Research Article

Cholesterol Degrading Potentiality of the Bacterial Isolates from Ghee

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ABSTRACT

The aim of the present study was to investigate the cholesterol degrading potentiality of the bacterial isolates from ghee. The bacterial strains were isolated by using five different media such as Nutrient agar, MacConkey agar, Mannitol salt agar, Eosin Methylene blue agar, Pseudomonas agar base and Lactobacillus MRS. The six isolates were isolated and tested for cholesterol degradation potentiality by plate assay method using cholesterol at different concentration. The cholesterol degrading bacteria were cultured in different concentrations of cholesterol, pH, temperature and incubation period to optimize the growth. The bacterial isolates such as G1, G5 and G6 were utilized cholesterol and grown well in medium containing cholesterol at 1 mg/ml. In the optimization study, G6 isolate was found to utilize 1.0mg/ml concentration of cholesterol at pH7, temperature at 35°C and 24 hrs incubation period. G6 isolate was able to utilize maximum quantity of cholesterol from the medium. The molecular identification of the isolates was confirmed the organism was *Bacillus subtilis* strain KAVK3 and the partial sequence was submitted to NCBI (Accession No: KP792774). Hence, *Bacillus subtilis* strain KAVK3has been reported as cholesterol degrading agent.

Keywords: Cholesterol, pH, Temperature, Incubation period and Ghee.

INTRODUCTION

Cholesterol is a vital substance in the human body. Excess of cholesterol may lead to atherosclerosis and may cause a major risk for developing cardiovascular diseases and colon cancer[1]. The cholesterol levels could be decreased using beneficial microorganisms.

The ability of bacteria to degrade cholesterol by members of the genera *Arthrobacter, Brevibacterium, Corenybacterium, Mycobacterium, Streptomyces, Rhodococcus, Pseudomonas, Comamonas, Burkholderia, Chromobacterium and Shizophyllum*[2-6]. Several researches indicated that consumption of dairy products with certain bacterial cultures could lower total plasma cholesterol and low-density lipoprotein cholesterol[1,7].

In human, consumption of fermented milk containing a Lactobacillus strain was reported to have a hypocholesterolemic (cholesterol-lowering) effect[8]. The dairy products fermented with the appropriate strains of bacteria might induce a decrease in the level of circulating cholesterol concentration[9].

Many experiments have been reported that *in vitro* and *in vivo* studies were to investigate the hypocholesterolemic effect of Lactic acid bacteria, especially strains of *Lactobacillus* and *Bifidobacterium*[10,11,1]. The degradation of cholesterol by a member of the genus *Bacillus* sp. has been reported recently.

The *Bacillus* genus possess various biological characters includes gut viability, resistance to bile and acid, production of metabolites such as antimicrobial compounds, enzymes, essential amino acids and vitamins. The other health benefits of *Bacillus* include decrease in the level of cholesterol in blood[12]. The purpose of this study was to isolate the bacteria from ghee and to evaluate its action on cholesterol degrading effect.

MATERIALS AND METHODS

Isolation and Identification of the Bacteria

The ghee sample was collected from the North Chennai, Tamil Nadu, India and stored at 4°C. It was spread plated using five different media such as Nutrient agar, MacConkey agar, Mannitol salt agar, Eosin Methylene blue agar, Pseudomonas agar base and Lactobacillus MRS agar and incubate at 37°C for 24 hours.

The six bacterial cultures were isolated and identified based on physical and biochemical characteristics[13].

Growth of the Bacterial Isolates in the Presence of Cholesterol

Nutrient medium containing cholesterol at various concentrations such as 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, 1.0mg/ml were prepared and the six isolates were inoculated and incubated at 37°C for 24 hours.

Nutrient medium without cholesterol was used as control plate.

The cholesterol degraded isolates at all the concentration were selected for optimization study.

Optimization for the Utilization of Cholesterol at Various Parameters

Optimization of G1, G5 and G6 bacterial isolates from ghee were optimized at various parameters such as different concentration of cholesterol, pH, temperature and incubation period. The cholesterol degrading ability

was checked by colorimetric reading, Optical density at 650nm.

The cholesterol degrading activity was calculated as follows:

$$Cholesterol\ Degrading\ Activity\ (OD) = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test}{Absorbance\ of\ Standard}$$

Cholesterol Degrading Activity at Different Concentration of Cholesterol

The cholesterol at different concentrations such as 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, 1.0mg/ml in 10 ml nutrient broth were prepared and a loop full of selected G1, G5 and G6 isolates were inoculated and incubated at 37°C for 24 hours.

