LWT - Food Science and Technology 74 (2016) 234–241

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Characterization of *Lactobacillus plantarum* strains for functionality, safety and γ-amino butyric acid production

Satyamitra L. Shekh [a], Jayantilal M. Dave [b], Bharatkumar Rajiv Manuel Vyas [a, *]

[a] Department of Biosciences, Saurashtra University, Rajkot 360 005, India
[b] 201, Shivam Vrindavan Society, Kalawad Road, Rajkot 360 005, India

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form
19 July 2016
Accepted 21 July 2016
Available online 25 July 2016

Keywords:
Probiotic
GABA
Mucin
BSH

ABSTRACT

Eight acid and bile tolerant *Lactobacillus plantarum* strains isolated from fruits and fermented foods were identified and characterized *in vitro* for probiotic attributes. The strains tolerated enzymes of simulated oro-gastro-intestinal fluids, pH extremes, bile and retained more than 50% viability during the simulated oro-gastro-intestinal transit. Isolates were able to adhere mucin and autoaggregate strain specifically. Most of the *L. plantarum* strains exhibited antimicrobial activity against indicator strains and produced health-promoting enzymes β-galactosidase and bile salt hydrolase (BSH). Strains OR, GP and HB produced γ-amino butyric acid (GABA) involved in various metabolic reactions. Safety evaluation assured the safe use of strains in food fermentations. The functional characterization of these strains isolated from fruits and fermented foods accentuated their potential as starter cultures in the manufacturing of functional probiotic products.

1. Introduction

Probiotics research has turned into lactic acid bacteria (LAB) research in recent years, focusing particularly on the *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* genera because of their potential health beneficial aspects. Interest in the use of LAB for the manufacturing of functional probiotic products is increasing day by day because of their long history of safe use in food fermentation and 'generally recognized as safe' status. FAO/WHO (2002) states probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit to the host". Probiotics should exert specific functional characteristics during their *in vivo* transit through human intrinsic environments. The tests used for *in vitro* functionality screening are survival in the conditions resembling pH and digestive enzymes of human oral cavity and gastro intestinal tract (GIT). LAB should survive in harsh GIT environments since 2.5 L of gastric juice having pH 2.5 (Kimoto, Ohmomo, Nomura, Kobayashi, & Okamoto, 2000) and 1 L of bile (Begley, Gahan, & Hill, 2005) are secreted into the human GIT every day. Other beneficial functional aspects include adherence to intestinal mucin layer, production of antimicrobial compounds and health promoting enzymes such as β-galactosidase and BSH (Collado, Isolauri, Salminen, & Sanz, 2009; Pithva, Shekh, Dave, & Vyas, 2014). It is a precondition for a strain to become a part of gastrointestinal microflora to exert these health benefits to the host. Organisms having ability to diminish existing microflora up to certain extent by producing inhibitory metabolite(s) are having competitive edge in establishing as dominant microflora in intestinal milieu. FAO/WHO recommends several tests for the safety of probiotic strains: assessment of antibiotic resistance, specific metabolic activities and enzyme activities having negative effects to the health and existing microflora.

GABA acting as inhibitory neurotransmitter in central nervous system, plays an important role in control of blood pressure (Inoue et al., 2003), diuretic effect (Stanton, 1963), tranquilizer effects (Jakobs, Jaeken, & Gibson, 1993), prevention of diabetic conditions (Hagiwara, Seki, & Ariga, 2004), and neuroprotective effect (Cho, Chang, & Chang, 2007). Glutamate decarboxylase enzyme (GAD; EC 4.1.1.15) converting glutamate into GABA is found in various *Lactobacillus* strains. The use of strains with GAD activity is being investigated intensively to develop novel fermented products with enhanced nutraceutical value.

Fruits and dairy products are used as natural sources for the isolation of novel probiotic organisms as extreme acidity, higher fiber content and antimicrobial agents present in these sources mimic traits of human GIT. Therefore these sources serve as niches

* Corresponding author.
E-mail address: brmvyas@hotmail.com (B.R.M. Vyas).

http://dx.doi.org/10.1016/j.lwt.2016.07.052
0023-6438/

rich in LAB with potential probiotic characteristics (Abo-Amer, 2011; Monteagudo-Mera et al., 2012; Tham, Peh, Bhat, & Liong, 2012; Vitali et al., 2012). In our previous report we evaluated probiotic potential of *Lactobacillus rhamnosus* strains of human origin for functional, safety and technological aspects (Pithva et al., 2014). But *Lactobacillus* strains isolated from fruits and traditional fermented foods are thought to be more suitable candidates because of their acquired adaptation to the probiotic products in which they are featured to be used. Therefore we characterized Lactobacilli of various food-origins for survival in human oro-gastro-intestinal juices, intestinal mucin adherence, antimicrobial activity towards intestinal pathogens and food spoilage organisms. Further screening of Lactobacilli for health beneficial aspects as well as safety was performed. *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* Fb were used as reference strains throughout the study.

2. Materials and methods

2.1. Strains and chemicals

Lactobacillus strains were isolated from various fruits and fermented foods (Table 1). Fruits purchased from local market and home-made butter were used for the isolation purpose. Raw cheese was obtained from Dairy Science College, Anand. Around 1 g of food sample was homogenized by vortexing in 0.85% NaCl, serially diluted and 0.1 mL was plated on De Man Rogosa Sharpe agar. MRS agar plates were incubated at 37 °C for 48 h (De Man, Rogosa, & Sharpe, 1960). Separable spindle shaped chalky white colonies were further streaked on MRS agar and examined by microscopy and biochemical tests. Gram-positive, catalase negative, non-spore forming rods were further identified on the basis of 16S rDNA sequence analysis. Pure *Lactobacillus* strains were preserved in 10% skimmed milk at 4 °C.

