly used fermented milk product in India since time immemorial. In this study lactic acid bacteria were isolated from curd and their cholesterol degradation potential was investigated. Total seven lactic acid bacteria were isolated from the curd sample out of which three isolates showed cholesterol degradation ability. This isolates were identified conventionally. The cholesterol degradation was estimated by CHOD-POD enzyme method. The results showed that *Lactobacillus rhamnosus* exhibits maximum cholesterol degradation (84.49% decreases in cholesterol concentration). Remaining two isolates viz. *Lactobacillus acidophilus* and *Lactobacillus plantarum* showed 63.53% and 49.23% decrease in cholesterol concentration. Hence, probiotics could be consumed for the maintenance of cholesterol level which will prevent occurrence of heart diseases.

**Key words:** Probiotic Bacteria, lactic acid bacteria, *Lactobacillus*, LDL, Cholesterol degradation

## Introduction

Lactic acid bacteria have been isolated from various sources such as dairy products. The medical world has long been interested in the nutritional value of curd. Lactic acid bacteria (LAB) are usually associated with fermented dairy products such as curd. Curd is fermented milk product which is also good source of lactic acid bacteria. The curd bacteria especially LAB considered GRAS (generally regarded as safe). The free online dictionary.

Defines curd as the part of milk that curds when milk is soured or treated with enzymes; curds are used to make cheese; and/or coagulated liquid such as curd. Curd is also a cheap and easily available source (Ghosh *et al.*, 2011).

Probiotics are live bacteria that are beneficial to the health of their hosts. Doctors have long been interested in the nutritional value of curd. Curd has been traditionally used as a fermented milk product in India since ancient times. In this study, lactic acid bacteria were isolated from curds and their probiotic potential was investigated (Shaikh and Shah 2013).

Probiotics are recommended to lower cholesterol through different mechanisms, including deconjugated coprecipitation of cholesterol and bile. Binds to the cell wall, cholesterol is incorporated into the cell membrane during growth, short chain fatty acids are produced and converted to coprostanols during fermentation (García *et al.* 2012; Lee) and Salminen, 1995; Lye *et al.*, 2010a, b; Pereira and Gibson, 2002).

This plays an important role in the health of the host (Buckenhusk 1993). Probiotics have many benefits, such as facilitating the absorption of nutrients and having antibacterial and antifungal properties that affect the body's general health and metabolism (Roblain *et al.* 2006). Probiotics are often used to improve animal and human health by altering the intestinal microbiota (Fuller R. 1989).

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Probiotics have many health benefits, such as improving intestinal microflora and/or inhibiting pathogenic microorganisms, promoting immunity, preventing cancer, promoting mineral absorption by choosing to stimulate the growth of probiotics, promoting bone and bone stability, treating irritation due to diarrhea, Bowel syndrome (Bielecka, M.; *et al.*, (2002), Schley, P.D.; *et al.*, (2002), Klinder, A.; *et al.*, (2004). Scholz, Ahrens, K.E., *et al.*, (2002), Cummings, J.H. *et al.*, (2002)).

Studies examining the efficacy of probiotics in reducing cholesterol often do not sufficiently address the mechanisms by which probiotics modulate hypocholesterolemic effects and the optimum dose, frequency, and duration of treatment for different probiotic strains. The present study deals with the identification of probiotic bacteria responsible for cholesterol degradation.

## Method and Materials

### Collection of Curd Sample

Curd sample were collected from local Dairy (AnanyaDhoodh Dairy) for the isolation cholesterol degradation lactobacillus. The samples were transported to the Department of Microbiology R.A.College, Washim.

After arriving, Sample was studied for further processing. Serial dilution method was employed by using test tube. In each test tube, precisely 10ml of distilled water was measured, and subsequently, 1 ml of the respective curd sample was added to achieve the desired dilution.

### Isolation of probiotic bacteria

For the isolation of probiotic bacteria selective media, such as MRS agar media, was used to isolate bacterial strain from the samples. After thoroughly mixing with 50 ml of sterile distilled water, de Mann Rogosa Sharpe (MRS) agar and incubated at 37°C for 2 days. The isolated colonies were transferred to MRS broth and purified by streaking twice on MRS agar plate (Mahantesh *et al.*, 2010).

### Primary screening for cholesterol degradation:

Bacterial isolates were screened on M9 salt agar plates containing 0.1% cholesterol as the only carbon source. Cholesterol plates were streaked with cultures and incubated in an incubator at 37° C for 7 days. The potentiality of bacteria to utilize cholesterol was evaluated via the growth of bacteria on these plates (Mohsin Shaikh & Gaurav Shah 2013)

### Identification of cholesterol degrading probiotic bacteria

Identification of cholesterol degrading probiotic bacteria by conventional method Viz; Gram Staining, IMViC, Catalase Test, Sugar Fermentation.

### Bile Salt tolerance test

The mean intestinal bile salt concentration is believed to be 0.3% and staying time of food is suggested to be 4 h. The experiment was applied at this concentration of bile for 4 h. For this purpose active cultures were used.

Cells were harvested by centrifugation and MRS broth containing 0.3% bile salts were added to pellets. During incubation of 4 h, at every hour inoculations were carried out in to MRS broths and they were incubated at 37°C for 48 h and growth was monitored after incubation at OD620.