The cholesterol degrading ability was observed by colorimetric reading, Optical density at 650nm.

Cholesterol Degrading Activity at Different pH

The cholesterol degrading activity was checked at different pH such as 2, 4, 5, 7 and 8 were used in 10 ml of nutrient broth containing cholesterol (1mg/ml).

A loop full of bacterial isolates such as G1, G5 and G6 were inoculated and incubated at 37°C for 24 hours.

The cholesterol degrading ability was measured by colorimetric reading, optical density at 650nm.

Cholesterol Degrading Activity at Different Temperature

The cholesterol degrading activity at different temperature such as 20°C, 25°C, 30°C, 35°C and 45°C were maintained in 10 ml nutrient broth containing cholesterol (1mg/ml).

A loop full of G1, G5 and G6 bacterial isolates were inoculated and incubated at respective temperature for 24 hours.

The cholesterol degrading ability at different temperature were recorded, optical density at 650nm.

Cholesterol Degrading Activity at Different Incubation Time

Different incubation time such as 1hr, 6hr, 12hr, 24 hr and 48hr were used to check the cholesterol degrading ability in 10 ml nutrient broth containing cholesterol (1mg/ml).

A loop full of G1, G5 and G6bacterial isolates were inoculated and incubated at 37°C for 24 hours. Colorimetric reading, optical density at 650nm was measured.

RESULTS AND DICUSSION

The bacterial isolates from Ghee were assessed for cholesterol degrading potentiality. Nutrient agar, MacConkey agar, Mannitol salt agar, Eosin Methylene blue agar, Pseudomonas agar base and Lactobacillus MRS agar were used to isolate bacteria (Figure 1 and Table 1).

The isolated bacteria were named as G1, G2, G3, G4, G5 and G6. The isolated bacteria were identified based on physical and biochemical characteristics (Table 2).

The administration of probiotic and prebiotic are effective in improving lipid profiles such as the reduction of serum total cholesterol, triglycerides, and low density lipoprotein[14,15].

Cholesterol degrading activities of the isolated bacteria were carried out in the nutrient agar medium containing cholesterol at different concentration (0.2mg/ml; 0.4 mg/ml; 0.6 mg/ml; 0.8 mg/ml; 1.0 mg/ml) (Figure 2).

According to Wang[1], during bacterial growth the cells can remove cholesterol from media both by binding of cholesterol to living cells and by uptake of cholesterol into living cells.

The isolate G1, G5 and G6 were able to utilize cholesterol at all the concentration and grown well in the nutrient medium were selected for further optimization study.

The optimizations at various parameters such as different concentration of cholesterol, pH, temperature and incubation period were used to check the isolates for cholesterol degrading potentiality.

G6 isolate was utilized cholesterol at 1mg/ml concentration as shown in Figure 3. At pH 7 maximum absorbance of cholesterol was observed for the isolate G6 as shown in Figure 4.

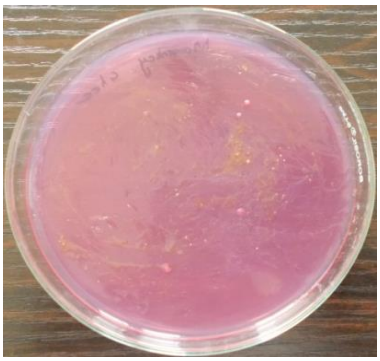
Temperature at 35°C and the incubation period was at 24 hrs were showed the maximum absorbance of cholesterol of the isolate G6 (Figure 5&6). The molecular identification of G6 isolate 16S rRNA partial sequence was confirmed the isolate was *Bacillus subtilis* strain KAVK3 and the sequence was submitted to NCBI (Accession No: KP792774).

During fermentation of flatfish, *Bacillus subtilis* SFF34 degraded cholesterol[16]. The result of the study proved that *Bacillus subtilis* strain KAVK3 was utilized cholesterol from the culture medium and it has been reported as cholesterol degrading agent.

