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Exopolysaccharide production and cholesterol reduction by Lactic Acid Bacteria isolated from traditionally fermented Indian foods

Gosavi Jyotsna, Shingade Sujata, Walhe Rajan*

Department of Microbiology, MES Abasaheb Garware Collage, Pune, MS, India
*Corresponding author: Walhe Rajan, Associate Professor and Head, Department of Microbiology, MES' Abasaheb Garware collage , Pune (India). E-mail: rawalhe@rediffmail.com

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ABSTRACT

Excess cholesterol is a major risk factor for the development of hypercholesterolemia, cardiovascular heart disease (CVD), colon cancer, etc. There is a necessity of easy way to reduce serum cholesterol concentration. It can be deal by probiotics, prebiotics and synbiotics therapies. LAB are present in fermented foods and may have probiotic properties such as cholesterol reduction and exopolysaccharides (EPS) production. In the present study, we isolated a total of 20 isolates of LAB from diverse traditionally fermented Indian foods. The LAB were morphotyped and primary tests for probiotics done. All 20 isolates were found to be non-hemolytic and able to tolerate acid at pH 2.0. Six isolates were able to tolerate bile at 0.3%. Selected isolates were screened for cholesterol reduction property by testing BSH activity on MRS agar and quantitative estimation of cholesterol in growth medium was determined by enzymatic method. Five isolates exhibited cholesterol reduction activity which is in the range of 35 to 70%. Isolates were also screened for exopolysaccharide (EPS) production which can be considered as beneficial characteristics. Thus, the isolated organisms can be considered as a putative probiotic candidates having cholesterol reduction activity.

Keywords: Fermented Foods, LAB, Cholesterol reduction, Probiotic, Exopolysaccharide

INTRODUCTION

Humans or animals consume food as nutritious substance to maintain life and growth. In many fermented foods lactic acid bacteria (LAB) mostly species of *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Weissella,* etc. are generally present (Tamang,2016). Along with commonly used microorganisms, LAB are considered as main categories of bacteria that are safe to use as starter cultures in fermented foods. Moreover, they are nonpathogenic, acid/ bile tolerant and suitable for industrial processes (Shehata, 2016). There are numerous LAB residing hut and benefiting host in the similar way as probiotics (Guan, 2017).

Probiotic is defined as, "live microorganisms that when administered in adequate amount confer a health benefit on the host" (Abedfar, 2016). Many species of *Lactobacillus,Bifidobacterium* and other genera mainly employ as probiotics. There is study report on LAB species that are able to synthesize exopolysaccharides (EPS) which are safe and benefit the host in many ways (Abedfar*,* 2016).

Elevated blood cholesterol lead to colon cancer and various heart related diseases including coronary heart disease, hypercholesterolemia causes death or disability in many countries (Wang*,* 2014). LAB can be used as cholesterol- lowering agent (Kim et al 2008). some mechanisms have been proposed to elucidate the cholesterol reducing activities associated with LAB including assimilation of cholesterol during growth, incorporation of cholesterol into the membrane of cells and binding of cholesterol to the cell surface, bile salt hydrolase activity (Kim ,2008). In the gastrointestinal tract cholesterol assimilation by probiotic bacteria permit the reduction of cholesterol absorption by enterocytes and excretion of the cholesterol from the host (Tomaro*.,*2014). Some strains of *Lactobacillus acidophilus* have bile salt hydrolase (BSH) activity which hydrolyzes conjugated bile salts and produces free bile salts and related amino acids that results diminished abortion from intestine as they (free bile salts) are lees soluble (Kim et al., 2008). LAB such as species of *Lactobacillus Bifidobacterium* possesses ability to metabolize cholesterol and thus reduce cholesterol level in human (Lim 2004). LAB shows prebiotic property due to ability of production of exopolysaccharides, moreover which have cholesterol lowering capability (Patel*,* 2012). There is a correlation between EPS production capacity and cholesterol removal. LAB which produce considerable amount of exopolysaccharides (EPS) have ability to reduce cholesterol (Tok*,* 2010).

