Food Bioscience 5 (2014) 27–33

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/fbio

Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products

Ami Patel[a,b,*], J.B. Prajapati[a], Olle Holst[b], Asa Ljungh[c]

[a]Department of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India
[b]Department of Biotechnology, Lund University, Lund, Sweden
[c]Department of Medical Microbiology, Lund University, Lund, Sweden

ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form
30 September 2013
Accepted 12 October 2013

Keywords:
Exopolysaccharides
BSH activity
Weissella
Dhokla
Idli batter
Antimicrobial activity

ABSTRACT

Exopolysaccharide producing lactic acid bacterial (LAB) were tested *in vitro* to select a candidate probiotic strain by testing their tolerance to low pH and bile, bile salt hydrolase (BSH) activity, antibiotics susceptibility pattern and antimicrobial activity. Results indicated that LAB isolates were highly sensitive against oxbile (0.3%) but could grow in the presence of sodium taurocholate (0.3%). Seven out of 9 isolates were found to be BSH positive. Two of the isolates, *Lactobacillus plantarum* 86 and *Weissella cibaria* 92 showed considerable antimicrobial activity against Gram-positive and Gram-negative pathogens. The study reveals that traditional fermented products of India could be an alternative and readily available resource for LAB starter cultures with interesting functional characteristics.

1. Introduction

Lactic acid bacteria (LAB) are associated with fermented foods where they flair to produce technologically important substances such as exopolysaccharides (EPS). EPS producing LAB are especially relevant in yoghurt, cheese, sour cream and other cultured dairy products by conferring beneficial rheological and functional properties as natural thickening agents, giving the product a suitable viscosity and reducing syneresis (Ruas-Madiedo & de los Reyes-Gavilán, 2005; De Vuyst & Degeest, 1999). EPS have been found to enhance gastrointestinal (GI) colonization of probiotic bacteria in the GI tract and thus, also play a significant role as prebiotics (Welman & Maddox, 2003).

Many of the LAB strains considered as probiotic are found to be effective against diverse GI exertions either by adhering to the gut mucosa, combating pathogens by virtue of producing antimicrobial compounds and/or exerting beneficial effects on human health (Servin, 2004). Before using bacterial strains as probiotics, they should be screened for certain imperative characteristics such as resistance to bile and low pH, antibiotic susceptibilities and antimicrobial activity

*Corresponding author at: Department of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. Tel.: +91 2692 640170.
E-mail address: amiamipatel@yahoo.co.in (A. Patel).

2212-4292/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fbio.2013.10.002

(Lee & Salminen, 2009; Kruszewska et al., 2002). Several probiotics bacteria are found to produce bile salt hydrolase (BSH) that helps to reduce serum cholesterol (Reynier et al., 1981) and hence BSH activity is also considered as an additional criterion for the selection of probiotics.

Traditional fermented products could be an alternative and readily available resource for LAB starter cultures with interesting functional characteristics and improved technological and probiotic properties. In this context, based on EPS producing trait, total 17 LAB strains were isolated from different vegetables such as carrot, cabbage, turmeric, cucumber and tomato and traditional fermented foods including dhokla batter, idli batter, dahi, and cabbage (Patel, Shah, & Prajapati, 2012). The present study is aiming at determining potential probiotic characteristics of these EPS producing LAB isolates that were, tested *in vitro* for tolerance to low pH and bile, bile salt hydrolase (BSH) activity, antibiotics susceptibility and antimicrobial activity.

2. Materials and methods

2.1. Bacterial strains—isolation, strain identification and growth conditions

All 17 EPS producing isolates were characterized biochemically and genotypically using 16S rDNA sequencing. The GenBank and EMBL accession numbers for reference 16S rRNA gene sequences are represented in Table 1. All 17 LAB isolates that were preserved at −20 °C on De Man, Rogosa and Sharpe (MRS, MERCK) broth containing 10% glycerol (v/v) and in freeze-dried form and were propagated twice prior to begin any experiment. The incubation temperature was maintained at 37 °C for lactobacillus spp. and *Weissella* isolates and 30 °C for *Pediococcus* spp. during the experiments whenever their normal growth was required. All isolates are available at the host institution and processed for submission in Microbial type culture collection-MTCC, Chandigarh, India.

