Polish Journal of Microbiology
2012, Vol. 61, No 4, 305–310

SHORT COMMUNICATION

Protein Profiles from Intact Cells as a Tool in *Bifidobacterium* Characteristics

ADAM WAŚKO[1], MAGDALENA POLAK-BERECKA[1] and MICHAŁ KALITA[2]

[1]Department of Biotechnology, Human Nutrition and Science of Food Commodities
University of Life Sciences in Lublin, Lublin, Poland
[2]Department of Genetics and Microbiology, Maria Curie-Sklodowska University, Lublin, Poland

Received 1 June 2012, revised 18 September 2012, accepted 19 September 2012

Abstract

In this study sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profiles were analysed and differences were confirmed by a unweighted pair group method with arithmetic average (UPGMA) analysis between bifidobacterial species, such as *B. infanis* ATCC1567, *B. bifidum* Bb-12, *B. longum* KN29, *B. catenulatum* KD14, and *B. animalis* BI30. Two dimensional electrophoresis separation profiles were compared, and the most characteristic spots were characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We propose proteins extracted from intact cells as an additional trait for bifidobacteria characterization, together with molecular techniques, which can be used to analyze bacterial protein polymorphism and to distinguish among species.

Key words: bifidobacteria, liquid chromatography-mass spectrometry (LC-MS/MS), 2D-electrophoresis, proteins

Bifidobacterium is a genus of Gram-positive, microaerophiles or strict anaerobes, some of which are commonly detected in the human gut. Bifidobacteria are phylogenetically grouped in the Actinomycete branch with a high G+C content, and currently 47 species are recognized within this genus (Matsuki *et al.*, 2003; Euzeby, 2007). Bifidobacteria are part of the resident microflora of the human large intestine and are beneficial to their host's health (Orrhage and Nord, 2000). Some bifidobacterial strains are widely used as health-promoting or probiotic components in functional food products (Lin, 2003). For these reasons, bifidobacteria are a subject of growing interest in the pharmaceutical and food industries.

Within the forty seven species currently recognized as belonging to the genus *Bifidobacterium*, only a few have been sequenced (Lukjancenko *et al.*, 2011). There is limited information on the polymorphism of bifidobacterial cell-wall proteins (BIFOP). Mattarelli *et al.* (1993) showed phenotypic differences among BIFOP on the basis of an examination of 150 strains of *Bifidobacterium globosum*. Proteomic analysis has been used to show differences among *Bifidobacterium longum* strains (Aires *et al.*, 2010). It has been demonstrated that SDS-PAGE of whole cell proteins is a reliable and specific method for the identification of the lactic acid bacteria down to the species level (Tae-Woon *et al.*, 2003). Hèbert *et al.* (2000) showed that SDS-PAGE fingerprinting of cell-wall proteins allowed to distinguish *L. helveticus* from *L. delbrueckii* subsp. *lactis.* Two subspecies of *Lactobacillus delbrueckii* were characterized by different SDS-PAGE cell-wall protein profiles. Gatti *et al.* (2001) have also shown that this method can be an efficient taxonomic tool. It has been reported that cell-wall proteins can be used as immunoreactive markers for the identification of some pathogenic microorganisms (Betts *et al.*, 2000; Duffs *et al.*, 2000; Enroth *et al.*, 2000).

The aim of the present study was to employ proteins extracted from intact cells for characterization of bifidobacterial species.

Bifidobacterium infantis ATCC1567 was obtained from the American Type Culture Collection (ATCC, Manassas, Va., U.S.A., http://www.atcc.org/). *B. bifidum* Bb-12, *B. animalis* BI30, *B. catenulatum* KD14, and *B. longum* KN29 were kindly provided by Prof. M. Bielecka (Department of Food Microbiology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland). Bacteria were cultured on Garche's medium (Rasic, 1990) (10 ml) at 37°C in anaerobic conditions. After 24 h, stock cultures were inoculated in a new medium using 5% (v/v) of inoculum. Extractions were performed in triplicate from three independent cultures.

