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Beneficial and Safety Properties of a *Corynebacterium vitaeruminis* Strain Isolated from the Cow Rumen

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Abstract *Corynebacterium vitaeruminis* MRU4 was isolated from the cow rumen and was differentiated from other isolates by rep-PCR and RAPD and identified by 16S rRNA sequencing. This strain presented higher survival rates for low pH and bile salts treatments, and it was able to survive and multiply in simulated gastric and intestinal environments. *C. vitaeruminis* MRU4 had a 53.2% auto-aggregation rate, 42.4% co-aggregation rate with *Listeria monocytogenes* Scott A, 41.6% co-aggregation rate with *Enterococcus faecalis* ATCC 19443, 10.0% co-aggregation rate with *Lactobacillus sakei* ATCC 15521, and 98.2% cell surface hydrophobicity rate. PCR analysis showed the presence of *EFTu* and *map* genes. The strain possessed positive results for deconjugation of bile salts (taurocholic acid, taurodeoxycholic acid, glycocholic acid, and glycodeoxycholic acid) and positive results for β-galactosidase activity and lactose assimilation activity (glucose of 8.15 ± 0.01 CFU/ml and lactose of 9.24 ± 0.02 CFU/ml). No virulence was observed by phenotypical tests. *C. vitaeruminis* MRU4 was resistant to oxacillin, gentamicin, erythromycin, clindamycin, sulfa/trimethoprim, and rifampicin by the disc diffusion method and showed resistance just for vancomycin by the Etest® strips test. The strain was negative for 50 tested virulence and resistance genes based on performed PCR. Based on our knowledge, this is the first report regarding the beneficial potential of one *C. vitaeruminis* strain.

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Keywords *Corynebacterium vitaeruminis* · Beneficial properties · Virulence factors

Introduction

Beneficial bacteria are responsible for a healthy environment in the gut ecosystem when they are present in adequate concentrations [1]. Evaluation of the beneficial potential and safety properties of such strains is important, as is their use in commercial dairy products [2]. The genus *Corynebacterium* consists of numerous species, some of which are increasingly recognized as important pathogens related to human and animal diseases [3]. However, some of the strains of this genus are safe and can even be considered beneficial. *Corynebacterium vitaeruminis* has already been shown to be safe and non-pathogenic [4, 5], but it has not been studied in greater detail. In the evaluation process of newly isolated strains with beneficial potential, the bacteria as well as their virulence potential need to be identified to make sure that these strains do not present risks to consumers. Thus, this communication aimed to present select beneficial properties and safety characteristics of *C. vitaeruminis* MRU4 isolated from the cow rumen.

Materials and Methods

Corynebacterium vitaeruminis MRU4

The strain *C. vitaeruminis* MRU4 was isolated from a cow rumen sample after plating on de Man Rogosa Sharpe (MRS) agar (Oxoid Ltd., Basingstoke, England) and incubating at 37 °C for 48 h. This isolate was characterized by Gram staining (positive) and the catalase test (positive), and it was

subjected to phenotypical tests to assess its resistance to gastric pH (2.0, 2.5, and 3.0; control 7.2) and bile (0.5 and 3%; control 0%) by plating and optical density, according to Argyri et al. [6]; results were compared by ANOVA and Tukey's test ($p < 0.05$) using XLSTAT 2016.01.26192 (AddinSoft, New York, NY, USA). The isolate was then differentiated from other isolated bacteria by rep-PCR and random amplification of polymorphic DNA-PCR (RAPD-PCR) [7]. Taxonomical identification was confirmed by sequencing the PCR-amplified 16S rRNA gene (Center for Human Genome Studies, Institute of Biomedical Sciences, University of São Paulo, Brazil). The resulting sequences were compared to known sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) [8]. In addition, selected enzymatic activities were detected by the API ZYM Kit (bioMérieux, Basingstoke, Hants) according to the manufacturer's instructions.

