International Journal of Food Microbiology 147 (2011) 97–104

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Evaluation of the functional potential of *Weissella* and *Lactobacillus* isolates obtained from Nigerian traditional fermented foods and cow's intestine

Funmilola A. Ayeni [a,b], Borja Sánchez [a], Bolanle A. Adeniyi [b], Clara G. de los Reyes-Gavilán [a], Abelardo Margolles [a], Patricia Ruas-Madiedo [a,*]

[a] Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Villaviciosa, Asturias, Spain
[b] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo State, Nigeria

ARTICLE INFO

Article history:
Received 27 September 2010
Received in revised form 18 March 2011
Accepted 22 March 2011

Keywords:
Weissella
Lactobacillus
Probiotic
Antibiotic resistance
Gastrointestinal transit
Adhesion epithelial cell culture

ABSTRACT

The characterisation of 24 lactic acid bacteria (LAB) isolates from Nigerian traditional fermented dairy foods, including some cow's intestine isolates, was conducted in order to select isolates for potential use as probiotics. LAB isolates were identified by partial sequencing the 16S rRNA gene as belonging to the species *Lactobacillus paracasei*, *Lactobacillus brevis* and mainly *Weissella confusa*. At the end of a characterisation process, 2 *L. paracasei* and 2 *W. confusa* isolates were selected, and their resistance to a simulated gastrointestinal digestion and their ability to adhere to eukaryotic cell lines were assessed. The survival to the simulated gastrointestinal passage was higher when bacterial suspensions were made in skimmed milk (2.0 ± 0.8 log units reduction) or at the simulated gastric juice pH 3 (2.7 ± 0.9 log units reduction) than at pH 2.0 (5.5 ± 0.7 log units reduction). Adhesion of LAB to both intestinal and vaginal epithelial models was comparable or higher than that of the reference *Lactobacillus rhamnosus* GG. However, some of the isolates increased the adhesion of the pathogen *Escherichia coli* LMG2092 to HT-29 and HeLa monolayers. Overall, isolates *L. paracasei* UI14 and *W. confusa* UI7 are good candidates for further studying potential benefits that support their use as probiotics. This is one of the few articles reporting the characterisation and the probiotic potential of *Weissella*, although more studies are needed in order to establish their safety for potential probiotic applications.

1. Introduction

Lactic acid bacteria (LAB) are being empirically employed from ancient times for the natural bio-preservation of raw materials. The development of food technologies involves the utilisation of specific, well-identified, and characterised LAB isolates to improve the safety as well as the organoleptical, nutritional or health properties of foods, i.e. this is the basis for the concept of "functional starter" (Leroy and De Vuyst, 2004). Due to the long history of use in human consumption, some genera of LAB are "Generally Recognized As Safe" (GRAS) by the United States Food and Drug Administration (FDA). Several species of these genera are also included in the list of taxonomic units proposed by the European Food Safety Authority (EFSA) for "Qualified Presumption of Safety" (QPS) status. Amongst LAB with QPS status, those commonly found in fermented foods are *Lactococcus lactis*, *Streptococcus thermophilus*, and several species of *Lactobacillus*, *Leuconostoc* and *Pediococcus* (EFSA, 2007).

Some specific LAB strains are considered as probiotics, which have been defined by the WHO/FAO as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO, 2006). The genera most commonly used as probiotics for oral delivery in human consumption are *Bifidobacterium* and *Lactobacillus* (Margolles et al., 2009). Even if some species of these genera are GRAS or have the QPS status, the working groups of the WHO/FAO recommend a proof that a given probiotic strain is safe. To check the safety, a series of *in vitro* tests are required such as, amongst others, the determination of the antibiotic resistant patterns and the production of toxic compounds. According to the guidelines proposed by these organisations, one of the criteria for the selection of probiotic strains is their ability to transiently colonise the human mucosa (WHO/FAO, 2006). This property could help to maintain or improve the health of the intestinal and vaginal environments and thereby the well-being of the consumer (Abad and Safdar, 2009; Lee and Salminen, 2009).

In developed countries, the search for new strains with functional properties is of great interest from both health and industrial points of view. In this way, the traditional fermented foods from non-industrialised countries constitute a reservoir to search for new strains with novel

* Corresponding author at: Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Carretera de Infiesto s/n, 33300 Villaviciosa, Asturias, Spain. Tel.: +34 985892131; fax: +34 985892233.
E-mail address: ruas-madiedo@ipla.csic.es (P. Ruas-Madiedo).

