

Bile Salt Hydrolase Activity Screening and Resistance to the Toxicity of Bile Salt by Indigenous Lactobacillus Isolates of Pakistan; A Research Article

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Citation

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Abstract

Objective: In this study 20 Lactobacillus strains isolated from different sources were selected for an account of their high bile-salt hydrolase (BSH) activity, bile-salt resistance, and tolerance to the toxicity of bile salts. **Method:** Bile salt hydrolase and resistance to toxic effects of bile salts were checked by assay for Deconjugation of bile salts and by toxicity of conjugated bile salt assay **Results:** A total of 20 isolates were tested for hydrolase activity of conjugated bile salts sodium salt taurocholic acid and glycocholic acid while toxicity of bile salts was in MRS broth supplemented various concentrations of bile salts. Out of 20 strains 2 strains displayed the largest precipitation zones were selected for toxicity assay. .

Conclusion: The present study suggested that the finally two indigenous isolated of Lactobacillus from Pakistan has an excellent hypocholesterolemic effects. They will be used as a Probiotics to prevent hypercholesterolemia from human health while the mechanisms of regulating serum cholesterol and the effect on serum cholesterol level in vivo needs further extensive investigations.

INTRODUCTION

Serum cholesterol is important from the public health because higher concentrations are often associated with a greater risk to cardiovascular diseases. Elevated level of cholesterol in ruminants could possibly due to their increased synthesis to meet the requirements for fat digestion and absorption in the intestine or slower rate of cholesterol disposal (Scott and Cook, 1975). Bile salts are synthesized mainly from cholesterol, conjugated with taurine or glycine in the liver (Batta et al., 1999).

Deconjugated bile acids are excreted more rapidly from the intestinal tract than are conjugated bile salts. While free bile acids are excreted more rapidly from the body, thus the synthesis of new bile salts from cholesterol can reduce the total cholesterol concentration in the body (Gilliland and Speck, 1977) while the enzyme responsible for the deconjugation activity, is bile salt hydrolase (BSH) (Grill et al. 1995). Some natural occurring microorganisms in human intestine are beneficial in terms of lowering cholesterol (Buck and Gilliland, 1994; Fernande, et al. 1987; Fukushima, et al. 1999; Mann and Spoerry, 1974).

It was also reported that Lactobacillus reduces blood cholesterol by direct breakdown of cholesterol and deconjugation of bile salts (Oh and Lee, 2000). Various attempts have shown that species of Lactobacilli were capable of lowering serum cholesterol and reducing the severity of hypercholesterolemia either by lowering total elevated serum cholesterol or reducing low density lipoproteins LDL.

Therefore, in vitro strain selection for hypercholesterolemic probiotic bacteria can base on bile tolerance and deconjugation of bile salts. This study also aimed to evaluate the potential hypocholesterolemic abilities of indigenous Lactobacillus species.

MATERIAL AND METHODS

MEDIA AND CHEMICALS

MRS (Oxoid) broth and agar were used in all the experiments. Sodium salt of Taurocholate (TCA) and Glycocholic acid (GCA) and Oxgall (Ox) were obtained from Sigma.

BACTERIAL STRAINS

20 of *Lactobacillus* strains were isolated from the human faeces, dairy or other sources of Pakistan were collected in the sterilized screw capped bottles (sterilized at 121 °C for 20 minutes in the autoclaved from different areas of Karachi. The samples were brought to the Center for Molecular Genetics, University of Karachi and stored immediately under refrigeration conditions for further processing. They were grown anaerobically in MRS broth at 37 C. Stock cultures at -80 C were prepared from overnight cultures grown in MRS to which 15% glycerol was added to adjust the prior to freezing.

QUALITATIVE ASSAY FOR DECONJUGATION OF BILE SALTS.