Beneficial Properties

Resistance to Simulated Gastric and Intestinal Conditions The resistance of *C. vitaeruminis* MRU4 to gastric and intestinal conditions was confirmed using an in vitro model according to dos Santos et al. [9]. Mean counts of log *C. vitaeruminis* MRU4 populations were compared by ANOVA and Tukey's test ($p < 0.05$) using XLSTAT 2016.01.26192 (AddinSoft).

Aggregation Properties Auto-aggregation and co-aggregation abilities of *C. vitaeruminis* MRU4 with co-aggregation partners *L. monocytogenes* Scott A, *Enterococcus faecalis* ATCC 19443 and *Lactobacillus sakei* ATCC 15521 were assessed according to dos Santos et al. [9].

Cell Surface Hydrophobicity The test for bacterial cell surface hydrophobicity, related to adhesion of the studied strain to hydrocarbons, was performed according to dos Santos et al. [9], with 37 °C as the incubation temperature.

Evidence for the Presence of Genes Related to Beneficial Properties DNA from *C. vitaeruminis* MRU4, cultured in MRS for 24 h at 37 °C, was isolated by the ZR Fungal/Bacterial DNA Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions, and the concentration was determined by spectrophotometry (NanoDrop, Thermo Scientific, Whaltam, MA, USA). DNA obtained from *C. vitaeruminis* MRU4 was subjected to PCR analysis for the presence of genes related to the bacterial adhesion characteristics. The target genes used were *EF*1249 (fibrinogen binding protein), *EF*2380 (membrane-associated zinc metalloprotease), *EF*2662 (choline binding protein), *prg*B (surface protein), *EFTu* (adhesion-like factor), and *map* and *mub* (mucus adhesion genes) [10].

Bile Salt Deconjugation The strain's ability to perform bile salt deconjugation was evaluated according to the method described by dos Santos et al. [9].

Lactose Assimilation The ability of the selected strain to metabolize lactose was tested according to Pelinescu et al. [11], using glucose as control. Mean counts of log *C. vitaeruminis* MRU4 populations were compared by ANOVA ($p < 0.05$) using XLSTAT 2016.01.26192 (AddinSoft).

Statistical Analysis All experiments were conducted in duplicate with three repetitions. Populations were compared by ANOVA ($p < 0.05$) using XLSTAT 2016.01.26192 (AddinSoft, New York, NY, USA).

Safety Characteristics

Phenotypical Evidence for Virulence *C. vitaeruminis* MRU4 was subjected to phenotypical tests to identify its hemolytic, DNase, gelatinase, and lipase activities, according to Barbosa et al. [12]. All tests were performed at 25 °C and 37 °C.

Biogenic Amine Production The production of biogenic amines was evaluated according to Bover-Cid, Holzapfel [13] at 25 °C and 37 °C.

Antibiotic Resistance The tested culture was subjected to phenotypical analysis of antibiotic resistance using antibiotic discs (Oxoid) and Etest® strips (bioMérieux SA, Marcy l'Etoile, France). The following antibiotics were used: oxacillin (1 μg/disc), sulfa/trimethoprim (25 μg/disc), tetracycline (30 μg/disc), imipenem (10 μg/disc), ampicillin (10 μg/disc), erythromycin (15 μg/disc), vancomycin (30 μg/disc), rifampicin (5 μg/disc), gentamicin (10 μg/disc), penicillin (10 U/disc), clindamycin (2 μg/disc), and chloramphenicol (30 μg/disc). The inhibition zones around the discs was measured and classified as presenting resistance (R) or sensitivity (S) according to the manufacturer's instructions and the recommendations of the European Committee on Antimicrobial Susceptibility Testing [14]. The presence of intermediate resistance was considered as resistant. In addition, the minimum inhibitory concentration (MIC) of five antibiotics (vancomycin, gentamicin, chloramphenicol, ampicillin, and rifampicin), representative of the important antibiotic classes, were determined. Considering the recorded MIC (μg/mL) for each antibiotic against *C. vitaeruminis* MRU4, the studied strain was classified as presenting resistance (R) or sensitivity (S), according to the manufacturer's instructions for rifampicin, and the recommendations of the European Committee on Antimicrobial Susceptibility Testing [14] for the other antibiotics tested.

