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International Journal of Food Microbiology 109 (2006) 205–214
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Lactobacillus spp. with in vitro probiotic properties from human faeces and traditional fermented products

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Received 28 April 2005; received in revised form 28 September 2005; accepted 2 January 2006

Abstract

Lactobacillus strains from traditional African fermented milk products, as well as human intestinal isolates were identified and investigated in vitro for their technological and functional characteristics as potential new probiotic strains. To test survival under gastrointestinal conditions, first the protective effect of milk and the effects of medium composition, lysozyme, pepsin, and pH of the medium on bacterial viability were assessed in vitro using the Plackett–Burman statistical model and the commercially used *L. johnsonii* LA1 probiotic strain. The use of either an artificial gastric electrolyte solution or MRS did not play a significant role in the viability of the cultures, while lysozyme, acidic conditions (pH 2.5), pepsin and the presence of milk significantly influenced the survival of the strain. Therefore, these parameters were selected as important test variables in a model stomach passage survival trial. Five strains identified as *L. plantarum* and two identified as *L. johnsonii* showed good survival under simulated gastrointestinal conditions. These selected strains also showed antimicrobial activity, probably due to production of organic acids. All strains exhibited bile salt hydrolase activity, while only the *L. plantarum* strains showed β-galactosidase activity.

Keywords: Probiotics; *Lactobacillus*; Acid and bile resistance; Bile salt hydrolase; β-galactosidase; Kule naoto; Kwerionik

1. Introduction

The intestinal microbial population is a dynamic ecosystem of high complexity, consisting of an estimated number of 10^{14} microorganisms including more than 400 bacterial species (Luckey and Floch, 1972). It plays a vital role by providing the host with enzymes necessary for assimilation and/or synthesis of certain nutrients, as well as in detoxification of harmful dietary compounds (Heneghan, 1988). In addition, the gastrointestinal microbiota represent a natural barrier against pathogens (Hentges, 1992) and stimulate bowel motility and the immune system (Holzapfel et al., 1998). 'Probiotic' refers to viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the gut (Holzapfel and Schillinger, 2002). Strains of *Lactobacillus* spp. occur naturally in the human intestine and for this reason, such intestinal strains are also preferentially developed for commercial use as probiotics.

According to the guidelines for the evaluation of probiotics in food reported by a Joint FAO/WHO working group (Chesson et al., 2002), two of the currently most widely used in vitro tests are resistance to gastric acidity and bile salts, as based on both survival and growth studies. The predictability of these in vitro tests is limited, but the use of sophisticated and dynamic, computer-controlled models of the gastrointestinal tract, like the one developed by Marteau et al. (1997), is beyond the scope of most laboratories. Other functional properties used to characterise probiotics are the production of antimicrobial compounds, the ability to modulate immune responses and adhesion to gut tissues (Saarela et al., 2000). The mechanism through which probiotics may antagonise pathogens involves production of antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide and bacteriocins. Even though *Lactobacillus* spp. are classified as GRAS (generally recognised as safe) microorganisms because of their long and safe use as starter cultures in fermented products, it is important to assess the safety

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doi:10.1016/j.ijfoodmicro.2006.01.029

of those microorganisms intended for use as food additives. Because of the serious concerns about the growing level of resistance to antibiotics in regular use in human medicine, one of the aspects which needs to be analysed is antibiotic resistance. Probiotic strains, as well as bacteria used in food, may harbour resistance genes which may be transferred to pathogenic bacteria (Teuber et al., 1999). Therefore, probiotic strains intended for the market should be screened for transferable resistance genes.

'Kule naoto' is a traditional fermented milk product of a nomadic community of southern Kenya and northern Tanzania called Maasai. As the Maasai rarely consume meat, fruits or grains, milk constitutes their principal nutritional source. Apart from its characteristic taste and aroma, people attribute to this product therapeutic properties such as prevention of diarrhoea and constipation (Mathara et al., 2004). The objective of this work was to investigate strains from traditional African fermented milk products, as well as human intestinal isolates, as potential probiotics in in vitro studies. Strains of *Lactobacillus* spp. were previously isolated in our institute from two traditional fermented milk products: the Kenyan Maasai 'Kule naoto' (Mathara et al., 2004) and 'Kwerionik' from Uganda. These and new isolates from children's faeces were identified and investigated for their technological and functional characteristics as potential new probiotic strains.