The indicator strains *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi*, *Shigella* sp., *Proteus vulgaris, Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Streptococcus mutans*, *Yersinia enterocolitica*, *Enterococcus faecalis, Bacillus* spp., and *Micrococcus luteus* were grown in Nutrient broth. *Listeria monocytogenes* and *Staphylococcus aureus* were grown in Brain Heart Infusion broth. *Aspergillus niger, Aspergillus flavus, Alternaria solani* and *Fusarium oxysporum* were cultured in Potato Dextrose Agar; *Aspergillus ochraceous* was grown in Czapek Dox Agar. The *Candida* strains were grown in Sabouraud Dextrose broth. All the bacterial and fungal indicator strains were grown at 37 °C for 18 h before assay.

All chemicals were purchased from Himedia, Mumbai, India if not indicated otherwise.

2.2. Preparation of cell suspension

Lactobacillus cultures were grown in 10 mL MRS broth for 24 h at 37 °C under static and aerobic condition. Next day, cells were centrifuged (5000*g*, 15 min, 4 °C), washed twice with phosphate buffer saline (PBS, 0.1 M, pH 7, 0.85% NaCl) and resuspended in 1 mL of phosphate buffer (0.1 M, pH 7) to prepare cell suspension (2×10^{10} cfu mL^{-1}).

Table 1
Sources of *Lactobacillus plantarum* strains with NCBI accession numbers.

L. plantarum strains	Source		Genbank Accession numbers
	Common name	Scientific name	
RC	Raw cheese	–	KF479380
GV	Guava	*Psidium guajava*	KF479381
SG	Sugarcane	*Saccharum officinarum*	KF479382
OR	Orange	*Citrus × sinensis*	KF479383
CK	Chiku	*Manilkara zapota*	KF479384
GP	Grapes	*Vitis vinifera*	KF479387
HB	Butter	–	KF479385
OP	Prickly pear	*Opuntia elaitor*	KF479386

2.3. Functional aspects

2.3.1. Determination of acid, bile, phenol and salt tolerance

Viability of *Lactobacillus* strains was assessed by the method of Jacobsen et al. (1999). 50 μL of cell suspension was inoculated in 3 mL of MRS broth with bile salt (2, 4%), NaCl (6%), pH (2, 3) and skim milk with phenol (0.6%) and incubated at 37 °C for 4 h. MRS broth and skim milk (10%) were taken as control. 0.1 mL aliquot was taken after 4 h, serially diluted, plated on molten MRS agar, and incubated at 37 °C for 48 h. Viable number of cells was expressed in the form of log cfu mL^{-1}.

2.3.2. Survival during oro-gastro-intestinal transit

Survival of cells in oral cavity was determined following Vizoso-Pinto, Franz, Schillinger, and Holzapfel (2006). To mimic the *in vivo* passage in salivary gland, 50 μL of cell suspension was mixed in simulated saliva (SS) solution comprising of sterile electrolyte solution containing lysozyme (Genei, Banglore; 100 mg L^{-1}). Electrolyte solution, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to Pithva et al. (2014). For sequential exposure to SS-SGF-SIF, 50 μL of cell suspension was incubated for 10 min with SS. Subsequently, cells pelleted by centrifugation were further evaluated for SS-SGF and SS-SGF-SIF passage tolerance as per the method of Charteris, Kelly, Morelli, and Collins (1998a). Bacterial suspension without enzymes of SS, SGF and SIF treatment was included as control. Survival (log cfu mL^{-1}) after each enzymatic exposure i.e. lysozyme, pepsin and pancreantin was determined by viable count method.

2.3.3. Mucin adherence assay

The adhesive capacity was determined by adhesion of Lactobacilli cells to immobilized porcine stomach type III mucin (Sigma-Aldrich, USA) in 96-well microtitre plates as described by Dhanani and Bagchi (2013). After serial dilution cfu mL^{-1} of adhered bacterial cells were calculated by plating on MRS agar.

2.3.4. Autoaggregation assay

Autoaggregation assay was performed as per the method of Del Re, Sgorbati, Miglioli, and Palenzola (2000) with minor modifications. Briefly, 0.1 mL cell suspension was mixed with 1.9 mL of phosphate buffer to prepare 2 mL system containing 10^9 cfu mL^{-1}, vortexed for 10 s to ensure proper mixing and incubated at 37 °C. 0.1 mL of the upper suspension was carefully collected, mixed with 0.9 mL PBS and A_{600} (UV 1601, Shimadzu, Japan) was measured. Autoaggregation %: $[(A_0 - A_t)/A_0] \times 100$, where A_0 is A_{600} at 0 h and A_t indicates A_{600} of cell suspension at different time intervals (2, 4 and 24 h).

2.3.5. Hydrophobicity assay

Bacterial adhesion to hydrocarbons was performed and results were expressed as per Rosenberg, Gutnick, and Rosenberg (1980).

2.3.6. Antimicrobial activity

The ability of *Lactobacillus* strains to inhibit the test bacteria was examined by the spot inoculation test (Schillinger & Lucke, 1989) with minor modifications as indicated in our previous report (Pithva et al., 2014). The antifungal activity of Lactobacilli against food spoilage fungi and pathogenic yeasts was evaluated as per the method described by Magnusson and Schnürer (2001) by