* Corresponding author: M. Polak-Berecka, Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland; phone.: +48 81 46 23 356; fax +48 81 46 23 400; **e-mail address: 3mj@wp.pl**

All species (0.5 l of liquid culture) were harvested by centrifugation (15 min, 4°C, 8 000 × g) at the early stationary growth phase. Cell-wall proteins were extracted using three protocols: the Mattarelli *et al.* (1993) method, a method using 8 M urea, and a method using 2 M guanidine hydrochloride (Rosenberg, 2005). Protein concentrations were estimated using the Bradford Assay Kit (Bradford, 1976). Proteins from all extracts were solubilised in loading buffer and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a final polyacrylamide concentration of 10% w/w (Laemmli, 1970).

Protein pattern profiles were scored manually for the presence (1) or absence (0) of protein bands. These binary data matrices were used for calculating Jaccard similarity coefficients (Sneath and Sokal, 1973) using the SIMQUAL (similarity for qualitative data) module of NTSYS-pc software version 2.02 g (Rohlf, 1998). Subsequently, similarity coefficients were used to construct UPGMA (unweighted pair group method with arithmetic average) dendrograms using SAHN (sequential, hierarchical, agglomerative and nested cluster methods) clustering implemented in NTSYS-pc 2.02g.

The samples of extracted proteins were prepared using a 2D Clean-Up kit (LG Healthcare) and two dimensional electrophoresis was performed according to Nezhad *et al.* (2012). Upon completion of 2D SDS-PAGE, the gels were stained with BioSafe Coomassie Stain. Stained protein spots were scanned on a GS-800 Calibrated Densitometer (Bio-Rad, USA). The 2D gels were calibrated using a 2D SDS-PAGE standard (Bio-Rad, USA). Spot detection and analysis was performed using PDQuest software version 8.0.1 (Bio-Rad, USA).

Gel slices were subjected to a standard in-gel tryptic digestion (Shevchenko *et al.*, 1996). The peptide mixtures obtained were then applied to a RP-18 precolumn (Waters) using a 0.1% (v/v) TFA solution as the mobile phase, and transferred to a nano-HPLC RP-18 column (Waters, length: 250 mm, bead diameter: 1.7 µm). The mass spectra obtained were preprocessed with the Mascot Distiller software (v. 2.3, Matrix Science) and searched against the non-redundant protein database from the NCBI (NCBInr, 14259576 sequences; 4884494093 residues) using the 8-processor on-site licensed MASCOT search engine (Mascot Server v. 2.2.03). The search parameters were set as follows: enzyme, Trypsin; fixed modifications, carbamidomethylation (C); variable modifications, oxidation (M); protein mass, unrestricted; peptide mass tolerance, ± 40 ppm; MS/MS fragment ion mass tolerance, ± 0.8 Da; max missed cleavages, 1. Only peptide hits exceeding a Mascot expectation value of 0.05 were accepted.

The acquired raw data were processed using the Mascot Distiller followed by a Mascot search (Matrix Science, London, UK, locally installed http://proteom.pl/mascot) against the NCBI nonredundant database. Search parameters for precursor and product ion mass tolerances were ± 40 ppm and ± 0.8 Da, respectively, with allowance made for one missed semi Trypsin, fixed modifications of cysteine through carbamidomethylation, and variable modification through lysine carbamidomethylation and methionine oxidation. The Mascot program reports an individual ion score for each assignment of an MS/MS spectrum to a database sequence, and groups correctly identified peptides into sets according to the corresponding database deposited proteins. The ion score is −10*Log(P), where P is the probability ($P < 0.05$) that the observed match is a random event.