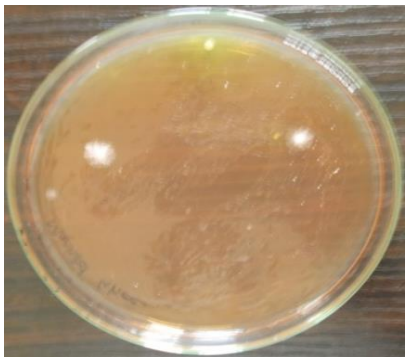
Table 1: List of Bacterial Isolates isolated from Different Medium

Number of Bacterial Isolates		
S. No.	**Media**	**Ghee**
1.	Lactobacillus MRS Agar	2
2.	MacConkey Agar	-
3.	Mannitol Salt Agar	1
4.	Eosin Methylene Blue Agar	1
5.	Pseudomonas Agar Base	1
6.	Nutrient Agar	1
Total number of bacterial isolates		6

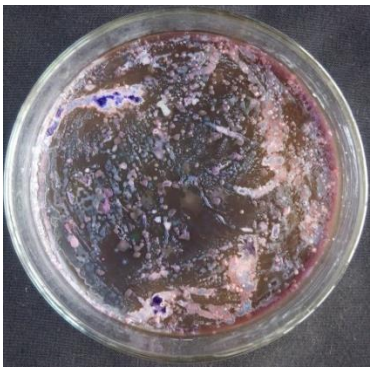
Lactobacillus MRS Agar



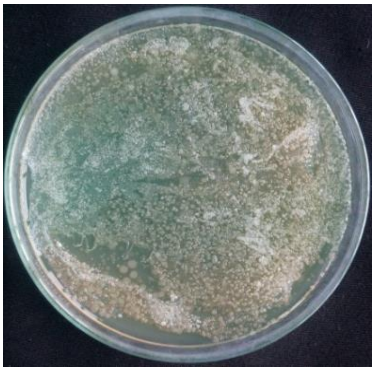
MacConkey Agar Media



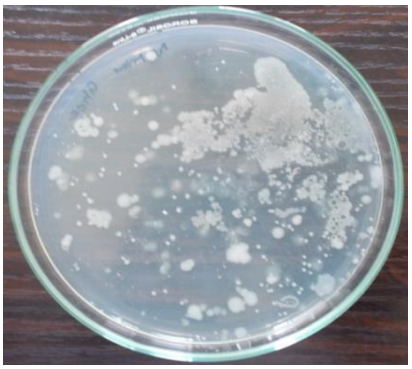
Mannitol Salt Agar



Eosin Methylene Blue Agar



Pseudomonas Agar Base



Nutrient Agar Media

Figure 1: Different Media used for the Isolation of Bacteria from Ghee Sample

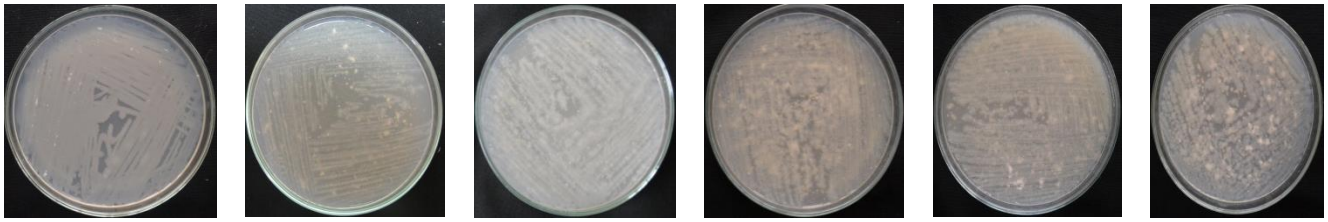
Table 2: Identification of Bacterial Isolates isolated from Ghee Sample

S. No.	Cultural Characteristics	G1	G2	G3	G4	G5	G6
1.	Colony Morphology	White to light yellow, smooth, round colonies	Yellow, circular, convex with entire margin	Pale colour, shiny, mucoid colonies which have entire margin	Green, mucoid colonies umbonate elevation	White, mucilaginous, irregular, round shape with flat elevation	Dry, flat, irregular, pale white colonies with lobate margin
2.	Gram's Staining	Gram Positive	Gram Positive	Gram Negative	Gram Negative	Gram Positive	Gram Positive
3.	Catalase Test	-	+	+	+	+	+
4.	Oxidase Test	-	+	-	+	+	-
5.	Indole Test	-	-	+	-	+	-
6.	Methyl Red Test	-	-	+	-	-	-
7.	Voges-Proskauer Test	-	+	-	-	-	+
8.	Citrate Utilization Test	-	+	-	+	+	
9.	Identification of the Organism	*Lactobacillus sp.*	*Micrococcus sp.*	*Escherichia coli*	*Pseudomonas sp.*	*Bacillus sp.*	*Bacillus sp.*

