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In vitro detection of bile salt hydrolase activity of probioticLactobacillus fermentum strains isolated from camel milk

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ABSTRACT: For satisfying the consumer demand in this era probiotic foods are preferred choice. In the situation during covid pandemic people have showed more interest for probiotic food products as they are proved to be good immunity boosters. Probiotic strains should possess the bile salt hydrolase (Bsh) activity for their effective application in lowering the serum cholesterol level in patients suffering from hypercholesterolemia. The present investigation was aimed to detect bsh gene activity in lactobacilli isolate on molecular basis for its further use as effective probiotic strain. A total of 43 isolates were recovered on MRS agar from 4 camel milk samples collected from Udaipur, Rajasthan, India. All 43 lactobacilli isolates showed varied response towards three different bile salts namely oxgall, sodium taurocholate and sodium taurodeoxycholatesupplemented at different concentrations such as 0.1, 0.2, 0.3, 0.4 and 0.5% in MRS medium. The highest degree of tolerance of lactobacilli isolates were seen against sodium taurodeoxycholate. Among 43 isolates subjected to molecular characterization 16 isolates showed 200bp product (specific for genus Lactobacillus) and only 3 among these showed sequence similarity confirming the species as L. fermentum. The Bsh activity was confirmed for two isolates namely Lactobacillus fermentum CMU 1 and CMU 7 which showed strong probiotic potential in this regard. These isolates can be potential probiotic candidates in future and can be explored for in vivo activity for further health benefits.

KEYWORDS: Bile salt hydrolase (Bsh), lactobacilli, Lacobacillus fermentum, bile salts, sodium taurodeoxy cholate

I. INTRODUCTION

Lactobacilli have been extensively used as probiotic bacteria for human beings. They are regarded as safe and provide several health benefits to the host [1-3]. Probiotic strains must survive in the gut in presence of bile during passage through gastrointestinal tract. Bile is released from gall bladder which digest the fat content of the food and facilitates its absorption. Cholesterol acts as is a precursor for bile synthesis. The free bile acid secreted in the gut are combined with amino acids such as glycine and taurine to produce conjugated bile salts [4].Bacterial bile salt hydrolases react upon conjugated bile salts and by deconjugation convert them to secondary bile acids [5].

Bile salts are also toxic in nature as they rupture the cell membrane of microorganisms leading to DNA damage. Therefore, its crucial for an probiotic bacterium to cope up with bile salts. To survive in the gut environment lactobacilli must have intrinsic mechanisms [6-9]. In recent years it has been the most important desirable trait that a probiotic bacterium should harborbsh gene contributing for bile tolerance ability and lowering the levels of cholesterol in individuals [10,11]. Research on lactobacilli for detecting Bsh activity has been extensively progressed in last five years and have increased during covid pandemic [12-14].

An attempt was made in present work to screen the Lactobacillusfermentum strains isolated from camel milk for probiotic potential and bile salt hydrolase activity.

II. MATERIALS AND METHODS

a. Isolation of Lactobacilli:

Camel milk samples were collected from Udaipur, Rajasthan, India. The samples were first inoculated for enrichment in MRS [15] broth and incubated at 37°C for 48 h. Then the samples were inoculated on MRS agar using standard pour plate method. Isolated colonies were recovered from the plates after an incubation period of 48 h at 37ºC. The individual colonies were selected and

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transferred into litmus milk and were further purified by successive streaking and sub-culturing on MRS medium.

b. Molecular Characterization:

The genomic DNA, from the test bacterium was extracted by following method of Pospiech& Neumann [16]. Molecular identification of the isolate was done using 16S rDNA amplification followed by sequencing. The semi-universal primers designated Lb1 (5'-AGAGTTTGATCATGGCTCAG-3') and Lb2 (5'-CGGTATTAGCATCTGTTTCC-3') designed by Klijn [17] were used for PCR amplification. The amplified products were subject to Bangalore geneipvt. ltd. Bangalore, India. Sequence data obtained after partial sequencing of 16S rDNA was analyzed by BLAST and was submitted to EMBL-EBI database under the accession number.