Detection of Virulence and Resistance Genes The presence of 50 virulence, antibiotic resistance and biogenic amine-related genes was investigated: *vanA, vanB, vanC1, vanC-1, vanC2,* and *vanC2/C3* (vancomycin resistance); *tet(K), tet(L), tet(M), tet(O),* and *tet(S)* (tetracycline resistance); *ermA, ermB,* and *ermC* (erythromycin resistance); *catA* (chloramphenicol resistance); *aph(2")-lb., ant(4')-la, aph(2")-ld, aph(2")-lc*and *aph(3')-llla* (aminoglycoside antibiotic family resistance); *aac(6')-le-aph(2")-Ia* (gentamicin and aminoglycoside resistance); *vat(E)* (streptogramin resistance); *bcrB, bcrD,* and *bcrR* (bacitracin resistance); *ant(6)-la* (streptomycin resistance); *mur-2ed* (specific for *E. durans*); *aac(6')-li* (specific for *E. faecium*); *mur-2* (specific for *E. hirae*); $Ddl_{E.\ faecalis}$ (specific for *E. faecalis*); *ace* (adhesion of collagen of *E. faecalis*); *asa1* (aggregation substance); *cyt2* (cytolysin and hemolytic endotoxins); *esp.* (enterococcal surface protein); *efaA* (endocarditis antigen); *cob, cpd,* and *ccf* (chemotactic for human leukocytes and facilitated conjugation); *sprE* (serine protease); *fsrA, fsrB,* and *fsrC* (*gelE* regulation); *gelE* (gelatinase production); *int* and *int-Tn* (transposon integrase gene); *odc* (ornithine decarboxylase); *tdc* (tyrosine decarboxylase); *hdc1* and *hdc2* (histidine decarboxylase); *hyl* (hyaluronidase) [15, 16].

Results and Discussion

Samples of raw milk, cow, and goat salivary and vaginal mucosa swabs; ruminal boluses; consumption water; and silage were screened for presence of beneficial bacteria in order to investigate their potential application as future probiotics and to ensure their safety. A collection of 500 isolates was built based on the preliminary screening, including survival at low pH and in the presence of bile. The rep-PCR and RAPD PCR were used as basic tools for the differentiation of isolated, potentially beneficial, strains. Based on the previous 16S rRNA sequencing, according to the BLAST database analysis, the isolated strain (encoded MRU4) presented 97% similarity to *C. vitaeruminis* strain DSM 20294 and was named *C. vitaeruminis* MRU4. To the best of our knowledge, this is the first report regarding the isolation of *C. vitaeruminis* from the cow rumen. As shown in Fig. 1, *C. vitaeruminis* MRU4 presented a high survival rate in the screening process for resistance to low pH and the presence of bile salts. Comparing the initial counts and after 3 h of different pH treatments, we observed that *C. vitaeruminis* MRU4 presented a slight decrease in the microbial population at pH 2.0, and the same behavior was observed in absorbance (A) at 650 nm (Fig. 1). In addition, the strain survived at the tested bile salts concentrations and exhibited good bile tolerance after 4 h of incubation (Fig. 1). Survival at different pH values and bile salts concentrations is mandatory for probiotic cultures, since this is related to survival of these bacteria in the passage through the gastrointestinal tract [17]. Based on its enzymatic profile (Table 1), *C. vitaeruminis* MRU4 generated positive results for the presence of esterase, esterase lipase, leucine arilamidase, α-chymotrypsin, acid phosphatase, naphthol phosphohydrolase and α- galactosidase. There was no activity for the 12 enzymes included in the API ZYM test of the total 19 present enzymes: alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

The beneficial and safety characteristics related to *C. vitaeruminis* MRU4 are also summarized in Table 1. The confirmatory test for resistance at different pH values and bile salts concentrations was conducted considering the gastrointestinal tract characteristics. *C. vitaeruminis* MRU4 was able to survive in the gastric phase with a survival rate of 99.6%. In addition, the strain was able to survive and even multiply in the intestinal phase with a survival rate of 100.9%. Many studies have shown survival rates of more than 98% for potential probiotic strains [18, 19].