### Enrichment and extraction of crude enzyme

The pure sub cultured colonies from all the samples and the commercially available strain of lactobacillus were further enriched in MRS broth at 37°C for 24hrs. The bacterial cells were removed from the culture broth by centrifugation at 4000 rpm for 20 min and the cell free extract was obtained. The cell free extract contains crude enzyme that was directly utilized for measuring cholesterol degradation. The Cholesterol degradation activity was determined by CHOD-POD enzymatic method (D.V.Plummer, (2006) and www.keegad .com)

### Result and Discussion: -

#### Isolation of probiotic bacteria

Table 1 shows the findings on isolation of probiotic bacteria from curd. Total seven isolates were purified and subjected to primary screening for cholesterol degradation. The isolates were screened on M9 salt agar plates containing 0.1% cholesterol as the only carbon source. The potentiality of bacteria to utilize cholesterol was evaluated via the growth of bacteria on these plates. Out of seven different strains only three bacterial strain shows growth on M9 salt agar plate.

**Table 1: Isolation of probiotic bacteria from curd**

Bacterial Isolates	Growth response
GW1	+
GW2	-
GW3	+
GW4	-
GW5	+
GW6	-
GW7	-

+: positive, - : negative

Table 2 shows the conventional identification of cholesterol degrading probiotic isolates. The isolates were found to be *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus acidophilus*.

**Table No. 2 Identification of probiotic cholesterol degrading bacteria**

Sr. No.	Characters	Screened Isolates		
		GW1	GW3	GW5
1	Gram staining	+ve Rod	+ve Rod	+ve Rod
2	Motility	Non-Motile	Non-Motile	Non-Motile
<b>Cultural Characteristics</b>				
3	Colony color Margin Opacity Margin	White Convex Opaque Entire	White Convex Opaque rough	Grey Convex Opaque Entire
<b>Biochemical Characteristics</b>				
4	Indole	- ve	- ve	- ve
5	Methyl red	+ ve	- ve	- ve
6	Vogas –proskauer	- ve	- ve	- ve
7	Citrate utilization	-ve	- ve	- ve
	Catalase	-ve	- ve	- ve
<b>Sugar Fermentation</b>				
1	Glucose	- ve	-ve	+ve
2	Mannitol	-ve	+ve	-ve
3	Sucrose	-ve	-ve	+ve
4	Maltose	+ve	+ve	+ve
	<b>Possible species</b>	<i>Lactobacillus plantarum</i>	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus acidophilus</i>

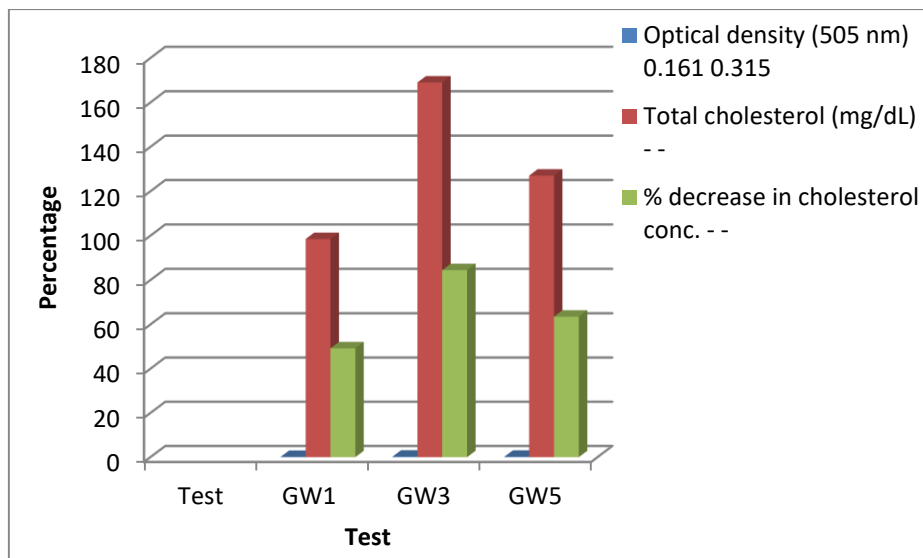
The studies on evaluating effect of cholesterol degrading bacteria on cholesterol concentration were conducted by CHOD- POD Method. The decreased level of cholesterol concentration by bacterial strain from curd sample was recorded and presented in (Table 3) and graphically presented in fig.(1).

From the results it was observed that, all the strain tested showed significant decreased in the level of cholesterol concentration out of which maximum and minimum decrease level were 84.49% and 49.23% by GW3 & GW1 isolates respectively as compared to standard cholesterol.

The GW5 isolate showed 63.53% decrease in cholesterol concentration. Hence, probiotic bacteria showed potential of cholesterol degradation.

**Table 3: Effect of cholesterol degrading bacterial isolates on cholesterol concentration**

Solution	Optical density (505 nm)	Total cholesterol (mg/dL)	% decrease in cholesterol conc.
Blank	0.161	-	-
Standard	0.3150	-	-
Test			
GW1	0.155	98.47	49.23
GW3	0.266	168.99	84.49
GW5	0.200	127.06	63.53

**Fig: 1 Effect of cholesterol degrading bacterial isolates on cholesterol concentration.**

### Conclusion

Probiotic bacteria from curd utilizes cholesterol as there carbon source. Remarkable decrease in cholesterol may enlighten the cholesterol degrading bacteria to be manifested for the development of probiotic specially to prevent the cardio artery diseases. Cholesterol degrading bacteria may be provided as probiotic supplement. However, in vivo studies in healthcare environment are recommended. Cholesterol degrading enzyme can also been isolated and characterized with respective to the development of management of coronary heart diseases.

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