Qualitative BSH activity of the isolated strains was evaluated using the procedure described by du Toit et al. (du Toit et al., 1998). Bile salt agar plates were prepared by adding 0.5% of sodium salt of taurocholate and glycocholate to MRS agar. After autoclaving and solidifying, the plates were placed in the GasPak anaerobic jar (Oxoid) for at least 48 h before use. Sterile filter disks were impregnated in an overnight culture of the test strain and placed on MRS agar plates supplemented with 0.5% (wt/vol) taurocholic acid sodium salt (TCA; Sigma), Sodium glycocholic acid (GCA; Sigma). The plates were incubated anaerobically at 37°C for 72 h. BSH activity was present when cholic acid precipitated in the agar medium below and around a colony. The diameters of the precipitation zones were measured. MRS agar plates without supplementation were used as controls. Each strain was tested in triplicate. Subsequently, diameters of the strains that displayed the largest precipitation zones were selected for further study.

TOXICITY OF CONJUGATED BILE SALT ASSAY

The isolates were tested for the capacity to resist the bactericidal activity of a conjugated bile salt (TCA) with an assay modified from the assay described by De Smet et al. (De Smet et al., 1998). A stationary-phase culture inoculum (1%) was added to MRS broth supplemented with TCA, GCA and oxgall at a concentration of 0%, 0.3%, 0.5% and 0.6%. At zero time and after 24, 48, and 72 h of anaerobic incubation at 37°C, dilutions of the bacterial suspensions were prepared. Aliquots of the dilutions were smeared onto MRS agar plates, which were then incubated anaerobically at 37°C for 48 h and 72h. Population estimates were made from viable counts.

RESULTS AND DISCUSSION

All 20 isolates were tested for hydrolase activity of conjugated bile salts GCA and TCA (Table 1). Five of the isolates exhibited GCA hydrolase activity, while six were able to express TCA hydrolase activity. One indigenous isolate CMGsaM135 was able to express hydrolase activity but unable to express TCA hydrolase activity. Conversely, indigenous isolate CMGsa168 and CMGsa212 were positive to produce opaque halo on TCA, unable to produce opaque halo on GCA plates. Opaque halo was considered as positive for Bile hydrolase activity (Table 1). The indigenous isolates were also determined for their resistance to toxicity of conjugated bile salts. Twelve isolates were able to resist the toxicity of GCA and eight were sensitive to GCA toxicity, while in case of conjugated bile salt TCA, nine have shown resistance to TCA toxicity and eleven were sensitive to TCA toxicity (Table 1). Two of the strains CMGsaM163 and CMGsaM268 are able to resist 0.5% GCA and TCA toxicity level for 48 to 72 hours (Table 2a and 2b) respectively while none of the strains are able to resist 0.6% toxicity of bile salts. Two of the strains CMGsaM163 and CMGsaM268 have also shown opaque halo of bsh in presence of 0.3% TCA.

There are increase evidence that *Lactobacilli* are able to provide a number of health benefits, including antimicrobial effects against pathogenic bacteria, anti-tumor effects, and protection against antibiotic-associated diarrhea or food allergy (Saxelin 1997; Orrhage and Nord 2000; Cummings and others 2001). *Lactobacilli* are reported to be acid and bile tolerant and are able to survive in the gastrointestinal tract. It was hypothesized that high BSH deconjugation activity associated with the stationary phase of culture was a result of reduced pH levels in the medium (Corzo & Gilliland, 1999). Eyssen has demonstrated that the increased excretion of bile salts can increase catabolism of cholesterol to bile acids more rapidly (Eyssen. 1973).

The ability of a specific probiotic strain to survive and reproduce in the hostile environment of the gut is the most relevant feature to be checked during the selection procedures, but production of metabolites resulting from effect of bile salts on bacterial cells is unknown (Mallory, et al. 1973), while in vivo testing is time-consuming and expensive. In vitro selection is therefore the first approach used to select few strains that can be further evaluated in vivo.

Nevertheless, results achieved are positive and promising.

The development of new probiotic products has produced new scientific achievements and a strong demand for improved and scientifically-based selection criteria.