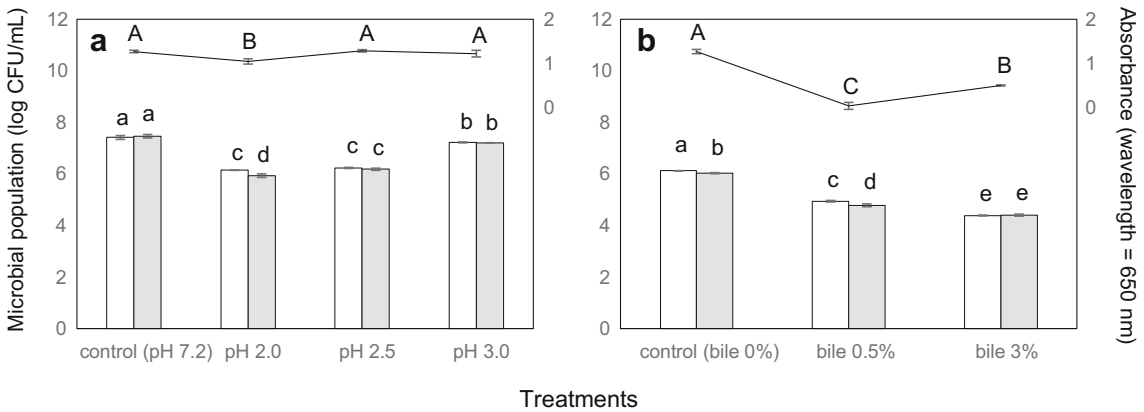

Fig. 1 Viability of *C. vitaeruminus* MRU4 at different pH values (**a** treatment of 3 h) and bile concentrations (**b** treatment of 4 h). Results obtained by plating (*white and gray bars*, indicating log CFU/mL counts at 0 h and 3 h/4 h, respectively) and optical density (*lines*, indicating recorded absorbances at 650 nm), with their respective standard deviations. *Values with different uppercase or lowercase letters* differs by ANOVA and Tukey's test ($p < 0.05$)

Table 1 Beneficial and safety properties of *Corynebacterium vitaeruminis* MRU4 isolated from the cow rumen

Properties of interest	Result for *C. vitaeruminis* MRU4
Beneficial properties	
Enzymatic activity	
Alkaline phosphatase	Negative
Esterase	Positive
Esterase lipase	Positive
Lipase	Negative
Leucine arilamidase	Positive
Valine arilamidase	Negative
Cistine arilamidase	Negative
Trypsin	Negative
α-chymotrypsin	Positive
Acid phosphatase	Positive
Naphthol phosfohydrolase	Positive
α-galactosidase	Positive
β-galactosidase	Negative
β-glucuronidase	Negative
α-glucosidase	Negative
β-glucosidase	Positive
N-acetil-β-glucosaminidase	Negative
α-manosidase	Negative
α-fucosidase	Negative
Gastric phase (survival rate)	99.6%
Intestinal phase (survival rate)	100.9%
Auto-aggregation	53.2%
Co-aggregation with	
Listeria monocytogenes Scott A	42.4%
Enterococcus faecalis ATCC 19443	41.6%
Lactobacillus sakei ATCC 15521	10.0%
Cell surface hydrophobicity	98.2%
Beneficial properties related genes[a]	
*EF*1249—fibrinogen binding protein	Negative
*EF*2380—membrane-associated zinc metalloprotease	Negative
*EF*2662—choline binding protein	Negative
*prg*B—surface protein	Negative
EFTu—adhesion-like factor	Positive
map—mucus adhesion	Positive
mub—mucus adhesion	Negative
Bile salt deconjugation	
Taurocholic acid (TC)	Positive
Taurodeoxycholic acid (TDC)	Positive
Glycocholic acid (GC)	Positive
Glycodeoxycholic acid (GDC)	Positive
β-galactosidase activity	Positive
Lactose assimilation*	Glucose (control): 8.15 ± 0.01^{b} CFU/ml
	Lactose: 9.24 ± 0.02^{a} CFU/ml
Safety properties	
Hemolytic activity (25 and 37 °C)	Negative
Gelatinase production (25 and 37 °C)	Negative
Lipase production (25 and 37 °C)	Negative
Deoxy ribonuclease activity (25 and 37 °C)	Negative
Biogenic amine production (25 and 37 °C)	
Lysine	Negative
Tyrosine	Negative
Histidine	Negative
Ornithine	Negative
Phenotypic antibiotic resistance	
Disc diffusion method	
Oxacillin	Resistant
Tetracycline	Sensitive
Imipenem	Sensitive
Ampicillin	Sensitive
Vancomycin	Sensitive
Gentamicin	Resistant
Penicillin	Sensitive
Erytromycin	Resistant
Clindamycin	Resistant
Chloramphenicol	Sensitive
Sulfa/trimethoprim	Resistant
Rifampicin	Resistant
Etest® strips	
Vancomycin	Resistant
Gentamicin	Sensitive
Chloramphenicol	Sensitive
Ampicillin	Sensitive
Rifampicin	Sensitive
Genotypic virulence and antibiotic resistance[a]	Negative for all 50 tested genes

