



Received on 18 July, 2014; received in revised form, 26 September, 2014; accepted, 01 December, 2014; published 01 March, 2015

CHOLESTEROL LOWERING PROPERTY OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM COW MILK

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Keywords:

Lactic acid bacteria, *Lactobacillus plantarum*, Cholesterol, Milk

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ABSTRACT: The aim of this study is to investigate the cholesterol lowering property of bacterial isolates. Different cultures of the bacteria were isolated from cow milk. Out of the cultured bacterial isolates *Lactobacillus* species, *Micrococcus luteus* and *Staphylococcus aureus* were screened. Three isolates such as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei* were identified based on morphological, physiological and biochemical characteristics. Sugar fermentation test was performed to identify *Lactobacillus* species. The sugars such as D- glucose, galactose, lactose, mannitol and D-fructose were used. The five isolates were inoculated on Nutrient agar medium with cholesterol (1mg/1ml) concentration and incubated for 24 hours to check the cholesterol utilization. Cholesterol lowering activities of these organisms were checked at different concentration of cholesterol, different pH, different temperature and different incubation time. The results of this study, suggests that *Lactobacillus plantarum* was an effective organism for cholesterol utilization at pH 7, temperature at 35°C and incubation time was at 24 hrs. Hence *Lactobacillus plantarum* has been reported as cholesterol lowering agent.

INTRODUCTION: Lactic acid bacteria (LAB) are the largest group of probiotic bacteria. Among the various LAB, *Lactobacillus* plays a vital role in both medical and also in food industry¹. A *Lactobacillus* species reported to have active benefits on nutrient, improved lactose utilization, anti-carcinogenic, anti-cholesterol and protection against disease^{2,3,4}. The anti oxidative activity was reported in lactic acid bacteria⁵. Most of the Lactic acid bacteria may show antagonistic activity against pathogenic bacteria^{6,7}. Consumption of large amount of milk fermented with a wild *Lactobacillus* strains and *Bifidobacteria* showed reduction in serum cholesterol level in human^{8,9}. Some *Lactobacilli* have reported *in vivo* studies in albino rats that can lower the cholesterol and low density lipoprotein deposition^{10,11}.

Lactobacillus plantarum 837 significantly decreased the high-density cholesterol level in Ross and Broiler chickens of both sexes¹². *Lactobacillus plantarum* ATCC 14917 was investigated for cholesterol assimilation in culture media under stimulated intestinal conditions¹³. *Lactobacillus plantarum* MDL 1118, isolated from milk was shown to remove cholesterol from broths of natural hen egg yolk and skimmed milk¹⁴. Strains of *Lactobacillus fermentum* such as NCIMB 5221, NCIMB 2797¹⁵ and *Lactobacillus plantarum* ATCC 14917¹⁶ were selected for investigation into cholesterol lowering property.

Probiotic strains could assimilate cholesterol in the range of 14-22 µg/ml¹⁷ and *Bifidobacterium bifidum* MB 109 assimilated 50 µg/ml of cholesterol in MRS broth¹⁸. According to Ning Xie (2011)¹⁹ *Lactobacillus plantarum* 9-41A may play role in fat metabolism in order to showing decreases in the body weight gain, liver and fat pad weight and adipocytes size. The probiotic potential of the strains such as *Lactobacillus plantarum* was able to assimilate cholesterol by *in vitro* method²⁰. *Lactobacillus*

QUICK RESPONSE CODE	DOI: 10.13040/IJPSR.0975-8232.6(3).1184-89
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(3).1184-89	

plantarum Lp09 and Lp45 were evaluated on plasma cholesterol in Sprague-Dawley rats ²¹.

Lactobacillus plantarum has antagonistic activity, bile resistance, bile salt deconjugation, β -galactosidase activity, antibiotic resistance activity and production of biogenic amines ²². The purpose of this study was to isolate and identify *Lactobacillus plantarum* Lactic acid bacteria from cow milk and to evaluate its action on cholesterol lowering effect.