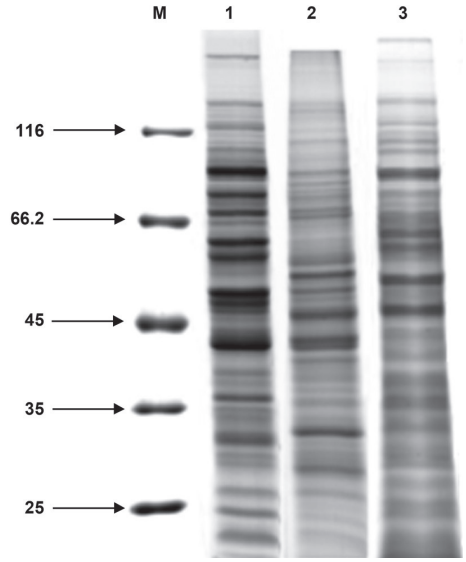
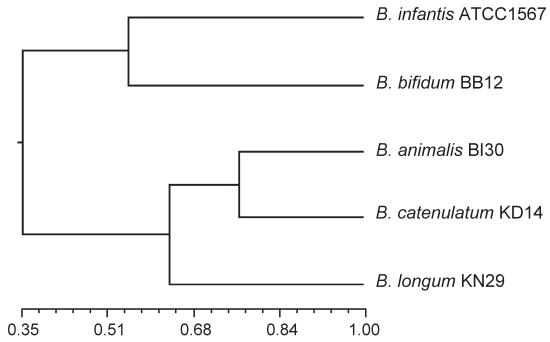


Fig. 1. Representative polyacrylamide gel showing cell-wall protein extracts obtained by different methods from *B. catenulatum* KD 14 cells. M: molecular mass markers (kDa). Lane 1, method by Mattarelli (1993); lane 2, method with 8 M urea; lane 3, method with 2 M guanidine hydrochloride.

The protein extraction methods were used to differentiate among the examined species of bifidobacteria. We showed that specific proteins isolated from intact cells could be species-specific. The different protein profiles obtained using the examined methods are shown in Fig. 1. The highest number of protein bands were identified using the extraction method by Mattarelli (1993). It can be assumed that the diversity of the collected proteins was due to ultracentrifugation, and it was only using this extraction method that low molecular weight proteins could be detected.

Dendrogram obtained by numerical comparison of the protein patterns of the investigated bifidobacterial species is shown in Fig. 2. A numerical analysis of the SDS-PAGE protein patterns grouped all the detected



	ATCC1567	BB12	BI30	KN29	KD14
ATCC1567	–				
BB12	0.55	–			
BI30	0.44	0.36	–		
KD14	0.39	0.23	0.76	–	
KN29	0.47	0.23	0.58	0.68	–

Fig. 2. UPGMA cluster analysis of *Bifidobacterium* spp. based on Jaccard's coefficients calculated from SDS-PAGE patterns of cell-wall proteins. Similarity matrices are shown next to each dendrogram.

patterns into two main branches. The phenotypes of the examined *Bifidobacterium* species were grouped into two clusters, 1) *B. infantis* ATCC1567 and *B. bifidum* Bb-12 and 2) *B. longum* KN29, *B. catenulatum* KD14, and *B. animalis* BI30, at similarity levels of 0.55 and 0.64, respectively. Canzi *et al.* (2005), who analyzed RAPD patterns of *B. bifidum* and *B. longum*, derived a dendrogram showing higher similarity levels (0.66 and 0.72) than those in our study. Also a phylogenetic analysis of all taxa included in the family *Bifidobacteriaceae* using 16S rRNA showed a similarity level of 0.9 (Sidarenka *et al.*, 2008). In another study, a sequence homology analysis of the 16S rRNA gene demonstrated very high similarities for some species groups: a *B. catenulatum* and *B. pseudocatenulatum* group (similarity 0.99), a *B. longum* and *B. infantis* group (similarity 0.99), and a *B. lactis* and *B. animalis* group (similarity 0.99) (Ward and Roy, 2005). Lukjancenko *et al.* (2011) have analyzed 19 genomes from 9 *Bifidobacteria* species. They reported high similarity (99.5%) between strains and the low level of similarity between species (28–55%). This statement is consistent with our results. However, in our study the level of similarities between species was higher (0.23–0.76) which can be explain by the same ecological niche sharing by different species of *Bifidobacteria* used in our study. Moreover, our study did not concern the whole proteome of bifidobacteria, but embraced the selected group of proteins extracted from intact cells. In this work we propose these proteins as additional trait for bifidobacteria characterization.