[a] See Materials and Methods for description of selected genes and performed PCR tests

*Average values ± standard deviations, three independent repetitions; values followed by different letters are significantly different by ANOVA and Tukey ($p < 0.05$)

The aggregation (auto-aggregation and co-aggregation) ability is the capacity of the strain to adhere and form biofilms on various surfaces, allowing the beneficial strain to persist in the gastrointestinal environment, which facilitates the beneficial effects for the host. The results showed that *C. vitaeruminis* RU4 had a 53.2% auto-aggregation rate.

C. vitaeruminis MRU4 showed the following results for co-aggregation: 42.4% with *L. monocytogenes* ScottA, 41.6% with *E. faecalis* ATCC 19443, and 10.0% with *Lb. sakei* ATCC 15521. Many studies have shown a large range for auto-aggregation and co-aggregation presented by probiotic bacteria, which is in agreement with our study [18].

C. vitaeruminis MRU4 showed 98.2% of cell surface hydrophobicity. Vinderola et al. [20] considered this feature to be a species-specific parameter. Moreover, some studies showed cell surface hydrophobicity rates of 5.4 to 79% for probiotic cultures [18, 21]. In addition, the selected strain generated positive results for the presence of two genes: *EFTu* and *map*. The first one is an adhesion-like factor gene that also aids in cell adhesion, and the second one is up-regulated in the presence of mucus [10].

The selected strain had a high ability to grow on MRS agar plates containing 0.5% (*w*/*v*) sodium salts of TC, TDC, GC, and GDC. This indicates a good capability to reduce cholesterol, and it is therefore desirable for use in probiotic products for human consumption [22]. In agreement with our study, many authors have reported the deconjugation capacity of probiotic cultures [18, 21].

Production of the β-galactosidase enzyme allows the probiotic culture to assimilate lactose and minimize lactose intolerance [19, 21]. *C. vitaeruminis* MRU4 showed no β-galactosidase activity in the API ZIM kit test. The ability of beneficial bacteria to assimilate lactose is a great advantage for use in probiotic foods targeted for lactose intolerant individuals. The results for the lactose assimilation test showed that *C. vitaeruminis* MRU4 presented better lactose assimilation (9.24 ± 0.02) than glucose (8.15 ± 0.01, $p < 0.05$) which was used as the control (Table 1).

C. vitaeruminis MRU4 did not express any virulence factors, such as for hemolytic activity, gelatinase production, lipase production, and deoxyribonuclease activity, in in vitro tests at both 25 °C and 37 °C. The same was verified for in vitro detection of biogenic amine production: *C. vitaeruminis* MRU4 was negative for lysine, tyrosine, histidine, and ornithine biogenic amines, as expected for safe strains [23]. Pisano et al. [24] highlighted the importance of the lack of these virulence factors for probiotic cultures.

