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Screening for lactic acid bacteria based on antihyperglycaemic and probiotic potential and application in synbiotic set yoghurt

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ABSTRACT

Twenty lactic acid bacteria were screened for the abilities of rat intestinal α-glucosidase inhibition, short-chain fatty acids production, utilization of prebiotics as well as gastrointestinal tract tolerance. Nine strains inhibited α-glucosidase, four of which, *Lactobacillus acidophilus* CCFM6, *Lactobacillus plantarum* CCFM47, CCFM232 and *Lactobacillus rhamnosus* GG (LGG) were tolerant to simulated gastrointestinal juices with survival rates up to 60% following simulated digestion. When grown on soybean oligosaccharides (SBOs), CCFM47 produced propionic (39.9 mM) and butyric (3.5 mM) acids while strain CCFM6 produced 17.2 mM propionic acid. Strains CCFM6 and CCFM47 were further tested for their viability and survival in set yoghurt supplemented with SBOs. SBOs enriched yoghurt improved probiotic survival in the simulated gastric juice and significantly improved α-glucosidase inhibition ($P < 0.05$). Thus strains CCFM47 and CCFM6 can be used as probiotics with antihyperglycaemic potential in the formulation of functional foods such as yoghurt.

1. Introduction

Diabetes has been recognized as one of the global epidemics (WHO/FAO, 2002), with obesity, high calorific diets and physical inactivity as some of the primary causes of type 2 diabetes (T2D) in people who are genetically predisposed (Everard & Cani, 2013). T2D is characterized by high postprandial blood glucose levels and can be managed through utilization of α-amylase or α-glucosidase inhibitors like acarbose and miglitol, which limit the hydrolysis of starch (Bischoff, 1994). Diets rich in fibre and prebiotics and low in saturated fat are recommended in nutrient therapy for diabetes patients (Afaghi, Kordi, & Sabzmakan, 2015; Baboota et al., 2013; Mata-Cases et al., 2015). Drugs like metformin that improve insulin activity and rapid insulin analogues to control postprandial hyperglycaemia are prescribed at advanced stages (Mazziotti, Gazzaruso, & Giustina, 2011). On the other hand, the influence of gut microbiota on the incidence of T2D is also of great importance as it has been reported to alter fatty acid metabolism in adipose tissue and levels of gut hormones like peptide YY (Cani et al., 2007). Other studies have shown differences in gut microbiota of both obese

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and lean subjects, indicating the role played by gut microbiota in the pathogenesis of obesity and T2D (Fei & Zhao, 2013; Turnbaugh et al., 2006).

Some reports have suggested that certain lactic acid bacteria (LAB) can inhibit α-glucosidase (Ankolekar, Pinto, Greene, & Shetty, 2012; Ramchandran & Shah, 2008) and therefore could be utilized to alleviate the effects of diabetes (Chen et al., 2014; Yun, Park, & Kang, 2009). It is therefore possible that an oral supplementation of probiotics with antihyperglycaemic properties might be beneficial to T2D patients. Besides α-glucosidase inhibitory properties, short-chain fatty acid (SCFAs) producing LAB are desirable dietary supplements for T2Dpatients. SCFAs alleviate the effects of a high-caloric diet by simulating the hormones involved in homeostasis such as leptin and glucagon-like peptide 1 (GLP-1) (Xiong et al., 2004; Yadav, Lee, Lloyd, Walter, & Rane, 2013). SCFAs can be produced through colonic bacterial fermentation of indigestible carbohydrates and undigested carbohydrates in the colon (Bergman, 1990).

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on their host (FAO/WHO, 2002). In the development of synbiotic foods, selection of the right food matrix for the delivery of the probiotics into their host not only ensures their viability throughout shelf life but also enables them to overcome physical and chemical barriers in the gastrointestinal tract (GIT) (Schillinger, Guigas, & Heinrich Holzapfel, 2005). Resistance to simulated gastric juice is an important prerequisite for probiotic strains to effectively colonize the colon of their host. Fermented milk products supplemented with prebiotics such as inulin have been shown to improve probiotic viability and technological characteristics like texture and stability (Akın, Akın, & Kırmacı, 2007; Buriti, Castro, & Saad, 2010). Combination of probiotics with ability to inhibit α-glucosidase and a prebiotic can be used to mitigate T2D and also protect the probiotics from adverse conditions of the GIT.

Therefore, the objective of this study was to screen LAB with abilities to inhibit α-glucosidase, GIT tolerance, SCFAs production and utilization of prebiotics. The selected probiotics with α-glucosidase inhibition properties were further explored for the production of a synbiotic fermented milk product in co-culture with *Streptococcus thermophilus* and their viability throughout the 21 days of storage at 4 °C.

