Brief communication

Identification of bifidobacteria isolated from Asian elephant (*Elephas maximus*)

VERA BUNESOVA[1], EVA VLKOVA[1,*], VOJTECH RADA[1], JIRI KILLER[1,3] and VLADIMIR KMET[2]

[1]*Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6-Suchdol 1652 1, Czech Republic*

[2]*Institute of Animal Physiology of Slovak Academy of Sciences, Soltesovej 4, Kosice 040 01, Slovakia*

[3]*Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague 4-Krč 14200, Czech Republic*

**Corresponding author (Fax, +420-2-24-38-27-55; Email, vlkova@af.czu.cz)*

Bifidobacteria are considered as one of the key genera in intestinal tracts of animals, and their species composition vary depending on the host. The aim of this study was to identify faecal bifidobacteria from Asian elephants (*Elephas maximus*), housed in Zoological gardens (Ostrava, Czech Republic). Using culturing, bifidobacteria were found in counts 7.60±0.56 log CFU/g. Twenty-six pure strains were isolated from faeces of Asian elephant. The isolates were clustered into two groups according to fingerprinting profiles and fermentation characteristic. Bacteria were identified by a combination of MALDI-TOF MS, PCR methods and sequencing as *B. boum* (12 isolates) and *B. adolescentis* (14 isolates). Elephant strains showed different fingerprinting profiles than type and collection strains. Since these two species are frequently isolated from gastrointestinal tract of herbivores, they seem to be typical of animals fed plant diets.

[Bunesova V, Vlkova E, Rada V, Killer J and Kmet V 2013 Identification of bifidobacteria isolated from Asian elephant (*Elephas maximus*). *J. Biosci.* **38** 239–243] **DOI** 10.1007/s12038-013-9322-y

1. Introduction

The gastrointestinal tract (GIT) of animals is a habitat of a complex collection of microorganisms, with large differences between individuals and animal species (Walter 2008). The largest surviving herbivores are elephants. The anatomy and physiology of the elephant gastrointestinal tract have many resemblances to what is found in the horse, where a relative simple stomach is followed by a voluminous small and large intestine required for the function of fermentative digestion through the metabolism of a complex microflora (Bojesen *et al.* 2006).

Bifidobacteria are considered as one of the important genera in the intestinal tracts of animals. They are common and autochthonous microflora of ruminant herbivores (Russell *et al.* 2011), and have also been isolated from faeces of marmoset and red-handed tamarin South Africa (Endo *et al.* 2010, 2012). Their presence in high numbers is associated with good health status of the host. Bifidobacteria are helpful in maintaining appropriate balance of the microbiota in the GIT, reducing the risk of pathogen infection. Bifidobacteria represent one of the most dominant groups, and some bifidobacterial species are frequently used as the probiotic ingredient for humans and also animals (Turroni *et al.* 2011; Russell *et al.* 2011).

The bifidobacterial occurrence and species composition in different animals is very variable. The objective of this study was to characterize and identify strains isolated from Asian elephant faeces by DNA fingerprinting techniques, biochemical testing and sequencing.

Keywords. 16S rRNA genes sequencing; bifidobacteria; *Elephas maximus*; herbivores; identification

Supplementary materials pertaining to this article are available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbiosci/jun2013/supp/bunesova.pdf*