There is evidence that proteomic analysis could be a tool for differentiation between some bacterial species. Two-dimensional electrophoresis has been used by other authors to analyze bacterial protein polymorphism and to distinguish among closely related pathogenic organisms. Proteomic comparison of membrane and extracellular proteins has been performed to identify biomarkers for *Helicobacter pylori* (Carlsohn *et al.*, 2006), *Listeria innocua* (Calvo *et al.*, 2005), and *Pseudomonas aeruginosa* (Nouwens *et al.*, 2002). Hitherto, 2D-electrophoresis has not been used to compare bifidobacteria. In the present study 2D-electrophoresis was employed in the analysis of the protein content of five *Bifidobacterium* species. A total of 29 to 53 spots were isolated, out of which only 12% to 41% were common for all the examined species. The analysis revealed that there were 149 spots which distinguished all the species, examples of them are shown in Figure 3. Identification of the most characteristic spots was performed using peptide mass fingerprinting and automated MS/MS analysis (Table I). The 18 identified proteins fell mainly into the following functional categories: metabolism-related proteins, especially membrane-related proteins; proteins involved in energy production and conversion; and proteins related to transcription and translation. Spots 4 and 13 were identified as ATP binding proteins that energize transport of sugars through the ABC transport system. Such activities can be associated with the cytoplasmic membrane (Gilad *et al.*, 2010). Spot 7 was recognized as a trigger factor. A trigger factor is involved in protein export. It is a ribosome-associated molecular chaperone, which is the first to interact with nascent polypeptide chains. It acts as a chaperone by maintaining the newly synthesized protein in an open conformation (Kramer *et al.*, 2004). The trigger factor

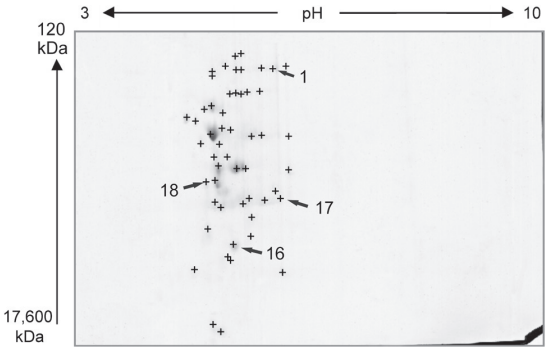


Fig. 3. Representative two-dimensional gel showing the proteins detected in the intact cells of *Bifidobacterium catenulatum* KD14.