+ Positive, - Negative

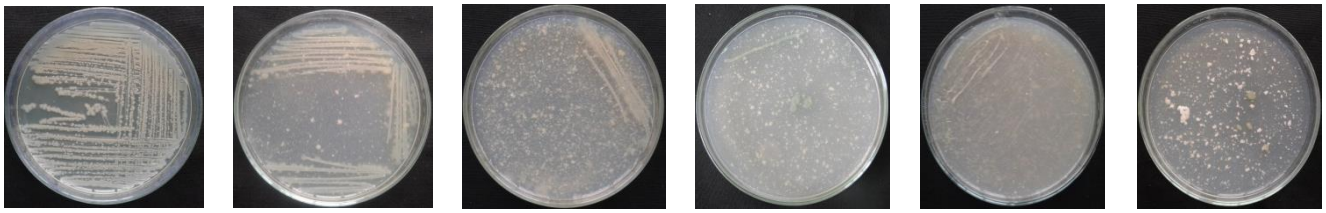
G1 - Isolate 1 from Lactobacillus MRS Agar Medium; G2 - Isolate 2 from Mannitol Salt Agar Medium
G3 - Isolate 3 from Eosin Methylene Blue Agar Medium; G4 - Isolate 4 from Pseudomonas Agar Base Medium
G5 - Isolate 5 from Nutrient Agar Medium; G6 - Isolate 5 from Nutrient Agar Medium

ISOLATE - G1



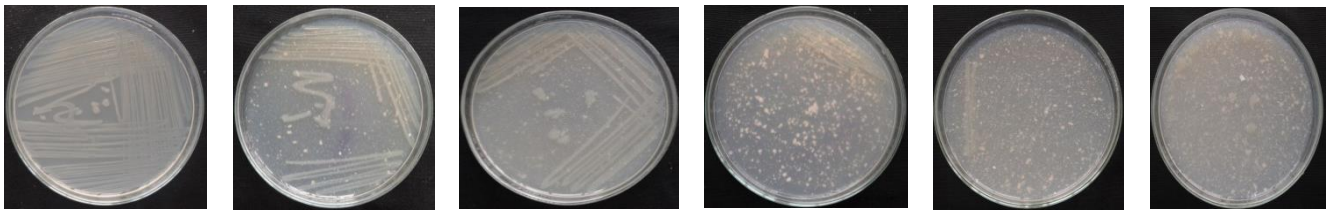
Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

ISOLATE - G2



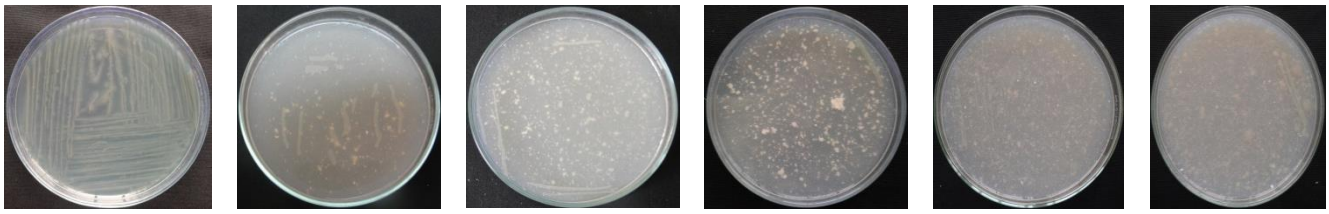
Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

ISOLATE - G3



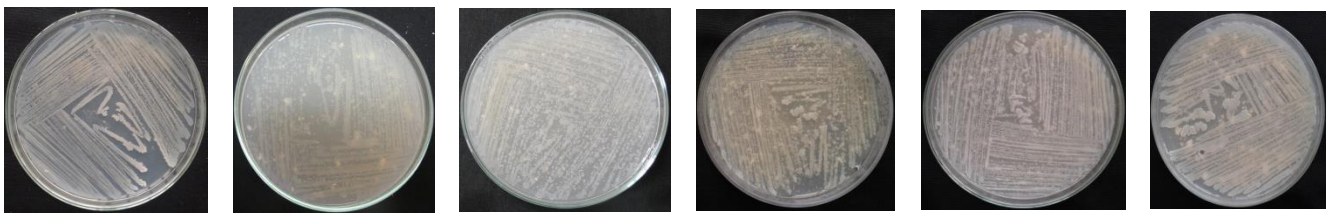
Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