MATERIALS AND METHODS:

Isolation and Identification of bacteria from cow milk: The cow milk was collected from the North Chennai, Tamilnadu, India and stored at 5 °C. The sample was pour plated on Nutrient agar medium in order to isolate organisms. It was incubated anaerobically for 24 hours at 37 °C. Based on the morphological, physiological and biochemical characterization each isolates were identified ²³. *Lactobacillus* were identified at species level, on the basis of sugar fermentation test²⁴. The following five sugars were used for fermentation test such as D- glucose, galactose, lactose, mannitol and D-fructose. The results of sugar fermentation test were evaluated with the Bergey's manual to identify the *Lactobacilli* species. Production of gas from glucose was observed by inoculating culture into inverted Durham tubes and incubating at 37 °C for 24-48 hours.

Growth of the isolates in the presence of cholesterol: Nutrient agar media with 1mg/1ml of cholesterol concentration was prepared, five isolates were inoculated and incubated anaerobically for 24 hours at 37 °C. Nutrient media without cholesterol as control was used in this study.

Screening of the isolates for Cholesterol lowering property:

Cholesterol lowering activity at different concentration of cholesterol: 0.2, 0.4, 0.6, 0.8, 1.0 (mg/ml) different concentration of cholesterol in 10 ml broth was used. Three isolates of *Lactobacilli*, two isolates such as *Micrococcus luteus* and *Staphylococcus aureus* were inoculated and incubated at 37 °C for 24 hours. Colorimetry

reading, Optical density at 650nm were recorded and compared with positive control.

Cholesterol lowering activity at different pH level: 10 ml of Nutrient broth at different pH such as 2,4,5,7 and 8 were used. Each isolates were inoculated and incubated at 37 °C for 24 hours. Colorimetry reading, Optical density at 650nm were recorded.

Cholesterol lowering activity at different temperatures: 10 ml of Nutrient broth at different temperature such as 20 °C, 25 °C, 30 °C, 35 °C and 45 °C were used. Each isolates were inoculated and incubated for 24 hours. Colorimetry reading, was taken Optical density at 650nm.

Cholesterol lowering activity at different incubation time: 10 ml of Nutrient broth at different incubation time such as 1hr, 6hr, 12hr, 24hr and 48hr were used. Each isolates were inoculated and incubated at 37 °C. Colorimetry reading, was taken Optical density at 650nm.

RESULTS: *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Micrococcus luteus* and *Staphylococcus aureus* were isolated and identified from cow milk were tabulated in **Table 1**. *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei* were subjected to sugar fermentation test for further identifications. Gas and acid producing positive *Lactobacillus* species were identified and listed out in **Table 2 and Figure 1**. These five isolates were assessed for cholesterol lowering property. Nutrient agar with cholesterol at 1mg/1ml concentration plates when inoculated with five isolates, cholesterol utilizing organism grown very well. Out of five isolates *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Micrococcus luteus* were shown maximum growth compared to *Lactobacillus casei* and *Staphylococcus aureus* were shown in **Figure 2**.

Lactobacillus plantarum grown maximum at (1g/1ml) concentration of cholesterol as shown in **Figure 3**. At pH 7 maximum absorbance was observed in *Lactobacillus plantarum* as shown in **Figure 4**. At 35°C for 24 hours of incubation period *Lactobacillus plantarum* gave maximum

absorbance at 650 nm that shows maximum growth of the organism (**Figure 5 & 6**). The results of present study suggest that the *Lactobacillus plantarum* was able to utilize cholesterol at 1g/1ml concentration of cholesterol at pH7 temperature at 35°C and incubation period was at 24 hours.