0168-1605/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2011.03.014

functional properties (Lee et al., 2005; Mathara et al., 2004; Mohammed et al., 2009; Thapa et al., 2006). From the point of view of developing countries, the study of the properties of the isolated strains could contribute to improve the safety, quality and industrial value of traditionally fermented foods and could also open the possibility of their use for different applications.

In a previous work, we have reported the antimicrobial ability of isolates from Nigerian fermented foods and animal intestines against clinical pathogen strains obtained from patients with urinary tract infections (Ayeni et al., 2009). The aim of the current work was to identify and characterise these LAB and select from them some with probiotic potential through the study of their ability to survive to simulated gastrointestinal digestion, to adhere to epithelial cell lines and to inhibit the adhesion of *Escherichia coli* LMG2092.

2. Material and methods

2.1. Identification of isolates and growth conditions

In this study, we used 24 LAB isolates (Table 1) from different dairy products [cheese, fermented skimmed milk (nono), whey and milk] and animal sources (cow's large intestine) in four geographic regions of western Nigeria (Ekiti, Osun, Ondo and Oyo). They were selected from 134 initial isolates using as a criterion their ability to inhibit the growth of uropathogenic *Staphylococcus aureus* strain (Ayeni et al., 2009). Selected LAB were grown in MRS (Biokar Diagnostics, Beauvais, France) broth for 24 h at 37 °C and 5% CO_2 in a Heracell® 240 incubator (Thermo Electron LDD GmbH, Langenselbold, Germany) and were spread on the surface of MRS (Biokar) agar plates to check purity. A single colony was picked up to prepare new stocks (stored at −80 °C in MRS with 20% glycerol) which were identified by partially sequencing the 16S rRNA gene using the Y1–Y2 primers as previously reported (around 350 bp) (Ruas-Madiedo et al., 2005). As standard procedure, LAB isolates from stocks were cultivated overnight at 37 °C, 5% CO_2 and used to inoculate (2%) fresh MRS media which were cultivated for 24 h under the same conditions.

2.2. Production of lactic acid and volatile compounds

The production of lactic acid in the supernatants of 24 h grown LAB cultures was measured by ion-exchange HPLC using a chromatographic system composed of an Alliance 2690 module injector, a Photodiode Array PDA 996 detector and the Empower software (Waters, Milford, MA, USA) under conditions previously described (Ruas-Madiedo et al., 2005). The volatile compounds were determined by means of head-space (HS) GC–MS. Samples (400 μL) of supernatants were mixed with cyclohexanone (0.36 mg/mL) as internal standard and were placed into 10-mL glass tubes sealed with rubber and metallic caps. The analysis was carried out in a 6890 N Agilent GC coupled with a HS automatic injector G1888 series and with a 5975B inert MS detector (Agilent Technologies Inc., Palo Alto, CA) using conditions previously reported (Salazar et al., 2009).

2.3. Antibiotic resistance pattern

The minimal inhibitory concentration (MIC) against several antibiotics was studied in 9 isolates selected according to their origin and belonging to different species. VetMic™ Lact-I microdilution tests (SVA, Uppsala, Sweden) were used to determine the MIC to gentamicin (concentration range tested: 0.5–256 μg/mL), kanamycin (2–1024 μg/mL), streptomycin (0.5–256 μg/mL), neomycin (0.5–256 μg/mL), tetracycline (0.12–64 μg/mL), erythromycin (0.016–8 μg/mL), clindamycin (0.03–16 μg/mL), and chloramphenicol (0.12–64 μg/mL). Additionally, hand-made plates were used for the following antibiotics: ampicillin (1–1024 μg/mL, Apollo Scientific Ltd., Cheshire, UK), ciprofloxacin (0.1–128 μg/mL, Sigma Chemical Co., St. Luis, MO, USA), trimethoprim–sulfamethoxazole (TMP–SMX, 0.25–256 μg/mL, Celtech Pharma S.A., Madrid, Spain), fosfomycin (3.13–3200 μg/mL, Pharmazam, Barcelona, Spain) and nitrofurantoin (0.25–256 μg/mL, Laboratorios ERN S.A., Barcelona, Spain). When necessary, the concentration of the excipient was subtracted for the calculation of the corresponding concentrations of each antibiotic. The LAB isolates were cultured overnight on agar LSM [90% Isosensitest (Oxoid) and 10% MRS (Klare et al., 2005)]. Individual