c.Determination of Bile Tolerance:

The method proposed by Sirilun [18] was used to detect the bile salt tolerance of isolates. MRS agar with and without bile salt was prepared. MRS agar without bile salts (control) and MRS agarcontaining 0.1 to 0.5 % (w/v) bile salts namely oxgall (Merck), sodium taurocholate(Chemika-Biochemika Reagents) and sodium taurodeoxycholate (Hi-Media) were streaked by the isloate and incubated at 37ºC for 48h. The plates were checked for the presence of growth to confirm bile tolerance.

d. Detection of BshActivity:

Detection of bsh gene was done using PCR assay. The primers designated LbBSHF/R 5' – ATCACCGCTACATTGGTTGG - 3', 5' – AGTCCGCCCATTCCTCTACT -3' [19] were used for amplification of the relevant gene.

III. RESULTS

a. Isolation of Lactobacilli:

A total of 43 isolates were obtained on MRS agar from 4 camel milk samples collected from Udaipur, Rajasthan, India. Pure colonies of lactobacilli isolate CMU 7 on MRS agar at 37ºC after 48h of incubation period is shown in Fig.1.

Fig.1: Pure colonies of lactobacilli isolate CMU 7 on MRS agar at 37º C after 48h of incubation period

b. Bile Tolerance of Lactobacilli:

To screen the bile tolerance of 43 lactobacilli isolates, MRS agar was supplemented with three different bile salts namely oxgall, sodium taurocholate and sodium taurodeoxycholate at different concentrations such as 0.1, 0.2, 0.3, 0.4 and 0.5% of each bile salt. A total of 43 isolates showed varied degree of growth when grown in MRS medium containing varying concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) of oxgall, sodium taurocholate and sodium taurodeoxycholate (Fig. 2). All lactobacilli isolates were subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of oxgall. Among 43 lactobacillistrains, 100% (43/43) isolates were able to grow at 0.1% oxgall. However, 34.88 % (15/43) isolates were able to grow upto 0.2 % oxgall. Only 11.62 % (5/43) isolates were able to grow upto 0.3% oxgall. At 0.4% and 0.5% concentration of oxgall, none of the strains showed growth. All lactobacilli strains were further subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of sodium taurocholate. Out of a total of 43 strains only 23.25% (10/43) strains were able to grow at 0.1% sodium taurocholate. However, 13.95% (6/43) strains were able to grow up to 0.2% sodium taurocholate. At 0.3%, 0.4% and 0.5% concentration of sodium taurocholate, none of the strain showed growth. A total of 43 strains were also subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of sodium taurodeoxycholate. Among 43 lactobacilli strains, 100% (43/43) isolates were able to grow at 0.1% of sodium taurodeoxycholate. However, 97.67% (42/43) strains were able to grow up to 0.2%. At 0.3% and 0.4%, 81.39% (35/43) and 20.93% (9/43) strains were grown, respectively. At 0.5 % concentration of sodium taurodeoxycholate, 9.3 % (4/43) of the strain showed growth.

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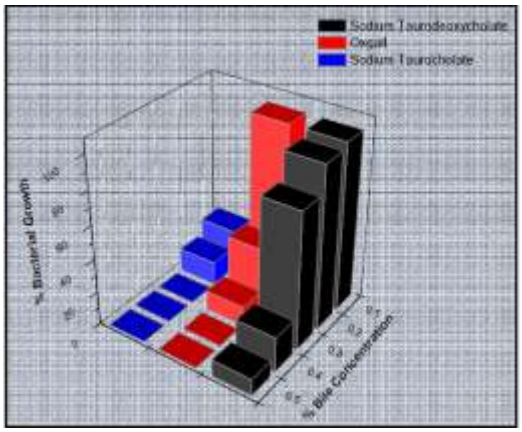

Fig. 2: Tolerance of lactobacilli isolates against different concentrations of oxgall, sodium taurocholate and sodium taurodeoxycholate

Among the three bile salts used in the study the lactobacilli isolated showed the highest tolerance towards sodium taurodeoxycholate. Among 43 isolates 9 isolates namely CMU 1, CMU7, CMU 12, CMU 15, CMU 19, CMU 20, CMU 26, CMU 33, CMU 40 showed growth in MRS agar supplemented with 0.4 % and 4 isolates namely CMU 1, CMU 7, CMU 15 and CMU 24 showed growth in MRS agar supplemented with 0.5 % sodium taurodeoxycholate.