Table I
Proteins identified in the examined *Bifidobacterium* species

Spot No. extracted from	Putative function	Bifidobacterial species	Accession No.	Theoretical molecular mass (kDa)	MS/MS	MWE	Signal peptides*	Anchoring*	PSORTb*
1. *B. catenulatum* KD14	Not identified	*Bifidobacterium catenulatum* DSM 16992	gi|212716076	92.43	46	1486	No	–	Unknown
2. *B. infantis* ATTC	Hsp60	*Lactobacillus plantarum*	gi|239759186	21.3	30	1567	No	–	Cytoplasmic
3. *B. animalis* BI30	protein grpE HSP-70 cofactor	*Bifidobacterium animalis* subsp. *lactis* HN019	gi|183602853	24.71	139	9065	No	–	Cytoplasmic
4. *B. animalis* BI30	ATP binding protein of ABC transporter for sugars	*Bifidobacterium animalis* subsp. *lactis* HN019	gi|183602647	41.38	67	2208	No	–	Cytoplasmic/ Membrane
5. *B. bifidum* Bb-12	Pyruvate kinase	*Bifidobacterium animalis* subsp. *lactis* BB12	gi|289178753	52.51	79	3657	No	–	Cytoplasmic
6. *B. bifidum* Bb-12	L-lactate dehydrogenase 2	*Bifidobacterium animalis* subsp. *lactis* HN019	gi|183602408	34.27	67	1855	No	–	Cytoplasmic
7. *B. bifidum* Bb-12	trigger factor	*Bifidobacterium animalis* subsp. *lactis* HN019	gi|183601396	49.92	77	2588	No	–	Cytoplasmic
8. *B. bifidum* Bb-12	hypothetical protein pLP9000_05	*Lactobacillus plantarum*	gi|21218447	16.35	21	758	No	–	Unknown
9. *B. infantis* ATTC	fructose-bisphosphate aldolase	*Lactobacillus plantarum* WCFS1	gi|28377250	31.02	48	2078	No	–	Cytoplasmic
10. *B. infantis* ATTC	pyruvate kinase	*Lactobacillus plantarum* WCFS1	gi|28378548	62.94	122	4112	No	–	Cytoplasmic
11. *B. infantis* ATTC	ribosome recycling factor	*Bifidobacterium animalis* subsp. *lactis* HN019	gi|183601689	18.98	81	2164	No	–	Cytoplasmic
12. *B. animalis* BI30	ribosome recycling factor	*Bifidobacterium longum* NCC2705	gi|23466062	20.12	30	1227	No	–	Cytoplasmic
13. *B. longum* KN29	ATP binding protein of ABC transporter for sugars	*Bifidobacterium longum* NCC2705	gi|23465255	40.76	24	1035	No	–	Cytoplasmic/ Membrane
14. *B. longum* KN29	COG0264: Translation elongation factor Ts	*Bifidobacterium longum* DJO10A	gi|23335170	29.96	38	939	No	–	Cytoplasmic
15. *B. longum* KN29	heat shock protein GrpE	*Bifidobacterium longum* NCC2705	gi|23465108	23.69	49	2185	No	–	Cytoplasmic
16. *B. catenulatum* KD14	hypothetical protein BIFPSEUDO_03766	*Bifidobacterium pseudocatenulatum* DSM 20438	gi|225352151	29.82	133	5765	No	–	Cytoplasmic
17. *B. catenulatum* KD14	hypothetical protein	*Bifidobacterium pseudocatenulatum*	gi|225351598	40.94	37	1808	No	–	Cytoplasmic/
	BIFPSEUDO_03195	DSM 20438							Membrane
18. *B. catenulatum* KD14	hypothetical protein BIFCAT_00580	*Bifidobacterium catenulatum* DSM 16992	gi|212715680	48.93	166	4755	No	–	Cytoplasmic

* Signal peptides and final subcellular localization were predicted using the PSORTb package (Gardy *et al.*, 2005).

is a ribosome-associated protein that interacts with the translation elongation protein and with a wide variety of polypeptides to catalyze their folding (Ventura *et al.*, 2003). Spots 11, 12, and 14 were identified as ribosomal proteins. These proteins are often exposed on the bacterial surface. Several surface-associated ribosomal proteins have been identified in *Streptococcus pyogenes* (Ventura *et al.*, 2003), *Bacillus subtilis* (Severin *et al.*, 2007), and *Lactobacillus rhamnosus* GG (Sanchez *et al.*, 2009). Spot 14 was also identified as a translation elongation factor. Spot 2 was identified as the cytoplasmic Hsp60 protein. It has been confirmed that the *hsp60* gene can be used for detection, characterization, and species identification of bifidobacteria (Ward and Roy, 2005). The diversity of the gene sequences indicates that the encoded proteins will differ from one another in various bifidobacterial species. Spots 5, 6, 9, and 10 were identified as cytoplasmic enzyme proteins involved in the sugar catabolism pathway.

The present study shows that the method of extraction of surface-associated proteins by Mattarelli *et al.* (1993) is the most suitable for the isolation of proteins from intact cells of bifidobacteria. The proteomic analysis revealed that these specific proteins make it possible to distinguish among *Bifidobacterium* species such as *B. infanis* ATCC1567, *B. bifidum* Bb-12, *B. longum* KN29, *B. catenulatum* KD14, and *B. animalis* BI30. This observation was borne out by SDS-PAGE and 2D electrophoresis and further confirmed by an MS/MS analysis, in which the most characteristic protein profiles were identified.