ISOLATE - G4



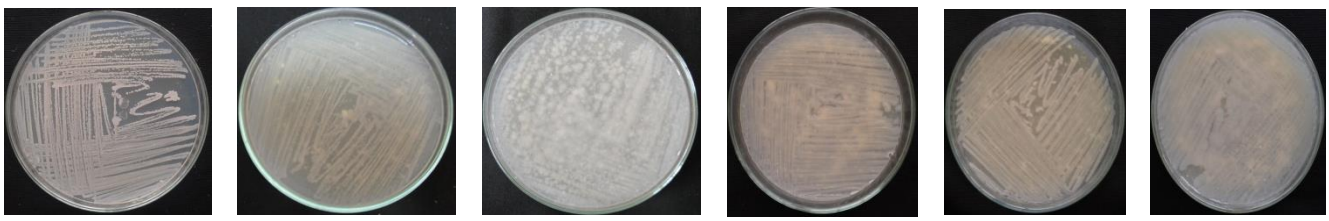
Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

ISOLATE - G5



Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

ISOLATE - G6



Control 0.2 mg/ml 0.4 mg/ml 0.6 mg/ml 0.8 mg/ml 1.0 mg/ml

Figure 2: Screening of the cholesterol degrading bacteria isolated from ghee sample by plate assay method

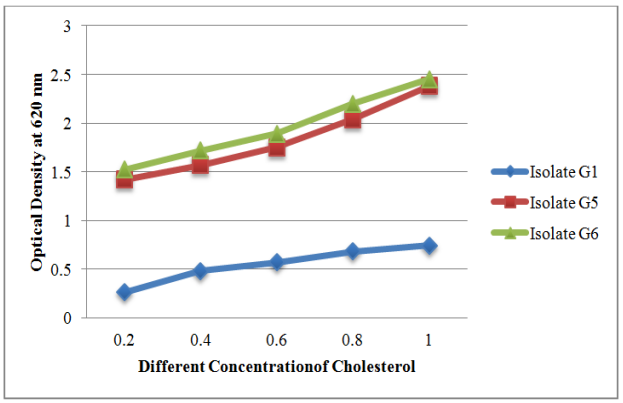


Figure 3: Cholesterol degrading activity of the isolates at different concentration of cholesterol

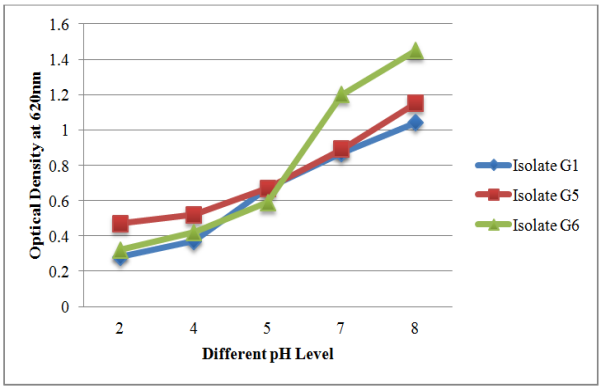


Figure 4: Cholesterol degrading activity of the isolates at different pH level

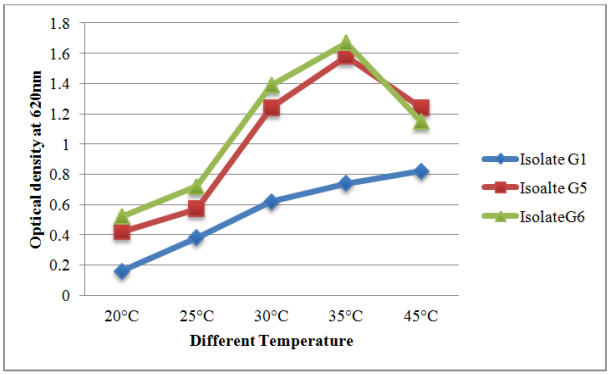


Figure 5: Cholesterol degrading activity of the isolates at different temperature

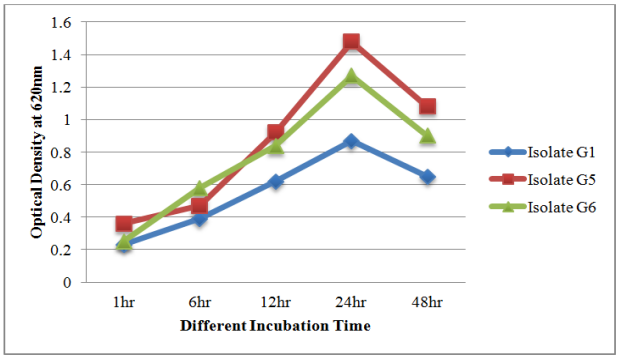


Figure 6: Cholesterol degrading activity of the isolates at different incubation time

CONCLUSION

Bacterial isolate from ghee were checked for cholesterol lowering potentiality.