TABLE 1: MORPHOLOGICAL, PHYSIOLOGICAL CHARACTERIZATION OF THE ISOLATES

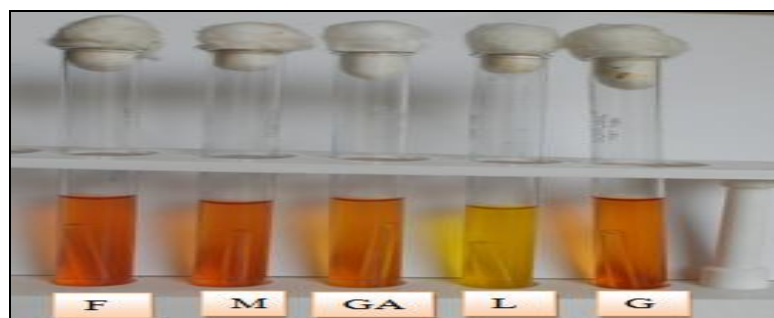
Characteristics	isolates 1	isolates 2	isolates 3	isolates 4	isolates 5
Morphological characteristics					
Colour	Pale white	Cream white	Off white	Yellow	Yellow
Shape	Rods	Rods	Rods	Cocci	Cocci
Gram Stain	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Physiological characteristics					
pH optimum	5.5-6.5	5.5-6.5	5.5-6.5	7-12	4.2-9.3
Temperature	30-37 °C	30-37 °C	30-37 °C	35-80 °C	6-48 °C
Catalase Activity	-	-	-	+	+
Oxidase Test	+	-	+	+	-
Indole Production	-	-	-	-	-
Urease	-	-	-	+	-
Motility test	-	-	-	-	-
Nitrate Reduction	-	-	-	-	+
H ₂ S Formation	-	-	-	-	-
Identified species	<i>Lactobacillus plantarum</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus casei</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus aureus</i>

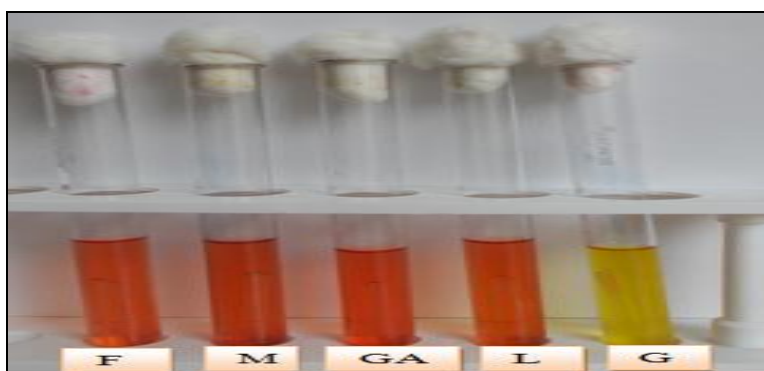
(+) Positive, (-) Negative

TABLE 2: CARBOHYDRATE FERMENTATION PATTERN

Substrate	<i>Lactobacillus plantarum</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus casei</i>
D-fructose	+A	+A	+A
Mannitol	+A,G	+A	+A
Galactose	+A,G	+A	+A
Lactose	+A	-	+A
Glucose	+A	+A	-