Table 1
Origin of LAB isolates from different traditional fermented dairy products and cow's intestine.[a]

Species	Isolate	Origin	Region	Mean ± SD			
				mg/mL	μg/mL		mg/mL
				Lactic acid	Acetaldehyde	Acetone	Ethanol
L. paracasei	UI1	Whey	Oyo	21.77 ± 2.93	13.32 ± 0.20	36.21 ± 5.73	0.012 ± 0.003
	UI2	Whey	Oyo	20.26 ± 0.97	15.77 ± 3.04	34.66 ± 4.75	0.026 ± 0.013
	UI9	Whey	Osun	19.74 ± 0.88	12.99 ± 2.94	32.06 ± 4.45	0.024 ± 0.015
	UI14	Whey	Oyo	19.68 ± 0.71	12.77 ± 2.43	38.06 ± 5.54	0.019 ± 0.006
	UI22	Whey	Ekiti	18.07 ± 4.36	12.56 ± 2.78	27.22 ± 7.97	0.010 ± 0.003
L. brevis	UI3	Whey	Oyo	16.54 ± 3.45	42.43 ± 9.11	3.99 ± 0.14	3.27 ± 1.22
	UI12	Cow intestine	Ekiti	13.70 ± 3.80	24.87 ± 2.42	7.48 ± 4.57	4.29 ± 0.37
W. confusa	UI4	Whey	Oyo	13.22 ± 2.04	51.84 ± 5.86	28.09 ± 4.09	4.03 ± 0.24
	UI5	Cow intestine	Oyo	13.39 ± 1.02	46.73 ± 7.02	23.88 ± 3.73	3.81 ± 0.28
	UI6	Cow intestine	Oyo	14.81 ± 1.94	49.72 ± 7.65	23.65 ± 4.88	3.47 ± 1.57
	UI7	Cheese	Oyo	13.61 ± 1.78	45.71 ± 10.06	23.07 ± 0.42	3.78 ± 0.12
	UI8	Whey	Ekiti	15.39 ± 3.57	49.58 ± 13.39	24.63 ± 1.30	4.01 ± 0.46
	UI10	Nono	Ondo	15.93 ± 3.22	39.32 ± 8.93	26.59 ± 8.27	2.68 ± 1.32
	UI11	Whey	Osun	13.37 ± 1.32	56.56 ± 12.06	30.33 ± 4.86	4.82 ± 0.61
	UI13	Whey	Ekiti	14.43 ± 2.05	54.78 ± 14.00	30.38 ± 4.20	4.70 ± 0.54
	UI15	Cow intestine	Oyo	14.14 ± 2.17	39.07 ± 6.23	28.30 ± 1.55	3.96 ± 0.21
	UI16	Milk	Ekiti	13.93 ± 2.30	64.02 ± 25.49	32.75 ± 16.83	5.03 ± 1.81
	UI17	Cow intestine	Oyo	15.00 ± 2.73	49.95 ± 14.15	26.49 ± 1.32	4.13 ± 0.50
	UI18	Cheese	Osun	14.26 ± 2.28	46.76 ± 14.35	24.81 ± 4.55	4.00 ± 0.54
	UI19	Cow intestine	Oyo	14.26 ± 1.97	45.88 ± 10.11	22.25 ± 2.02	3.89 ± 0.26
	UI20	Cow intestine	Oyo	15.01 ± 2.32	44.92 ± 9.08	16.91 ± 1.14	4.07 ± 0.16
	UI21	Milk	Ekiti	13.73 ± 2.10	47.01 ± 6.75	19.70 ± 5.81	3.81 ± 0.50
	UI23	Nono	Ondo	13.59 ± 1.38	35.05 ± 16.78	15.80 ± 7.76	3.94 ± 0.54
	UI24	Cow intestine	Oyo	14.14 ± 2.49	55.90 ± 15.70	21.17 ± 0.50	4.91 ± 0.83

[a] Production of metabolites (lactic acid, acetaldehyde, acetone and ethanol) in MRS broth after 24 h of cultivation.