In the present study various traditionally fermented foods were used to isolate LAB with primary probiotic characteristics and attempt was made to check their beneficial activities like cholesterol reduction and EPS production.

MATERIALS AND METHODS

Isolation of LAB from traditionally fermented Indian food samples:
Lactic acid bacteria were isolated from different traditionally fermented Indian food (or / and batter) samples (*idali, dhokala, amboli, chakuli, kanji,* Kimchi*, sel roti,* etc.).

Isolation of LAB:
Fermented Indian food Samples mixed with sterile distilled water, diluted and steaked on sterile de Man Rogos Sharpe (MRS) agar plate, plates were incubated at 37ºC for 24-48 hours under microaerophilic condition. The Gram staining of isolates was performed. Only Gram positive and catalase negative isolates were maintained as glycerol stocks and used for further studies.

Screening of preliminary primary probiotic characterization: The isolates were characterized for their primary probiotic potentials on the basis of their primary probiotic characters.

Hemlolytic activity Test: The test was done as per Hosseini (2009) with some changes. Saline washed fresh growth of culture isolates were spot inoculated onto the sterile blood agar (MRS agar with 5% blood) plate and incubated under microaerophilic condition at 37ºC for 24-48 hours. Isolates not showing zone of hemolysis were selected for further probiotic characterization

Acid/ Bili tolerance: Acid and bile tolerance test was performed as per Jamaly, 2011 with modifications. Bacterial cells from MRS cultures were collected by centrifugation. Washed (PBS) bacterial cells were resuspended in buffers of pH 2.0, pH 3.0 and pH 4.0; incubated at 37ºC for various time intervals. After centrifugation, bacterial cells resuspended in PBS, washed and inoculated in fresh medium. The results were checked after incubation, positive results indicated by turbidity and colour change. The acid tolerant isolates were selected for further studies.

The sterile MRS broths at concentration of 0.1%, 0.2%, 0.3% bile salt (sodium taurocholate) were inoculated with fresh growth of isolates and incubated under microaerophilic condition at 37ºC for 24-48 hours. The isolates were checked for turbidity and color change.

Biochemical / physiological properties for probiotics:

Homo or Hetero fermentation test: The fresh growth of isolates were inoculated in sterile MRS broth tubes with inverted Durham's tube and incubated under microaerophilic condition at 37ºC for 24-48 hours. The isolated tubes were checked for acid and gas production (Nikita, 2012).

Catalase test/Oxidase test:Test was perform as per Cruickshank ,1975. Isolates were inoculated in sterile MRS broth for fresh growth and incubated under microaerophilic condition at 37ºC for 24-48 hours. With a glass pipette or droper, slide a few drops of 3% hydrogen peroxide along the inner walls of the test tube onto the growth on the agar slant tube. Tubes were checked for effervesces of H_2O_2 to check catalase activity. For oxidase, a loopful of fresh growth was taken on a strip of Whatman No. 2 filter from the sterile MRS slant of isolates tubes. When placed freshly prepared several drops of oxidative reagent on it, the positive test showed colonies colour changed to purple colour.

Screening of isolates for their cholesterol reduction activity:

Bile salt hydrolases (BSH) activity: (Qualitative test):The fresh growth of isolates were inoculated on sterile BSH agar (Sterile MRS agar with 0.5% bile salt and CaCl2) plates in triplicate and plates were incubated under microaerophilic condition at 37ºC for 24-48 hours. After incubation, plates were checked for zone of precipitation zone diameter noted as average of three with SD (Shehata , 2016).

Quantitative evaluation of cholesterol reduction: The isolates were checked for their cholesterol reduction activity by using cholesterol (Delta cholesterol kit). The fresh growth of isolates were inoculated in sterile MRS broth containing filtered sterilized cholesterol and incubated under microaerophilic condition at 37ºC for 24-48 hours. Followed by centrifugation, the assay of cholesterol reduction activity was performed by using delta cholesterol kit. Average of three readings with SD noted. The enzymatic method followed as per Wu CC (2015) with modification.