(+) Positive reaction, (A) acid, (G) gas

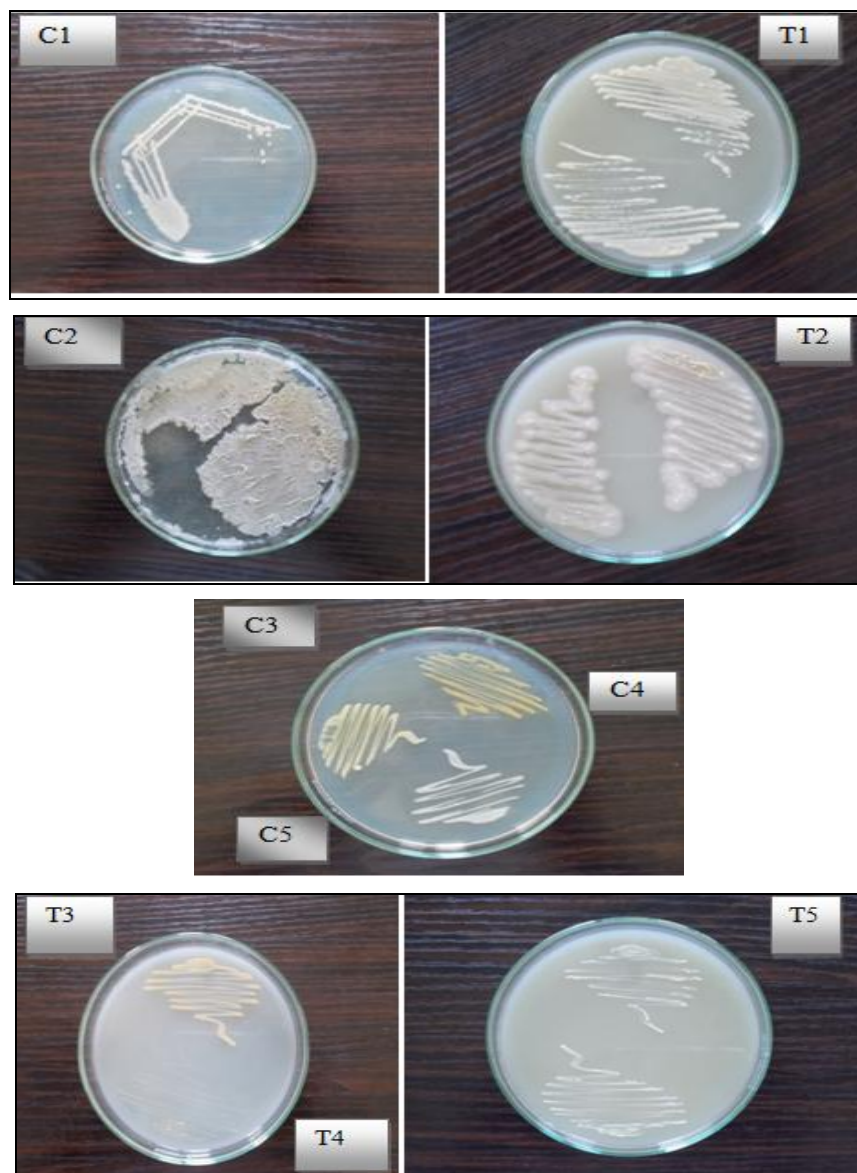
**LACTOBACILLUS PLANTARUM****LACTOBACILLUS FERMENTUM**



LACTOBACILLUS CASEI

F, D-fructose; M, Mannitol; GA, Galactose; L, Lactose; G, D-glucose

FIGURE1: CARBOHYDRATE FERMENTATION TEST FOR SPECIES IDENTIFICATION



C- Control plate containing medium without cholesterol C1- *Lactobacillus plantarum* , C2- *Lactobacillus fermentum* , C3- *Micrococcus luteus*, C4- *Staphylococcus aureus* , and C5- *Lactobacillus casei*;
 T- Test plate containing medium with cholesterol T1- *Lactobacillus plantarum* , T2- *Lactobacillus fermentum* , T3- *Micrococcus luteus*, T4- *Staphylococcus aureus* , and T5- *Lactobacillus casei*;

