LWT - Food Science and Technology 87 (2018) 478–487

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

In-vitro investigation into probiotic characterisation of *Streptococcus* and *Enterococcus* isolated from camel milk

Mutamed Ayyash [a, *], Aisha Abushelaibi [a], Suheir Al-Mahadin [a], Mohamed Enan [b], Khaled El-Tarabily [b], Nagendra Shah [c]

[a] Food Science Department, College of Food and Agriculture, United Arab Emirates University (UAEU), PO Box 1555, Al Ain, United Arab Emirates
[b] Department of Biology, College of Science, United Arab Emirates University (UAEU), PO Box 1555, Al Ain, United Arab Emirates
[c] Food and Nutritional Science, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong

ARTICLE INFO

Article history:
Received 18 February 2017
Received in revised form
8 September 2017
Accepted 15 September 2017
Available online 18 September 2017

Keywords:
Camel milk
Probiotics
Streptococcus
Enterococcus
Cholesterol lowering

ABSTRACT

The objectives of this study were to isolate lactic acid bacteria (LAB), namely *Streptococcus* and *Enterococcus*, on M17 agar from raw camel milk and investigate their probiotic characteristics. Physiological properties, cell surface properties (hydrophobicity, autoaggregation, co-aggregation), acid and bile tolerance, bile salt hydrolysis, cholesterol removing ability, exopolysaccharide (EPS) production, hemolytic and antimicrobial activities, resistance toward lysozyme and six antibiotics, and fermentation profile (growth, pH, and proteolysis) were examined. rDNA sequencing was carried out to identify the isolates and to acquire Genbank accession numbers. LAB isolates showed cholesterol lowering and pathogens inhibition properties. Hydrophobicity and autoaggregation results revealed strong attachment capabilities of the isolated LAB. Resistance of LAB isolates to lysozyme activity and to 60 °C were also high. Identified LAB exhibited promising fermentation profile. This study reveals that the isolated LAB isolates especially *E. faecium* KX881783 and *S. equinus* KX881778 may be excellent candidates to produce functional foods to promote health benefits.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

The need to prevent or alleviate symptoms of serious diseases including inflammatory bowel disease, irritable bowel syndrome, constipation, antibiotic-associated and acute diarrhea, allergy related conditions, hypertension, and diabetes is always on demand (Souza, Cocco, Sarni, Mallozi, & Solé, 2010; Weichselbaum, 2009). Moreover, promotion of human health via functional food is in high demand (Tripathi & Giri, 2014). Natural approaches including use of probiotics and functional foods are important means having no side effects (Gionchetti et al., 2010). Functional foods are currently produced mainly (but not limited) by the aid of probiotic microorganisms (Tripathi & Giri, 2014). FAO/WHO (2002) has defined probiotics as “living microorganisms which, when administrated in adequate numbers, confer a health benefit to the host.” Consequently, several criteria are being implemented to examine probiotic characteristics of a newly isolated microorganism including tolerance to low acid and bile conditions, to assimilate cholesterol in food and human intestine, to hydrolyze bile salt, having no haemolytic activity, ability to possess antimicrobial properties, and ability to survive during the fermentation process. The potential probiotic should demonstrate resistance to acidic condition and bile salts, sensitivity to antibiotics, ability to inhibit pathogens, inability to show haemolytic activity, ability to lower cholesterol, ability to attach to epithelial cells and strong fermentation profile when used in foods (Khan, 2014; Naidu, Bidlack, & Clemens, 1999).

Potential probiotics are isolated from food matrices in which those microorganisms are used (Das, Khowala, & Biswas, 2016). Due to fact that camel milk contains a greater amount of natural antimicrobial compounds than bovine milk (Elagamy, Ruppanner, Ismail, Champagne, & Assaf, 1996), camel milk is a potential source from which LAB can be isolated with high probiotic potential (Abushelaibi, Al-Mahadin, El-Tarabily, Shah, & Ayyash, 2017a). Food & Agriculture Organization (2008) has reported Somalia, Saudi Arabia and United Arab Emirates (UAE) as being the highest camel milk producing countries. Several attempts have been carried out to isolate LAB from camel milk and its products. *Lactobacillus* spp. was the major group has been isolated from camel milk (Abushelaibi,

* Corresponding author.
E-mail address: mutamed.ayyash@uaeu.ac.ae (M. Ayyash).

https://doi.org/10.1016/j.lwt.2017.09.019
0023-6438/© 2017 Elsevier Ltd. All rights reserved.