2. Materials and methods

2.1. Microorganisms and growth conditions

The 20 LAB strains used in this study belonged to the culture collection of Jiangnan University and are listed in Table 1. Lactobacilli were grown in routine without agitation in De Man–Rogosa–Sharpe (MRS) broth culture media whereas streptococci were grown in M17 broth at 37 °C for 18 h (Ashraf & Shah, 2011).

2.2. Preparation of cell-free extract and cell-free supernatants

After incubation, the growth media was centrifuged at 804 *g* for 15 min at 4 °C to remove bacterial cells. The pH of the supernatant was adjusted to pH 7.4 using 1 mol/L NaOH and filtered through 0.22-μm membrane filters to obtain the cell-free supernatant (CFS-MRS) that was kept in an ice bath. The intact cells were washed three times with phosphate-buffered saline (PBS) which contained 0.80% NaCl, 0.02% KCl, 0.02% KH_2PO_4, and 0.22% Na_2HPO_4 pH 7.4 solution after which the cells

Table 1 – Taxon and rat intestinal α-glucosidase inhibition (%) of LAB strains.

Strain	Taxon	Inhibition of rat intestinal α-glucosidase		
		CFE	CFS-MRS	CFS-RSM
LGG	*Lactobacillus rhamnosus*	$8.4 \pm 0.3^{c,d}$	$13.5 \pm 1.3^{b,c}$	$6.1 \pm 0.4^{a,b}$
CCFM4	*Lactobacillus bulgaricus*	11.8 ± 0.4^{e}	12.8 ± 0.9^{b}	18.6 ± 1.2^{g}
CCFM6	*Lactobacillus acidophilus*	$9.9 \pm 1.7^{d,e}$	$18.7 \pm 0.4^{d,e,f}$	$8.7 \pm 0.3^{b,c,d}$
CCFM10	*Lactobacillus plantarum*	12.0 ± 1.5^{e}	$22.9 \pm 0.8^{g,h}$	$14.1 \pm 1.9^{e,f}$
CCFM16	*Bifidobacterium bifidum*	ND	$25.0 \pm 0.7^{h,i}$	$19.7 \pm 2.0^{g,h}$
CCFM29	*Lactobacillus bulgaricus*	$8.5 \pm 0.9^{c,d}$	$22.8 \pm 0.9^{g,h}$	5.3 ± 0.7^{a}
CCFM47	*Lactobacillus plantarum*	$4.6 \pm 0.7^{a,b}$	$21.7 \pm 0.8^{f,g}$	$25.9 \pm 0.6^{j,k}$
CCFM137	*Lactobacillus acidophilus*	ND	$15.9 \pm 2.0^{c,d}$	$17.3 \pm 0.3^{f,g}$
CCFM147	*Streptococcus thermophillus*	$6.1 \pm 0.9^{b,c}$	27.9 ± 0.5^{i}	$23.2 \pm 0.3^{i,j}$
CCFM218	*Streptococcus thermophillus*	ND	$25.8 \pm 0.6^{h,i}$	$22.4 \pm 3.1^{h,i}$
CCFM231	*Lactobacillus plantarum*	ND	9.5 ± 0.5^{a}	$16.4 \pm 1.9^{f,g}$
CCFM232	*Lactobacillus plantarum*	$5.6 \pm 0.5^{a,b}$	9.8 ± 1.0^{a}	27.4 ± 0.6^{k}
CCFM236	*Lactobacillus casei*	ND	$17.5 \pm 2.4^{d,e}$	18.1 ± 0.4^{g}
CCFM237	*Lactobacillus rhamnosus*	ND	$20.6 \pm 1.0^{f,g}$	$11.3 \pm 1.9^{d,e}$
CCFM240	*Lactobacillus plantarum*	3.3 ± 0.9^{a}	$25.4 \pm 0.2^{h,i}$	$32.9 \pm 1.^{l}$
CCFM241	*Lactobacillus plantarum*	ND	$19.7 \pm 2.2^{e,f}$	$16.6 \pm 0.9^{f,g}$
CCFM307	*Lactobacillus plantarum*	ND	26.8 ± 0.2^{i}	$23.9 \pm 1.1^{i,j}$
CCFM308	*Lactobacillus plantarum*	ND	$20.8 \pm 1.2^{f,g}$	$23.5 \pm 0.3^{i,j}$
CCFM309	*Lactobacillus plantarum*	ND	12.9 ± 0.9^{b}	$7.9 \pm 0.6^{a,b,c}$
CCFM311	*Lactobacillus rhamnosus*	ND	$10.8 \pm 0.4^{a,b}$	$10.4 \pm 0.4^{c,d}$

Each value in the table is the mean ± standard deviation ($n = 3$). Means in the same column with different superscript letters indicate significant differences among strains ($P < 0.05$). CFE, cell-free extract; CFS-MRS, cell-free supernatant De Man–Rogosa–Sharpe; CFS-RSM, cell-free supernatant reconstituted skim milk; LAB, lactic acid bacteria; ND, not detected.