Regarding antimicrobial resistance, *C. vitaeruminis* MRU4 was resistant to oxacillin, gentamicin, erythromycin, clindamycin, sulfa/trimethoprim, and rifampicin based on the disc diffusion method. Considering the Etest® strips, *C. vitaeruminis* MRU4 was resistant just to vancomycin. This vancomycin resistance can be due to an intrinsic characteristic of the studied bacteria. This is in agreement with the observation that based on the performed PCR, we could not detect the presence of genes related to vancomycin resistance. Studies of antimicrobial resistance in probiotic cultures have shown that resistance is species-specific and there is no pattern for resistance with the tested antibiotics [25].

Based on the performed PCR screening for the presence of virulence related genes, *C. vitaeruminis* MRU4 was negative for the 50 tested genes. The results obtained in this study agree with those obtained by other authors, and they show that these results are species and strain-specific [21]. The absence of antibiotic resistance or virulence genes suggests that there might be a new virulence mechanism that can occur by either acquiring genes or by mutation of endogenous genes [26].

In summary, *C. vitaeruminis* MRU4 demonstrated safety and potential beneficial functions in in vitro tests. However, it is necessary to emphasize the importance of additional studies regarding the safety of this strain, as well as confirmation of the benefits through in vivo testing in animal models and humans.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Ahire JJ, Mokashe NU, Patil HJ, Chaudhari B (2013) Antioxidative potential of folate producing probiotic *Lactobacillus helveticus* CD6. J Food Sci Technol 50(1):26–34
2. Dimitrellou D, Kandylis P, Petrović T, Dimitrijević-Branković S, Lević S, Nedović V, Kourkoutas Y (2016) Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk. LWT Food Sci Technol 71:169–174. doi:10.1016/j.lwt.2016.03.007
3. Dobinson HC, Anderson TP, Chambers ST, Doogue MP, Seaward L, Werno AM (2015) Antimicrobial treatment options for granulomatous mastitis caused by *Corynebacterium* species. J Clin Microbiol 53(9):2895–2899. doi:10.1128/jcm.00760-15
4. Al-Dilaimi A, Albersmeier A, Kalinowski J, Rückert C (2014) Complete genome sequence of *Corynebacterium vitaeruminis* DSM 20294 T, isolated from the cow rumen as a vitamin B producer. J Biotechnol 189:70–71. doi:10.1016/j.jbiotec.2014.08.036
5. Bechdel SI, Honeywell HE, Dutcher RA, Knutsen MH (1928) Synthesis of vitamin B in the rumen of the cow. J Biol Chem 80(1):231–238
6. Argyri AA, Zoumpopoulou G, Karatzas KG, Tsakalidou E, Nychas GE, Panagou EZ, Tassou CC (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiol 33(2):282–291. doi:10.1016/j.fm.2012.10.005
7. Todorov SD, Wachsman M, Tomé E, Dousset X, Destro MT, Dicks LMT, Franco BDGM, Vaz-Velho M, Drider D (2010) Characterisation of an antiviral pediocin-like bacteriocin produced by *Enterococcus faecium*. Food Microbiol 27(7):869–879. doi:10.1016/j.fm.2010.05.001