Screening of Exopolysaccharide (EPS) producing LAB strains: The fresh growth of isolates were inoculated in sterile ESM (Exopolysaccharide selective media) and incubated under microaerophilic condition at 37ºC for 24-48 hours. Isolates were checked for viscosity of medium (Ruas and Gavilan, 2005).

RESULTS AND DISCUSSION

A total of 20 LAB were isolated from traditionally fermented Indian foods. All 20 isolates were screened for primary probiotic properties after morphotyping (all were found to be gram positive rods or cocci, results not shown here) and nonhemolytic and hence can be considered as nonpathogenic.

All 20 isolates tolerated acidic pH 2.0, exposed for time interval up to 120 minutes. Bile tolerance characteristics of organisms were tested for these isolates. Six out of 20 isolates tolerate bile at the concentration of 0.3% bile (Table 1). So these results were similar with Tokat et al 2015 as tolerance to gastric acidity (pH around 2.5) and high intestinal bile concentration (around 0.3%) was tolerated.

Thus these isolate can tolerate the gastrointestinal conditions of acid (pH aroud 2.0) and bile concentration upto 0.3%. These isolates were selected for their cholesterol reduction activity and EPS production.

Biochemical tests were performed for 6 isolates. Five out of 6 isolates were catalase, oxidase negative and showed homofermentative mode of fermentation and were considered as Lactic Acid Bacteria. Five isolates were short-listed for cholesterol reduction activity and EPS production activity. Qualitative estimation of cholesterol reduction activity of LAB was determined by using bile salt hydrolase test (Figure 1). All isolates were showed zone of precipitation on sterile BSH test agar plate. In quantification of cholesterol reduction, range seen was from 35 (by isolate AV2) to 75% (by isolate GF -1); which is higher as compared (45 to 55% reduction) to the study reported on LAB from dairy products by Albano et al., 2018.

Four out of five short-listed isolates were showed EPS production activity seen by change in viscosity of medium as compare to control (Figure 3.). As Tok and Aslim, 2010 have stated that EPS of LAB have cholesterol reducing property; our isolate could have that potential as well.

Table 1: Tolerance LAB to bile at different concentration

Sr.No	Isolate	Food sample	Concentration of bile in growth medium					
			0.1%		0.2%		0.3%	
			24h	48h	24h	48h	24h	48h
1	AB	*Amboli*	+	+	+	+	+	+
2	WB	Meduwada batter	-	-	-	-	-	-
3	DB	Dhokala batter	+	+	-	-	-	-
4	MAB-2	Manglore bun batter	+	+	+	+	+	+
5	BB	Babru batter	-	+	-	-	-	-
6	IB-1	Idli batter	+	+	-	-	-	-
7	IB-2	Idli batter	+	+	-	-	-	-
8	SK	Sauerkraut	-	-	-	-	-	-
9	PB-1	Panta Bhat	+	+	-	-	-	-
10	GF-1	Fermented ginger juice	+	+	+	+	+	+
11	WW-1	Wheat water (fermented)	+	+	+	+	-	-
12	WW-2	Wheat water (fermented)	+	+	-	-	-	-
13	PB2-(I)	Panta Bhat	+	+	+	+	+	+
14	FCR-1	Kimchi	+	+	-	-	-	-
15	FCR-2	Kimchi	+	+	-	-	-	-
16	SH-1(II)	*Sel roti*	-	-	-	-	-	-
17	CH-1(II)	*Chakuli*	-	-	-	-	-	-
18	FCB	*Kangi*	+	+	+	+	+	+
19	SH-1(I)	*Sel roti*	+	+	+	+	i	i
20	AV-2	Fermented Alovera	+	+	+	+	-	+