Published online: 26 April 2013

2. Material and methods

Faecal samples of two Asian elephanta *(Elephas maximus)* from Ostrava Zoo (The Czech Republic) were investigated. Fresh faecal samples were collected and aseptically transferred to a tube with Wilkins-Chalgren broth (Oxoid) and transported immediately to the laboratory. Wilkins-Chalgren-Soya pepton-Mupirocin agar (WSPmup) was used for the bifidobacteria enumeration and isolation. This medium is modified Wilkins-Chalgren agar (Oxoid) supplemented with soya peptone (5 g/L, Oxoid), L-cystein (0.5 g/L, Sigma), Tween 80 (1 mL/L, Sigma), mupirocin (100 mg/L, Merck) and glacial acetic acid (1 mL/L) according to (Rada and Petr 2000). Bifidobacteria were cultivated in anaerobic jars (Anaerobic Plus System, Oxoid) at 37°C for 2 days. Thirteen colonies were isolated from each elephant sample. The detection of fructose-6-phosphate phosphoketolase activity (Orban and Patterson 2001) and PCR with primers Bif 164/Bif662 (Roy and Sirois 2000) was used for the genus identification of the isolates. DNA was isolated from the bacterial cells by the PrepMan®Ultra protocol (Applied Biosystems, USA). All strains were further clustered according to their fingerprinting profiles and morphology. RAPD-PCR amplification was performed using the primer 173 as described by Sakata *et al.* (2002), primer OPV – 07 (Mayer *et al.* 2007) and primer $(GTG)_5$ was used for REP-PCR according to Gevers *et al.* (2001). The fermentations characteristics of the isolates were obtained by ANAEROtest 23 (Erba Lachema, Czech Republic) and API 50CHL (BioMériux, France). Two representative strains from each group were identified using the sequencing of 16S rRNA genes according to Killer *et al.* (2010). The specific-species primer pair for *B. adolescentis* Bi-ADO1 and Bi-ADO2 (Bonjoch *et al.* 2004) was used for comparison the sequencing results. Elephant strains were identified also by matrix assisted laser desorption/ionization time of flight mas spectrometry (MALDI-TOF MS) according to Kmet' and Drugdová (2012). Strains from culture collection *B. adolescentis* DSM 20083 (isolated from intestine of human adult), *B. adolelescentis* DSM 20087 (bovine rumen) and *B. boum* DSM 20432 (bovine rumen) were used as a control.

3. Results and discussion

Bifidobacteria were found in the faecal samples of two elephants in counts of 7.60±0.56 log CFU/g. A total of 26 strains were isolated from WSPmup and identified as *Bifidobacterium* sp. Strains were divided into two groups according to their morphology and fingerprinting profiles. Two representative strains from each group were identified by sequencing. Resulted sequences were compared with 16S rRNA gene sequences of the most related type bifidobacterial strains using the jPHYDIT program (*http://plaza.snu.ac.kr/~jchun/jphydit/index.php*) based on results of BLAST (Basic Local Alignment Search Tool) system (table 1). Isolates from the first group were identified as *B. adolescentis* (14 isolates) and as *B. boum* strains clustered into second group (12 isolates; figure 1). Identification results were confirmed by MALDI-TOF MS analysis, when isolates from first group were also identified as *B. adolescentis* and strains from second group as *B. boum*. The fermentation characteristics of tested strains obtained by biochemical testing were compared with type and collection strains (table 2). Elephant isolates identified as *B. boum* showed similar fermentation profiles to the type strain *B. boum* DSM 20432; nevertheless, elephant isolates were not able to utilize lactose. The fermentation profile of *B. adolescentis* isolated from elephant faeces differ from the collection strain (table 2). Additionally, *B. adolescentis* DSM 20083 and DSMZ 20087 were able to utilize a different substrate (table 2). All elephant origin isolates identified as *B. boum* showed identical fingerprinting profiles, and also the isolates in the

Table 1. Results of bifidobacteria identification

Strain	Origin	PCR BiADO	16S rRNA sequencing		
			Identified as	Similarity to most related type strain (GenBank)	%
Group 1	Elephant faeces	+	*B. adolescentis*	*B. aolescentis* ATCC 15703 T (AP009256)	98.30
		+	*B. adolescentis*	*B. aolescentis* ATCC 15703 T (AP009256)	98.70
Group 2		−	*B. boum*	*B. boum* JCM 1211 T (EU127549)	99.71
		−	*B. boum*	*B. boum* JCM 1211 T (EU127549)	99.80
DSM 20083	Intestine of adult	−	*B. adolescentis*	Type strain DSM	
DSM 20087	Bovine rumen	+	*B. adolescentis*	Collection strain DSM	
DSM 20432	Bovine rumen	−	*B. boum*	Type strain DSM	

DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

ATTC, American Type Culture Collection.

JCM, Japan Collection of Microorganisms.