To perform antimicrobial assay, the clinical isolates of *Escherichia coli* ESBL and methicillin-resistant *Staphylococcus aureus* (MRSA) were obtained from the department of Medical Microbiology, Lund University, Lund (Sweden). They were propagated in brain heart infusion (BHI, Oxoid) broth and maintained as frozen stocks at −20 °C in BHI broth containing 10% (v/v) glycerol. Before use, frozen cultures were grown on blood agar plate followed by two successive transfers into BHI broth.

2.2. Tolerance to low pH

Tolerance to low pH was determined using the method of Chou and Weimer (1999) with some modifications. The active strains grown in MRS broth were inoculated (1.5%) in 10 ml of fresh MRS broth adjusted to pH 3.0 with hydrochloric acid (1.0 M) for 2.5 h at 37 °C. Samples were withdrawn at 0 h and at the end of 2.5 h of incubation to measure the initial bacterial population and residual cell population by plating suitable dilutions on MRS agar plates. The plates were incubated at 37 °C/30 °C for 48 h and the number of colonies grown was counted.

2.3. Bile resistance

The ability of isolated LAB strains to grow in presence of two different biles, namely Oxbile and sodium-taurocholate was

Table 1 – Genetic and phenotypic characterization data for the isolates and EPS production in semi-defined media.

Isolate code	Source of isolation	Similarity based on 16S rRNA	Sequence length (no. base pairs)	% Similarity	Gene accession number	EPS production (mg/l)
AD1	Dahi	*L. fermentum*[a]	1114	99	JN792470	360
AI2	Dhokla batter	*L. fermentum*[b]	1017	99	JN792468	570
AI3	Dhokla batter	*L. fermentum*[c]	1357	98	JN792457	280
AV2	Fermented cabbage	*L. fermentum*[a]	1397	99	JN792461	250
AV3	Carrot	*L. fermentum*[a]	1041	98	JN792462	600
AV4	Cabbage	*L. fermentum*[b]	1031	98	JN792463	680
138	Fresh turmeric	*L. fermentum*[a]	1395	97	JN792459	350
AD29	Dahi	*L. plantarum*[d]	985	99	JN792465	390
86	Dahi	*L. plantarum*[d]	1027	98	JN792454	960
AI10	Idli batter	*W. confusa*[e]	1445	96	JN792460	610
AV1	Fermented cabbage	*W. cibaria*[f]	932	99	JN792467	500
85	Dahi	*W. cibaria*[f]	1375	97	JN792458	570
92	Idli batter	*W. cibaria*[f]	1109	99	JN792466	570
142	Cucumber	*W. cibaria*[f]	1141	98	JN792456	570
145	Cabbage	*W. cibaria*[f]	938	99	JN792455	480
AI1	Idli batter	*P. parvulus*[g]	1100	99	JN792469	470
AV5	Tomato	*P. parvulus*[g]	825	100	JN792464	410

[a] % Sequence similarity of 16S rDNA gene with the type strains *L. fermentum* CIP 102980^T.
[b] % Sequence similarity of 16S rDNA gene with the type strains *L. fermentum* OMZ 1117^T.
[c] % Sequence similarity of 16S rDNA gene with the type strains *L. fermentum* IFO 3956^T.
[d] % Sequence similarity of 16S rDNA gene with the type strains *L. plantarum* CIP 103151^T.
[e] % Sequence similarity of 16S rDNA gene with the type strains *W. confusa* JCM 1093^T.
[f] % Sequence similarity of 16S rDNA gene with the type strains *W. cibaria* NRIC 0136^T.
[g] % Sequence similarity of 16S rDNA gene with the type strains *P. parvulus* DSM 20331^T.