Al-Mahadin, El-Tarabily, Shah, & Ayyash, 2017b; Amina et al., 2014; Ashmaig, Hasan, & Gaali, 2009; Belkheir, Centeno, Zadi-Karam, Karam, & Carballo, 2016; Biratu & Seifu, 2016; Fguiri et al., 2016; Mahmoudi et al., 2016; Soleymanzadeh, Mirdamadi, & Kianirad, 2016; Wang, Zhou, Xia, Zhao, & Shao, 2016; Yateem, Balba, Al-Surrayai, Al-Mutairi, & Al-Daher, 2008). These reports have major drawbacks in the procedure section. For example, the study by Yateem et al. (2008) lacks probiotic characterization including their acid and bile tolerance abilities, cholesterol removal ability, hemolytic pattern, and antimicrobial activity and use of old non-DNA based methods for identification of isolates. Soleymanzadeh et al. (2016) lack many probiotic parameters to provide an evidence that the isolated LABs have probiotics characteristics. Likewise, several attempts have been made to isolate and characterize *Streptococcus* and *Enterococcus* strains from camel milk with major demerits (Abdelgadir, Nielsen, Hamad, & Jakobsen, 2008; Akhmetsadykova, Baubekova, Konuspayeva, Akhmetsadykov, & Loiseau, 2014; Davati, Yazdi, Zibaee, Shahidi, & Edalatian, 2015; Ghali, Scott, Alhadrami, & Al Jassim, 2011; Hamed & Elattar, 2013; Hassaïne, Zadi-Karam, & Karam, 2007; Jans, Bugnard, Njage, Lacroix, & Meile, 2012; Kadri et al., 2014; Kadri, Spitaels, et al., 2015; Kadri, Vandamme, et al., 2015; Mahmoud, Montaser, Al Zhrani, & Amer, 2014; Soleymanzadeh et al., 2016). The characterization procedure of probiotics properties of the isolated *Streptococcus* and *Enterococcus* spp. was incomprehensive. For instance, Jans et al. (2012), Kadri et al. (2014), Kadri, Vandamme, et al. (2015), and Ghali et al. (2011) have isolated *Streptococcus* and *Enterococcus* strains with no information regarding the probiotic characterizations. Therefore, the objectives of this study were to isolate lactic acid bacteria (LAB) on M17 agar from raw camel milk and to provide more comprehensive investigation for their probiotic characteristics such as physiological properties, cell surface properties (hydrophobicity, autoaggregation, co-aggregation), acid and bile tolerance abilities, bile salt hydrolysis, cholesterol removing property, exopolysaccharide (EPS) production ability, hemolytic and antimicrobial activities, resistance toward lysozyme and six antibiotics, and fermentation profile (growth, pH, and proteolysis) and rDNA sequencing to identify those isolates.

2. Materials and methods

2.1. Sample collection

One-hundred samples of raw camel milk were collected in sterilized bottles from different camel farms in Abu Dhabi emirate, UAE. Attention was given to obtain samples from healthy one-humped camels. Due to the distance between farms and UAE University, samples were transported on ice and directly tested in our food microbiology lab at UAE University upon arrival. All chemicals in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise.

2.2. Isolation of lactic acid bacteria

LABs were isolated by the spread-plate method on M17 agar (Oxoid, Thermo-Fisher Scientific Inc., Hampshire, UK). Plates were incubated at 37 °C for 24 h aerobically. One-hundred colonies showing different morphologies were subjected to Gram stain and catalase test. Gram-positive and catalase-negative colonies were sub-cultured in M17 broth (Oxoid) to maintain purity. Glycerol stocks (50% v/v) were prepared of each colony and stored at −80 °C.

2.3. Pathogenic strains

Pathogens *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* 02–8423, *Escherichia coli* O157:H7 1934, and *Staphylococcus aureus* ATCC 15923 were obtained from Prof. Richard Holley Laboratory, University of Manitoba, Canada. These pathogens were selected due to high association with foodborne outbreaks (World Health Organization (WHO) (2015).

2.4. Evaluation of probiotic characterization

2.4.1. Tolerances to acid and bile

Acid and bile tolerances of pure isolates were carried out, aerobically, according to methods detailed by Abushelaibi et al. (2017b). M17 broth and M17-agar were used instead of MRS. The percentage of growth suppression by bile salt was calculated using the following formula:

$$\%\,of\,suppresion = \frac{Growth\,in\,Control\,broth - Growth\,in\,bile\,broth}{Growth\,in\,control\,broth} \times 100$$

2.4.2. Autoaggregation

Autoaggregation was performed according to methods described by Collado, Meriluoto, and Salminen (2008). Autoaggregation percentage was calculated based on $\left[1 - \frac{At}{A0}\right] \times 100$, where A_t represent absorbance at time t and A_0 represent absorbance at $t = 0$.

2.4.3. Hydrophobicity

Hydrophobicity was tested against three hydrocarbons (n-hexadecane, xylene, and octane). Hydrophobicity assay and calculations (%) were carried out according to the previous method (Mishra & Prasad, 2005).

2.4.4. Co-aggregation

Co-aggregation of LAB isolates as well as four pathogens was assayed at 20 °C and 37 °C during incubation for 4 h according to methods detailed (Zuo et al., 2016). Co-aggregation percentage was expressed as. $\% = \frac{A0-At}{A0} \times 100$

2.4.5. Antibacterial activity

Antibacterial activity of cell-free supernatant of LAB isolates was tested according to (Mishra & Prasad, 2005).

2.4.6. Antibiotic susceptibility

Antibiotic resistant was performed according to the method of (Das et al., 2016). Penicillin (PEN; 10 μg), trimethoprim (TRI; 25 μg), ampicillin (AMP; 10 μg), clindamycin (CLI; 2 μg), vancomycin (VAN; 30 μg), and erythromycin (ERY; 15 μg) were employed. Antibiotic disks and cartridge dispenser were from Oxoid. M17 agar was used instead of MRS agar.

2.4.7. Haemolytic activity

Haemolytic activity of LAB isolates was examined on Colombia blood agar (Himedia, Mumbai, India) according to Angmo, Kumari, Savitri, and Bhalla (2016). Haemolysis was categorized into: no clear halos (γ-haemolytic or non-haemolytic), clear haemolysis zone (β-haemolytic or completely haemolytic) and a greenish halo (α-haemolytic or partially haemolytic).

2.4.8. Exopolysaccharide production

Ability to produce exopolysaccharide test (positive/negative) for LAB isolates was carried out according to the method of (Angmo

Download English Version:

https://daneshyari.com/en/article/5768636

Download Persian Version:

https://daneshyari.com/article/5768636

Daneshyari.com