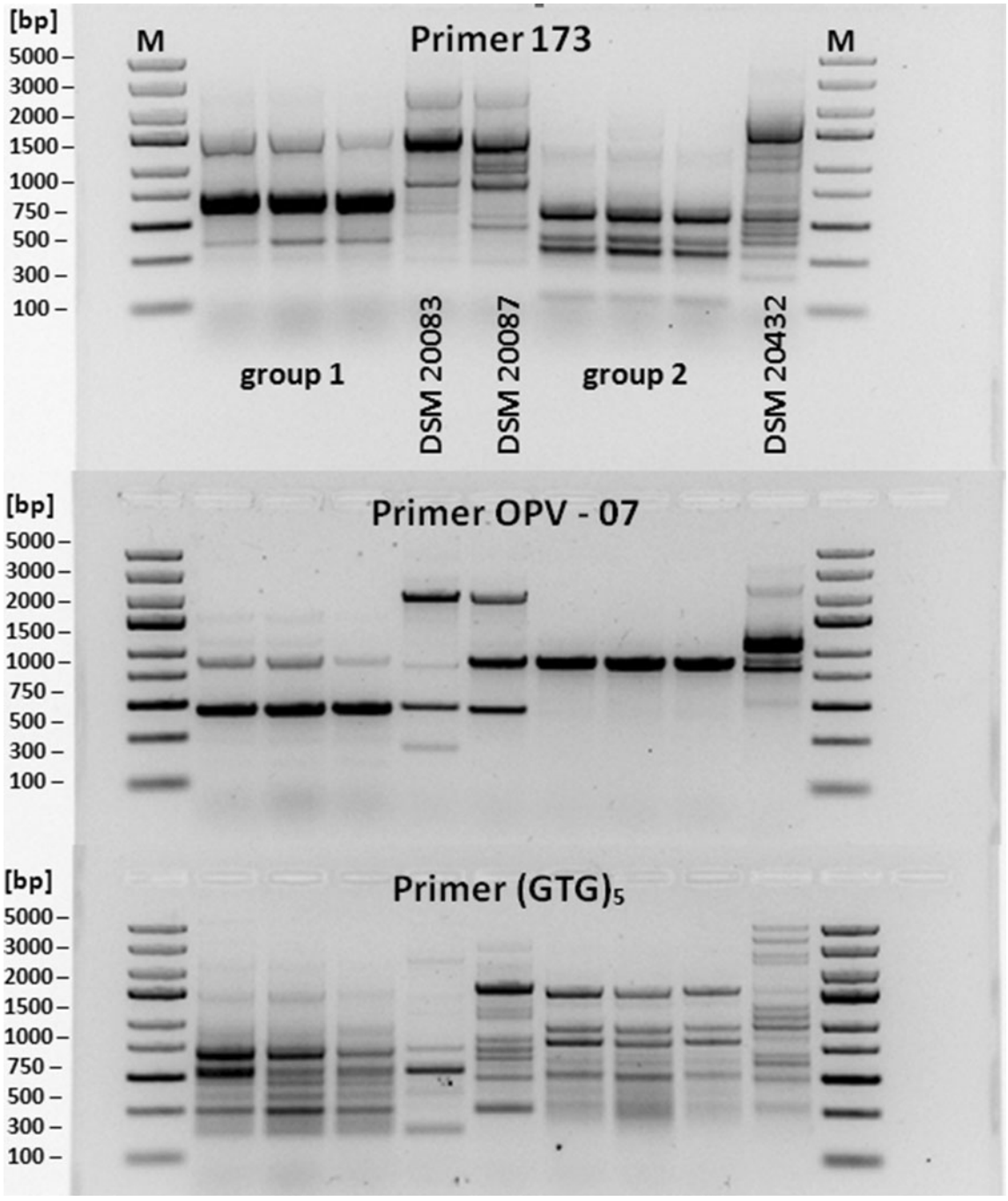

Figure 1. Fingerprinting profiles of tested *Bifidobacterium* strains based on REP-PCR and RAPD-PCR (for description of the strains, see table 1). DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

second group (*B. adolescentis*) exhibit identical bands. However, the profiles of elephant origin, collection and type strains were different depending on the primer (figure 1). Samples included in group *B. adolescentis* had a positive reaction with the species-specific primers Bi-ADO1 and Bi-ADO2. The primers for *B. boum* species are not available.

Examination the fermentation characteristics and DNA fingerprinting revealed genetic diversity among the type, collection and elephant origin strains. RAPD and REP-PCR assays are useful for strain differentiation (Sakata *et al.* 2002; Mayer *et al.* 2007; Gevers *et al.* 2001). An ecological survey of the bifidobacteria population associated with animal faeces