Among the six isolates, *Bacillus subtilis* strain KAVK3 (G6) isolate was able to utilize cholesterol from the culture medium at various optimized parameters.

Hence we suggest that *Bacillus subtilis* strain KAVK3 has cholesterol degrading potentiality.

REFERENCES

1. WangJ, ZhangH, ChenX, ChenY and BaoQ. Selection ofpotential probiotic Lactobacilli for cholesterol-lowering propertiesand their effect on cholesterol metabolism in rats fed a high lipid diet. *J. Dairy Sci*. 95(4), 2012, 1645-1654.
2. Fernandez de las Heras L. ChoG is the main inducible extracellular cholesterol oxidase of *Rhodococcus ruber* strain CECT3014. *Microbiol. Res*. 166, 2011, 403-418.
3. Doukyu N. Characteristics and biotechnological applications of microbial cholesterol oxidae. *Appl. Microbiol. Biotechnol*. 83, 2009, 825-837.
4. Pawar K and Bhatt M. Accumulation of pharmacologically important 17- ketosteroid during side chain cleavage of cholesterol by *Pseudomonas putida* MTCC 1259. *World J Sci Tech*. 1(5), 2011, 62-65.
5. Srisawasdi P, Jearanaikoon P, Wetprasit N. Application of *Streptomyces* and *Brevibacterium* cholesterol oxidase for total serum cholesterol assay by the enzymatic kinetic method. *Clin. Chim. Acta*. 372, 2006, 103-111.
6. Wang C, Cao Y, Sun B. Preparation and some properties of cholesterol oxidase from *Rhodococcus sp.* R142. *World J. Microbiol. Biotechnol*. 24, 2008, 2149-2157.
7. Wang CY, Wu SJ, Fang JY, Wang YP and Shyu YT. Cardiovascular and intestinal protection of cereal pastes fermented with lactic acid bacteria in hyper lipidemic hamsters. *Food Res Int*, 48, 2012b, 428-434.
8. Mann GV and Spoerry A. Studies of a surfactant and cholesteremia in the Maasai. *Am. J. Clin. Nutr*. 27(5), 1974, 464–469.
9. Pereira DIA and Gibson GR. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Crit. Rev. Biochem. Mol. Biol*. 37(4), 2002, 259-281.
10. Pan DD, Zeng XQ and Yan YT. Characterisation of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *J. Sci. Food Agri*. 91(3), 2011, 512-518.
11. Oner O, Aslim B and Aydas SB. Mechanisms of cholesterol lowering effects of *Lactobacilli* and *Bifidobacteria* strains as potential probiotics with their *bsh* gene analysis. *J. Mol. Microbiol. Biotech*. 24(1), 2014, 12-18.
12. Kim KP, Rhee CH, Park HD. Degradation of cholesterol by *Bacillus subtilis* SFF34 isolated from Korean traditional fermented flatfish. *Lett. Appl. Microbiol*. 35, 2002, 468-472.
13. Williams and Wilkins., 9th Edition. Bergey's Manual of determinative bacteriology, 1994.
14. Watanabe S, Katsube T, Hattori H, Sato H, Ishijima T, Nakai Y. Effect of *Lactobacillus brevis* isolated from Tsudakabu red turnips on cholesterol levels in cholesterol administered rats. *J.Biosci.Bioeng*. 116, 2013, 45-51.
15. Ooi LG, Ahmad R, Yuen KH, Liong MT. *Lactobacillus acidophilus* CHO-220 and inulin reduced plasma total cholesterol and low density lipoprotein cholesterol via alteration of lipid transporters. *J. Dairy Sci*. 93, 2010, 5048-5088.
16. Kwan-Pil Kim, In-Koo Rhee and Heui-Dong Park. Degradation of cholesterol by *Bacillus subtilis* SFF34 in flatfish during fermentation. *J.Microbiol*. 41(4), 2003, 284-288.

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