Further research and comparative analyses are needed to develop appropriate proteomic profiles for different species and to find the specific cell-wall proteins, their sequences and characteristics. If characteristic profiles of these proteins are known, scientists will have an additional tool for accurate identification of bifidobacteria. Interesting possible application could be in MALDI-TOF mass spectrometry. This technique is cost effective and allow for highly accurate identification of bifidobacteria in a faster way than traditional methods.

Literature

Aires J., P. Anglade, F. Baraige, M. Zagorec, M. Champomier-Vergès and M. Butel. 2010. Proteomic comparison of the cytosolic proteins of three *Bifidobacterium longum* human isolates and *B. longum* NCC2705. *BMC Microbiol.* 10: 29.

Betts J.C., P. Dotson, S. Quan, A.P. Lewis, P.J. Thomas, K. Dunkan and others. 2000. Comparison of the proteome of *Mycobacterium tuberculosis* strain H37Rv with clinical isolate CDC 1551. *Microbiol.* 146: 3205–3216.

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.

Calvo E., M.G. Pucciarelli, H. Bierne, P. Cossard and others. 2005. Analysis of the *Listeria* cell wall proteome by two-dimensional nano-liquid chromatography coupled to mass spectrometry. *Proteomics* 5: 433–443.

Canzi E., S. Guglielmetti, D. Mora, I. Tamagnini and C. Parini. 2005. Conditions affecting cell surface properties of human intestinal bifidobacteria. *Antonie van Leeuwenhoek* 88: 207–219.

Carlsohn E., J. Nystrom, H. Karlsson, A.M. Svennerholm and C.L. Nilsson. 2006. Characterization of the outer membrane protein profile from disease-related *Helicobacter pylori* isolates by subcellular fractionation and nano-LC FT-ICR MS analysis. *J. Proteome Res.* 5: 3197–3204.

Duffs F., P. Jenoe and P. Boyaval. 2000. Use of two-dimensional electrophoresis to study differential protein expression in divercin V 41-resistant and wild type strains of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66: 4318–4324.

Enroth H., T. Akerlund, A. Sillen and L. Engstrand. 2000. Clustering of clinical strains of *Helicobacter pylori* analyzed by two-dimensional gel electrophoresis. *Clin. Diagn. Lab. Immunol.* 7: 301–306.

Euzeby J.P. 2007. List of Prokaryotic names with standing in nomenclature – genus *Bifidobacterium*. J.P. Uzeby SBSV, http://www.bacterio.cict.fr.

Gardy J.L., M.R. Laird, F. Chen, S. Rey, C.J. Walsh, M. Ester and F.S. Brinkman. 2005. PSORTb v. 2.0: expanded prediction of bacterial proteins subcellular localization and insights gained from comparative proteome analysis. *Bioinformatics* 21: 617–623.

Gatti M., M.E. Fornasari and E. Neviani. 2001. Differentiation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus delbrueckii* subsp. *lactis* by SDS-PAGE of cell-wall proteins. *Lett. Appl. Microbiol.* 32: 352–356.

Gilad O., S. Jacobsen, B. Stuer-Lauridsen, M.B. Pedersen, C. Garrigues and B. Svensson. 2010. Combined transcriptome and proteome analysis of *Bifidobacterium animalis* subsp. *lactis* BB-12 grown on xylo-oligosaccharides and a model of their utilization. *Appl. Environ. Microbiol.* 76: 7285–7291.

Hèbert E.M., R.R. Raya and G.S. de Giori. 2000. Use of SDS-PAGE of cell-wall proteins for rapid differentiation of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus helveticus* in the human gastrointestinal tract. *Sys. Appl. Microbiol.* 26: 572–584.

Kramer G., A. Rutkowska, R.D. Wegrzyn, H. Patzelt, T.A. Kurz, F. Merz, T. Rauch, S. Vorderwulbecke, E. Deuerling and B. Bukau. 2004. Functional dissection of *Escherichia coli* trigger factor: unraveling the function of individual domains. *J. Bacteriol.* 186: 377–784.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 227: 680–685.