2. Materials and methods

2.1. Bacterial strains used in this study

2.1.1. Bacterial strains

For isolation of lactobacilli from children's faeces, 1 g of freshly collected faeces from a 3-year-old and a 4-year-old healthy child, were suspended (10^{-1} dilution) in quarter-strength Ringer solution (QRS) and further diluted in a ten-fold dilution series with QRS. One hundred microliters of suitable dilutions were spread plated onto Rogosa Agar (Merck, Darmstadt, Germany) and de Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) adjusted to pH 6.5 to enumerate bacteria and isolate predominant colonies. Plates were incubated in anaerobic jars with Anaerocult® (Merck, Darmstadt, Germany) at 37 °C for 48 h. Representative colonies were picked from plates and pure cultures were obtained. Cultures were kept at −80 °C in MRS broth (Merck) containing 20% (v/v) glycerol. Twenty Gram-positive, catalase-negative, rod-shaped isolates from infant faeces and fifteen isolates (from BFEL culture collection) from two traditional fermented milk products 'Kule naoto' (Kenya) and Kwerionik (Uganda) were used as probiotic candidate strains in this study.

2.1.2. Probiotic control strains, culture media and growth conditions

The well-studied probiotic strains *Lactobacillus johnsonii* LA1 (deposited in our BFEL culture collection as BFE 663), and *Lactobacillus paracasei* (BFE 675, isolated from Actimel, Danone) and the non-probiotic *Lb. plantarum* ATCC 8014 strain (Table 1) were all used as reference strains. The potential probiotic strains used in this study are also listed in Table 1. All strains were cultivated in MRS broth using a 1% inoculum and aerobic incubation at 37 °C for 24 h.

Table 1
Strains included in this study

Origin	Strain	Phenotypical characterisation	API CH50	Rep-PCR
Kule naoto (African fermented milk product, strains isolated in Kenya and deposited into BFEL culture collection)	BFE 6128	*L. acidophilus*	*L. crispatus*	*L. johnsonii*
	BFE 6154	*L. acidophilus*	*L. acidophilus*	*L. johnsonii*
	BFE 5878	*L. plantarum*	*L. plantarum*	*L. plantarum*
	BFE 5092	*L. plantarum*	*L. plantarum*	*L. plantarum*
Kwerionik (African fermented milk product, strains isolated in Uganda and deposited in BFEL culture collection)	BFE 5759	*L. plantarum*	*L. paracasei*	*L. plantarum*
Children's faeces BFEL culture collection	BFE 1684	*L. plantarum*	*L. plantarum*	*L. plantarum*
	BFE 1685	*L. plantarum*	*L. paracasei*	*L. plantarum*
LC-1 Nestlé BFEL culture collection	BFE 663 (LA1)	n.d.	n.d.	*L. johnsonii*
Actimel-Danone BFEL culture collection	BFE 675	n.d.	n.d.	*L. paracasei*
American Type Culture Collection	ATCC 8014		*L. plantarum*	
	ATCC 43895		*Escherichia coli*	
Bundesanstalt für Fleischforschung	S 514		*Salmonella typhimurium*	
Deutsche Sammlung von Mikroorganismen und Zellkulturen	DSM 15590		*Enterococcus faecium*	
	DSM 20409		*Enterococcus faecalis*	
	DSM 6178		*Streptococcus mutans*	
	DSM 20011		*L. casei*	
Weihenstephan Institute	WS 2258		*Listeria innocua*	

n.d. Not determined.
[a]These strains have been previously identified and are part of the BFEL Culture Collection.

2.2. Characterisation of potentially probiotic strains

2.2.1. Phenotypic characterisation.

The 35 candidate probiotic strains were tested for their ability to survive a successive passage through solutions mimicking saliva, gastric juice and intestinal juice (see below). Only those strains which survived the simulated gastrointestinal passage in detectable numbers were unequivocally identified and further investigated for in vitro probiotic properties. Strains were characterised phenotypically by testing their growth in MRS broth at 15 and 45 °C, production of CO_2