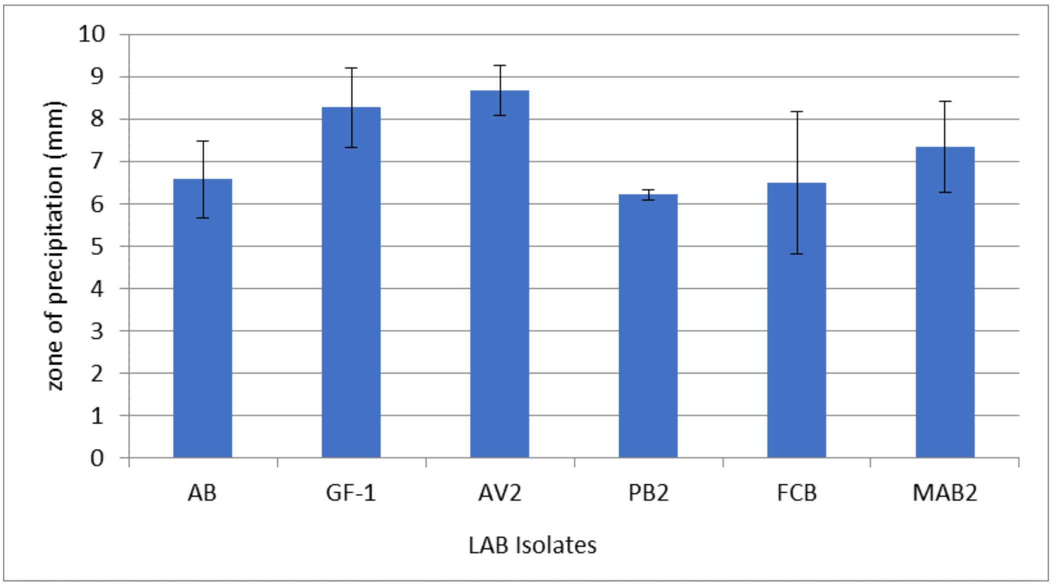

Figure1: Graphical representation of BSH Activity

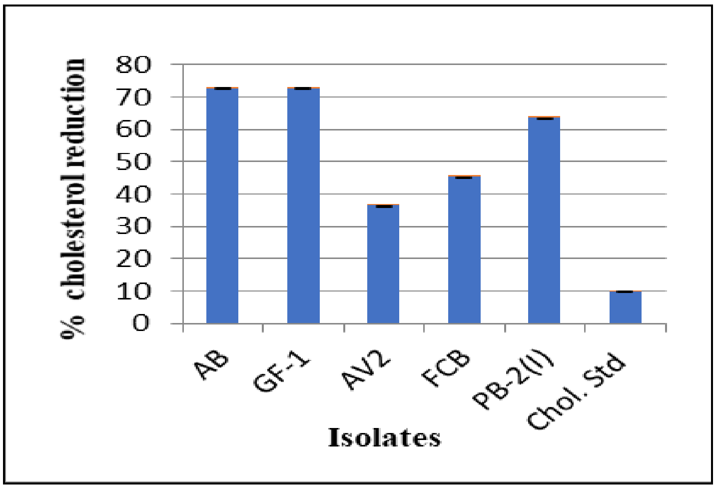

Figure 2: Graphical representation of cholesterol reduction activity

Figure 3: Qualitative test for EPS production; change in viscocity.

CONCLUSION

It can be concluded that the isolated LAB from used traditionally fermented Indian foods in the present study present as a putative probiotics as seen from acid-bile tolerance, hemolytic activity testing. Also, beneficial effects were shown by these bacteria ie ability to produce EPS which can be considered as prebiotic potential and having cholesterol reduction activity offers added advantages to the LAB isolates. More extensive study is required to prove their probiotic status and additive in food for functional potential. Since these isolates are from traditionally fermented food in practice it can be said that they are safe to achieve benefit though more scientific investigations are required.

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Conflicts of interest: The authors stated that no conflicts of interest.

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