Lin D.C. 2003. Probiotics as functional foods. *Nutr. Clin. Pract.* 18: 497–506.

Lukjancenko O., D.W. Ussery and T.M. Wassenaar. 2011. Comparative genomics of Bifidobacterium, Lactobacillus and related probiotic genera. *Microb. Ecol.* DOI 10.1007/s00248-011-9948-y.

Matsuki T., T. Watanabe and R. Tanaka. 2003. Species- and species-specific PCR primers for the detection and identification of bifidobacteria. *Curr. Issues Intest. Microbiol.* 4: 61–69.

Mattarelli P., B. Biavati, F. Crociani, V. Scardovi and G. Prati. 1993. Bifidobacterial cell-wall proteins (BIFOP) in *Bifidobacterium globosum*. *Res. Microbiol.* 144: 581–590.

Nezhad M.H., M. Knight and M. L. Britz. 2012. Evidence of changes in cell surface proteins during growth of *Lactobacillus casei* under acidic conditions. *Food Sci. Biotechnol.* 21(1): 253–260.

Nouwens A.S., M.D. Willcoks, B.J. Wolsh and S.J. Cordwell. 2002. Proteomic comparison of membrane and extracellular proteins from invasive (PAO1) and cytotoxic (6206) strains of *Pseudomonas aeruginosa*. *Proteomics* 2: 1325–1346.

Orrhage K. and C.E. Nord. 2000. Bifidobacteria and lactobacilli in human health. *Drugs Exp. Clin. Res.* 26: 95–111.

Rasic J.L. 1990. Culture media for detection and enumeration of the bifidobacteria in fermented milk products. *Bulletin of IDF* 252: 24–31.

Rohlf F.J. 1998. *Ntsys-pc: Numerical taxonomy: the principles and practice of numerical classification.* W.H. Freeman and company, San Francisco.

Rosenberg I.M. 2005. *Protein Analysis and Purification. Benchtop Techniques*. 2nd ed. Birkhäuser, Boston.

Sánchez B., P. Bressolier, S. Chaignepain, J.M. Schmitter and M.C. Urdaci. 2009. Identification of surface-associated proteins in the probiotic bacterium *Lactobacillus rhamnosus* GG. *Int. Dairy J.* 19: 85–88.

Severin A., E. Nikbarg, J. Wooters, S.A. Quazi, J.V. Matsuoka, E. Murphy, I.K. Moutsastsos, R.J. Zagursky and S.B. Olmsted. 2007. Proteomic analysis and identification of *Streptococcus pyogenes* surface-associated proteins. *J. Bacteriol.* 189: 1514–1522.

Shevchenko A., M. Wilm, O. Vorm, O.N. Jensen, A.V. Podtelejnikov, G. Neubauer, A. Shevchenko, P. Mortensen and M. Mann. 1996. A strategy for identifying gel-separated proteins in sequence databases by MS alone. *Biochem. Soc. Trans.* 24: 893–896.

Sidarenka A.V., G.J. Novik and V.N. Akimov. 2008. Application of molecular methods to classification and identification of bacteria of the species of *Bifidobacterium*. *Microbiol.* 77: 251–260.

Sneath P.H.A. and R. Sokal. 1973. *Numerical Taxonomy.* Freeman, San Francisco, CA.

Tae-Woon K., S. Jung, J. Leem, S. Choim, S. Park, J. Jo and H. Kim. 2003. Identification of lactic acid bacteria in kimchi using SDS-PAGE profiles of whole cell proteins. *J. Microbiol. Biotechnol.* 13: 119–124.

Ventura M., C. Canchaya, V. Meylan, T.R. Klaenhammer and R. Zink. 2003. Analysis, Characterization, and loci of the *tuf* genes in *Lactobacillus* and *Bifidobacterium* species and their direct application for species identification. *Appl. Environ. Microbiol.* 69: 6908–6922.

Ward P. and D. Roy. 2005. Review of molecular methods for identification, characterization and detection of bifidobacteria. *Lait* 85: 23–32.