3. Molecular Characterization:

All 43 isolates were subjected to PCR assay using genus specific primers Lb1 and Lb2. A total of 16 isolates out of 43 showed 200 base pair product confirming that they belong to genus Lactobacillus. Remaining isolates didn't show the 200 bp product. These 16 isolates were subjected to sequencing analysis. Sequence data obtained after partial sequencing of 16S rRNA revealed that 3 isolates namely CMU 1 (93%), CMU 6 (99%) and CMU 7 (99%) showed similarity to Lactobacillus fermentum. The assession numbers for CMU 1, CMU 6 and CMU 7 was LK985320, LM994029 and LM994030 respectively. Remaining isolates didn't show no sequence similarity to species L. fermentum.

4. Presence of bshGene Activity:

To detect bsh gene activity all 7 Lactobacillus isolates CMU 1-7 were subjected to PCR assay using bsh gene specific primer LbBSHF/R. Among 7 isolates, only 2 isolates namely Lactobacillus fermentum CMU 1 and Lactobacillus fermentum CMU 7 produced 231 bp amplified product (Fig.3). These 2 isolates were found to be Bsh positive strains confirming their strong probiotic potential.

Fig. 3: PCR based detection of bshgene in Lactobacillus isolates. Lane 1 = 100 bp ladder, Lane 2 = Lactobacillus isolate CM1 (Positive control), Lane 3-8 = Lactobacillus isolates CMU 1, CMU 2, CMU 4, CMU 5, CMU 6 and CMU 7

IV. DISCUSSION

During its passage through gastrointestinal tract probiotic organisms are exposed to various stressful conditions such as low pH of stomach and then high concentration of bile salt in colon [20]. The normal level of bile salt in the intestine is around 0.3% [21]. An efficient probiotic organism must grow well in presence of high concentrations of bile ranging from 0.1 to 0.5% [22]. In the present study Lactobacillus fermentum CMU7 proved to be the best as it showed tolerance upto 0.5 % of sodium taurodeoxycholate.

The substrate specifity for a probiotic organism vary for the different bile salts. The workers showed wide variety of responses towards sodium glycocholate and sodium taurocholate and taurodeoxycholate [23, 24]. In the finding of present research all lactobacilli showed high degree of tolerance to sodium taurodeoxycholate among the three bile salts tested namely oxgall, sodium taurocholate and sodium taurodeoxycholate.

Bile salt hydrolases help bacterial strains to tolerate bile in the gastrointestinal tract released during digestion process. Several workers have used different primers for detection of bsh gene [25, 26]. Many researchers have reported the presence of Bsh activity in L. fermentum strains [27, 28]. Amplified products of 231 bp size were obtained for Lactobacillus fermentum CMU1 and CMU 7 isolates. These results are in complete agreement with Kaushik [19] who proposed that presence of bsh gene gives 231 bp amplified product with primers LpBSHR/F.

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V. CONCLUSION

Lactobacillus fermentum CMU 7isolated from camel milk sample exhibited strong probiotic potential in regard to possessing bile tolerance and Bsh activity. The isolate survived in the MRS broth supplemented withsodium taurodeoxycholateupto 0.5% concentration and with other bile salts to a variable extent. The degree of tolerance to bile exhibited by the isolate is important in governing their effectiveness in growing in the intestinal tract particularly in the upper intestinal tract. Further studies can be carried out to explore the potential of isolate to provide health benefits in special regard to lowering of cholesterol.

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