Figure 1

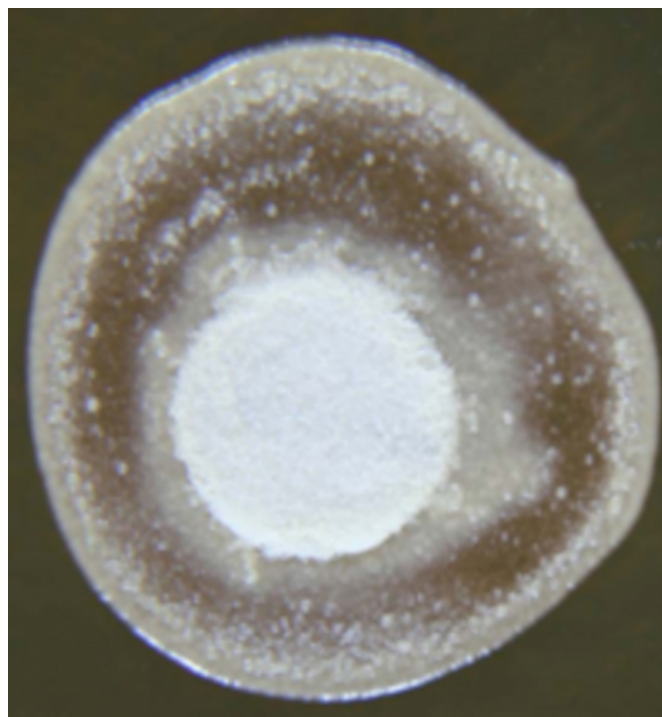
TABLE 1: Resistance and Hydrolase activity for conjugative bile salts GCA and TCA.

TABLE 1: Resistance and Hydrolase activity for conjugative bile salts GCA and TCA.					
S. No.	Strains Code	GCA Hydrolase activity	TCA Hydrolase activity	Resistance to GCA Toxicity	Resistance to TCA toxicity
1	CMGsa120	N	N	S	S
2	CMGsa122	N	N	R	S
3	CMGsaM135	P	N	R	S
4	CMGsa148	N	N	R	S
5	CMGsa154	N	N	S	S
6	CMGsaM156	N	N	R	S
7	CMGsa158	N	N	R	S
8	CMGsaM163	P	P	R	R
9	CMGsa164	N	N	R	R
10	CMGsa168	N	P	S	R
11	CMGsa179	P	P	R	R
12	CMGsa181	N	N	S	S
13	CMGsa182	N	P	S	R
14	CMGsa183	N	N	S	R
15	CMGsa228	N	N	R	S
16	CMGsa261	N	N	S	S
17	CMGsa264	N	N	S	S
18	CMGsaM269	P	P	R	R
19	CMGsaF186	P	P	R	R
20	CMGsaF188	P	P	R	R
21	CMGsa1548	N	P	S	S
22	CMGsaE168	N	P	R	S
23	CMGsaL241	N	P	R	S
24	CMGsaS306	N	P	R	S

Estimated by examining bacterial populations growing for 0, 24, 48 and 72 h in MRS containing TCA or GCA at concentration of 0.3%, 0.5% and 0.6%.
GCA, glycocholic acid; TCA, Taurocholic acid.

Figure 2

Figure 1a:



Bile Salt Hydrolase (BSH) activity in Lactobacillus strain CMGsaM163 in presence of 0.3% TCA

Figure 3

Figure 1b:

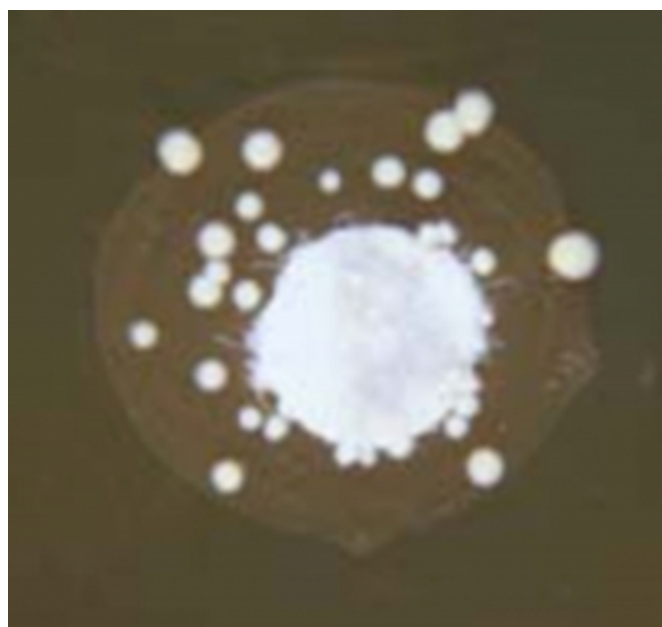
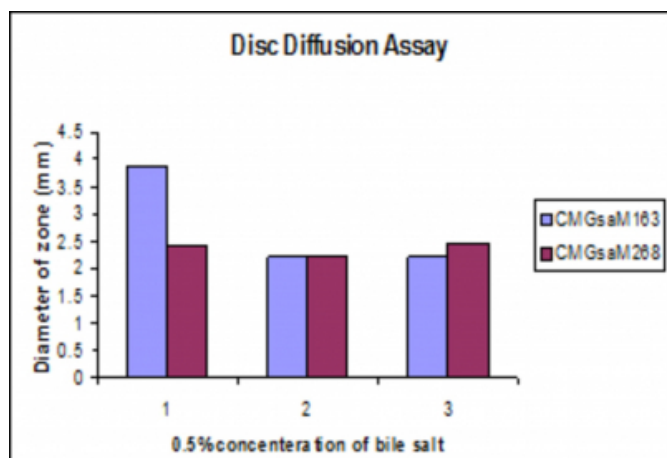


Figure 4

Figure 2:

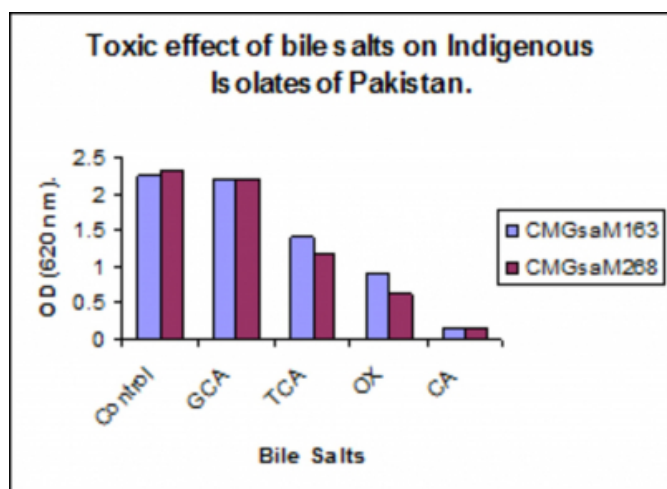


Bile Salt Hydrolase (BSH) activity in Lactobacillus strain CMGsaM268 in presence of 0.3% TCA

Disc Diffusion Assay for Indigenous isolates CMGsaM163 and CMGsaM268 at 0.5 % concentration of bile salts.

Figure 5

Figure 3:



Toxic effect of various bile salts (0.5%gm of Glycocholic acid, Taurocholic acid, Oxgall, Cholic acid) on indigenous isolates CMGsaM163 and CMGsaM268, after 48h of incubation. Each bar represents average of three.