FIGURE 2: GROWTH OF ISOLATES ON THE NUTRIENT MEDIUM CONTAINING WITH AND WITHOUT THE PRESENCE (CONTROL) OF CHOLESTEROL

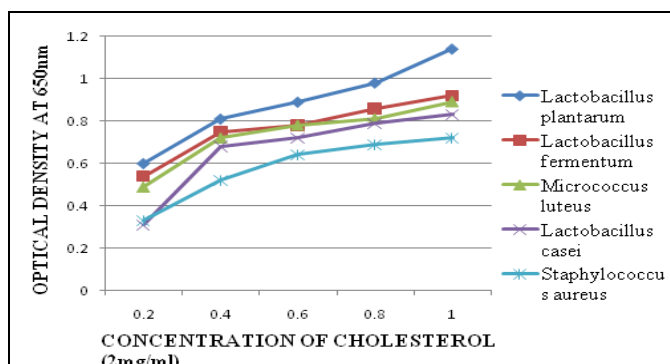


FIGURE 3: CHOLESTEROL LOWERING ACTIVITY AT DIFFERENT CONCENTRATION OF CHOLESTEROL

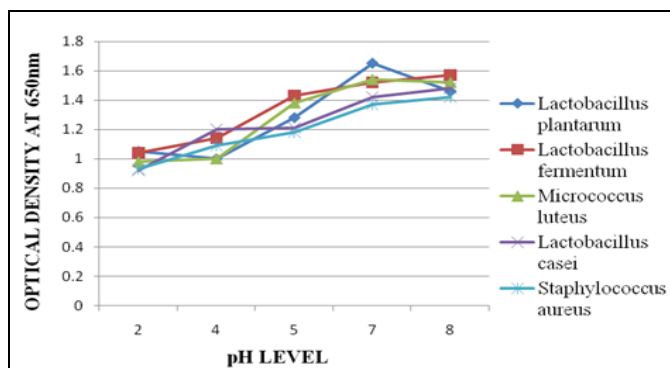


FIGURE 4: CHOLESTEROL LOWERING ACTIVITY AT DIFFERENT PH LEVEL

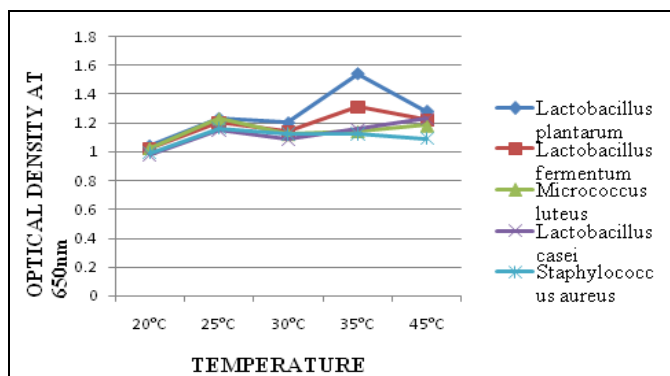


FIGURE 5: CHOLESTEROL LOWERING ACTIVITY AT DIFFERENT TEMPERATURES

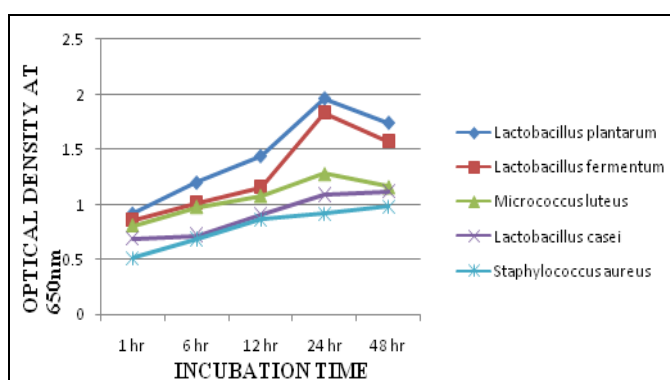


FIGURE 6: CHOLESTEROL LOWERING ACTIVITY AT DIFFERENT INCUBATION TIME

DISCUSSION: Five isolates such as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Micrococcus luteus* and *Staphylococcus aureus* were isolated from cow milk. Bergey's Manual was used to identify five isolates. Dora *et al.* (2002)²⁵ reported cholesterol assimilation of lactic acid bacteria. Nguyen *et al.* (2007)²⁶ evaluated *Lactobacillus plantarum* as potential probiotic with cholesterol lowering effect. Sasithorn Sirilun *et al.* (2010)²⁷ selected four strains of *Lactobacillus plantarum* showing probiotic potential in cholesterol lowering property. Kuda *et al.* (2013)²⁸ reported *Lactobacillus plantarum* can be used as a profitable starter organism and probiotic. In my present study *Lactobacillus plantarum* shown higher utilization of cholesterol from the medium. At different concentration of cholesterol at different pH, temperature, incubation time *Lactobacillus plantarum* proved its stability in utilization of cholesterol. 1g/1ml concentration of cholesterol was observed by *Lactobacillus plantarum* for its optimal growth at pH7, temperature 35 °C and incubation time at 24 hours.

CONCLUSIONS: *Lactobacillus plantarum* was able to utilize cholesterol from the culture medium at optimum pH, temperature, incubation time and different cholesterol concentration. *Lactobacillus plantarum* has optimum absorbance of cholesterol was about 1g/1ml hence this has been reported as cholesterol lowering agent.

ACKNOWLEDGEMENT: This research was supported by Vels University Research grant.

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How to cite this article:

Amutha K and Kokila V: Cholesterol Lowering Property of *Lactobacillus Plantarum* Isolated from Cow Milk. Int J Pharm Sci Res 2015; 6(3): 1184-89. doi: 10.13040/IJPSR.0975-8232.6(3).1184-89.

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