8. Basic local alignment search tool (2015) http://www.ncbi.nlm.nih.gov/BLAST. Accessed 2015, August 25
9. dos Santos KMO, Vieira ADS, Buriti FCA, do Nascimento JCF, de Melo MES, Bruno LM, Borges MF, Rocha CRC, Lopes ACS, Franco BDGM, Todorov SD (2015) Artisanal Coalho cheeses as source of beneficial *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. Dairy Science & Technology 95(2):209–230
10. Ramiah K, van Reenen CA, Dicks LMT (2007) Expression of the mucus adhesion genes *Mub* and *MapA*, adhesion-like factor *EF-Tu* and bacteriocin gene *plaA* of *Lactobacillus plantarum* 423, monitored with real-time PCR. Int J Food Microbiol 116(3):405–409. doi:10.1016/j.ijfoodmicro.2007.02.011
11. Pelinescu D, Chifiriuc MC, Ditu LM, Sarbu I, Bleotu C, Vassu I, Stocia I, Lazar V, Corcionivoschi N, Sasarman E (2011) Selection and characterization of the probiotic potential of some lactic acid bacteria isolated from infant feces. Romanian Biotechnological Letters 16(3):6178–6189
12. Barbosa J, Gibbs PA, Teixeira P (2010) Virulence factors among enterococci isolated from traditional fermented meat products produced in the north of Portugal. Food Control 21(5):651–656. doi:10.1016/j.foodcont.2009.10.002
13. Bover-Cid S, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. Int J Food Microbiol 53(1):33–41. doi:10.1016/S0168-1605(99)00152-X
14. EUCAST (2016) European Committee on Antimicrobial Susceptibility Testing. http://www.eucast.org/ast_of_bacteria/. Accessed Accessed 27 April 2016 2016
15. Favaro L, Basaglia M, Casella S, Hue I, Dousset X, Franco BDGM, Todorov SD (2014) Bacteriocinogenic potential and safety evaluation of non-starter *Enterococcus faecium* strains isolated from home made white brine cheese. Food Microbiol 38:228–239
16. Fortina MG, Ricci G, Borgo F, Manachini PL, Arends K, Schiwon K, Abajy MY, Grohmann E (2008) A survey on biotechnological potential and safety of the novel *Enterococcus* species of dairy origin, *E. italicus*. Int J Food Microbiol 123(3):204–211. doi:10.1016/j.ijfoodmicro.2008.01.014
17. Leite AMO, Miguel MAL, Peixoto RS, Ruas-Madiedo P, Paschoalin VMF, Mayo B, Delgado S (2015) Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. J Dairy Sci 98(6):3622–3632. doi:10.3168/jds.2014-9265
18. Caggia C, De Angelis M, Pitino I, Pino A, Randazzo CL (2015) Probiotic features of *Lactobacillus* strains isolated from Ragusano and Pecorino Siciliano cheeses. Food Microbiol 50:109–117. doi:10.1016/j.fm.2015.03.010
19. Vidhyasagar V, Jeevaratnam K (2013) Evaluation of *Pediococcus pentosaceus* strains isolated from idly batter for probiotic properties in vitro. J Funct Foods 5(1):235–243. doi:10.1016/j.jff.2012.10.012
20. Vinderola G, Capellini B, Villarreal F, Suárez V, Quiberoni A, Reinheimer J (2008) Usefulness of a set of simple in vitro tests for the screening and identification of probiotic candidate strains for dairy use. LWT Food Sci Technol 41(9):1678–1688. doi:10.1016/j.lwt.2007.10.008
21. dos Santos KMO, Vieira ADS, Salles HO, Oliveira JS, Rocha CRC, Borges MF, Bruno LM, Franco BDGM, Todorov SD (2015) Safety, beneficial and technological properties of *Enterococcus faecium* isolated from Brazilian cheeses. Braz J Microbiol 46(1):237–249. doi:10.1590/s1517-838246120131245
22. Begley M, Hill C, Gahan CGM (2006) Bile salt hydrolase activity in probiotics. Appl Environ Microbiol 72(3):1729–1738. doi:10.1128/aem.72.3.1729-1738.2006
23. Vankerckhoven V, Huys G, Vancanneyt M, Vael C, Klare I, Romond M, Entenza JM, Moreillon P, Wind RD, Knol J, Wiertz E, Pot B, Vaughan EE, Kahlmeter G, Goossens H (2008) Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. Trends Food Sci Technol 19(2):102–114. doi:10.1016/j.tifs.2007.07.013
24. Pisano MB, Viale S, Conti S, Fadda ME, Deplano M, Melis MP, Deiana M, Cosentino S (2014) Preliminary evaluation of probiotic properties of *Lactobacillus* strains isolated from sardinian dairy products. Biomed Res Int 2014:9. doi:10.1155/2014/286390
25. Han J, Chen D, Li S, Li X, Zhou W, Zhang B, Jia Y (2015) Antibiotic susceptibility of potentially probiotic *Lactobacillus strains*. Italian Journal of Food Science 27(3):8. doi:10.14674/1120-1770/ijfs.v270
26. EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Panel on Additives and Products or Substances used in Animal Feed, vol 10. EFSA Journal. doi:10.2903/j.efsa.2012.2740