CONCLUSION

In general, probiotic strain must colonize in GTI of host and must have acid, bile salt – tolerance (Du Toit, et al. 1998; Gilliland, et al. 1985). And from present result, it was suggested that the finally indigenous isolated Lactobacillus strains had an excellent hypocholesterolemic effects. They

will be used as a Probiotics to prevent hypercholesterolemia from human health while the mechanisms of regulating serum cholesterol and the effect on serum cholesterol level in vivo needs further extensive investigations

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References

- Batta AK, Salen G, Arora R, Shefer S, Batta M, and Perseon: Side chain conjugation prevents bacterial 7-dehydroxylation of bile acids. J. Biol. Chem; 1990; 265:10925-10928.
- Buck LM, Gilliland SE. Comparison of freshly isolated strains of Lactobacillus acidophilus of human intestinal origin for ability to assimilate cholesterol during growth. Journal of Dairy Science; 1994; 77:2925 – 2933.
- Corzo G and Gilliland SE. Bile salt hydrolase activity of three strain of Lactobacillus acidophilus. Journal of dairy Science; 1999; 82:472-480.
- Cummings JH, Macfarlane GT and Englyst HN. Prebiotic digestion and fermentation. American Journal of Clinical Nutrition; 2001; 2073: 415S-420S.
- De Smet I, van Hoorde L, De Saeyer N, van de Woestyne M, Verstaete W. In vitro study of bile-salt hydrolase (BSH) activity of BSH isogenic Lactobacillus plantarum 80 strains and estimation of cholesterol lowering through enhanced BSH activity. Micro. Ecol. Health Disease; 1994; 7: 315-29.
- De Smet I, De Boever P and Verstraete W. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. British Journal of Nutrition; 1998; 79:185-194.
- Du Toit M, Franz CMAP, Dicks LMT, Schillinger U, Haberer P, Warlies B, Ahrens F, Holzapfel WH. Characterization and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faecesmoisture content. International Journal of Food Microbiology; 1998; 40:93-104.
- Eyssen HJ, Parmentier GG, Compennolle FC, De Pauw G, Piessens-Denef M. Biohydrogenation of sterols by Eubacterium ATCC 21,408--Nova species. European Journal of Biochemistry; 1973; 16; 36(2):411-421.
- Fernandes C.F., K.M. Shahani.. Anticarcinogenic and immunological properties of dietary lactobacilli. Journal of Food Protection; 1990; 53:704 – 710.
- Fukushima M, Yamada A, Endo T, Nakano M Effects of a mixture of organisms, Lactobacillus acidophilus or Streptococcus faecalis on delta6-desaturase activity in the livers of rats fed a fat and cholesterol-enriched diet. Nutrition; 1999; 15:373-378.
- Gilliland SE and Speck ML. Deconjugation of bile acids by intestinal lactobacilli. Applied and Environmental Microbiology; 1977; 33:15-18.
- Gilliland SE, Nelson CR and Maxwell C. Assimilation of cholesterol by Lactobacillus acidophilus. Appl. Environ. Microbiol; 1985; 49:377-381.
- Gilliland SE. Acidophilus milk products: a review of potential benefits to consumers. Journal of Dairy Science; 1989; 72:2483-2494.
- Grill JP, Schneider F, Crociani J, Ballongue J Purification and characterisation of conjugated bile salt hydrolase from Bifidobacterium longum BB536. Appied

Environmental Microbiology; 1995; 61: 2577–2582

15. Mallory A, Kern F, Smith J, and Smith JG. Patterns of bile acids and microflora in the human small intestine. I. Bile acids. *Gastroenterology*; 1973; 64:26-36.

16. Mann GV, Spoerry A. Studies of a surfactant and cholesteremia in the Maasai. *American Journal of Clinical Nutrition*; 1974; 27:464-469

17. Oh CY, Lee WK. Cholesterol lowering effect of lactic acid bacteria isolated from the human intestine. *Korean Journal of Veterinary Public Health*; 2000; 24, 181-188.

18. Orrhage K, Nord CE. Bifidobacteria and Lactobacilli in human health. *Drugs Experimental and Clinical. Research*; 2000; 26(3):95–111.

19. Saxelin M. Lactobacillus GG—a human probiotic strain with thorough clinical documentation. *Food Rev Int.* ; 1997; 13(2):293–313

20. Scott TW and Cook LJ .Digestion and metabolism in the ruminants", *Proc. 4th Int. Symp. on Ruminants Physiology*, Sydney, Univ. of New England, Armidale;1975; p. 150,

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