Table 2. Phenotypic characteristics of tested *Bifidobacterium* strains

Substrate	*B. adolescentis*			*B. boum*	
	DSM 20083	DSM 20087	Elephant isolates	DSM 20432	Elephant isolates
Ribose	+	+	+	−	−
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Mannose	+	−	+	−	−
Sorbitol	−	+	+	−	−
Amygdaline	−	−	+	−	−
Arbutine	−	−	+	−	−
Esculine	+	+	+	−	−
Salicine	−	+	+	−	−
Maltose	+	+	+	+	+
Lactose	+	+	+	+	−
Melibiose	+	+	+	+	+
Saccharose	+	+	+	+	+
Trehalose	−	−	+	−	−
Rafinose	+	+	+	+	+
Amidon	+	+	+	+	+
Glycogene	+	+	+	+	+
Gentiobiose	+	+	+	−	−
D turanose	+	+	+	+	+

DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

revealed a broad distribution of bifidobacteria in the gut of a wide variety of animals as herbivore (Lamendella *et al.* 2008). The species *B. boum* was first isolated by Scardovi *et al.* (1979) from bovine rumen and piglet faeces. The species *B. adolescentis* was found in more ecological niches. Reuter (1963) isolated this strain from the intestine of human adults. According to Russell *et al.* (2011), *B. adolescentis* was further identified in bovine rumen, human vagina and sewage. The last niche is likely to be the results of contaminations from their original natural source (Turroni *et al.* 2011). However, no results have been published on isolating and identifying bifidobacteria in elephant GIT. Feeding practices and the composition of animal diets can influence the microbial balance and composition of microflora in the gastrointestinal tract (Chaucheyras-Durand and Durand 2010). According to Russell *et al.* (2011), bifidobacteria were identified also in the faeces of other animals' primary ingesting plants. *B. ruminantium* and *B. merycicum* was found in bovine rumen, *B. cuniculi* and *B. saeculare* are typical for rabbits, and *B. pseudolongum* ssp. *pseudolongum* was isolated from bovine rumen and rabbit faeces. Bunešová *et al.* (2012) identified *B. pseudolongum* ssp. *pseudolongum*, *B. animalis* ssp. *animalis*, *B. pseudocatenulatum* and *B. choerinum* in lamb faeces.

4. Conclusion

To our knowledge, this study is the first description of bifidobacteria from elephant faeces. The isolates were identified as *B. boum* and *B. adolescentis*, and seem to be common species occurring in the GIT of herbivores.

Acknowledgements

The study was supported by grant 523/08/1091 of the Grant Agency of the Czech Republic.

References

Bojesen AM, Olsen KEP and Bertelsen MF 2006 Fatal enterocolitis in Asian elephants *(Elephas maximus)* caused by *Clostridium defficile*. *Vet. Microbiol.* **116** 329–335

Bonjoch X, Ballesté E, Blanch AR 2004 Multiplex PCR with 16S rRNA gene-targeted primers of *Bifidobacterium* spp. to identify sources of fial pollution. *Appl. Environ. Microbiol.* **70** 3171–3175

Bunešová V, Vlková E, Killer J, Rada V and Ročková Š 2012 Identification of *Bifidobacterium* strains from faeces of lambs. *Small Rum. Res.* **105** 355–360

Endo A, Futagawa-Endo Y and Dicks LMT 2010 Diversity of *Lactobacillus* and *Bifidobacterium* in feces of herbivores, omnivores and carnivores. *Anaerobe* **16** 590–596

Endo A, Futagawa-Endo Y, Schumann P, Pukall R and Dicks LMT 2012 *Bifidobacterium reuteri* sp. nov., *Bifidobacterium callitrichos* sp. nov., *Bifidobacterium saguini* sp. nov., *Bifidobacterium stellenboschense* sp. nov. and *Bifidobacterium biavatii* sp. nov. isolated from feces of common marmoset (*Callithrix jacchus*) and red-handed tamarin (*Saguinus midas*). *Syst. Appl. Microbiol.* **35** 92–97

Gevers D, Huys G and Swings J 2001 Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol. Lett.* **205** 31–36

Chaucheyras-Durand F and Durand H 2010 Probiotics in animal nutrition and health. Beneficial Microbes **1** 3–9

Killer J, Kopečný J, Mrázek J, Havlík J, Koppová I, Benada O, Rada V and Kofroňová O 2010 *Bombiscardovia coagulans* gen. nov., sp. nov., a new member of the family *Bifidobacteriaceae* isolated from the digestive tract of bumblebees. *Syst. Appl. Microbiol.* **33** 359–366

Kmet' V and Drugdová Z 2012 Antimicrobial susceptibility of microflora from ovine cheese. *Folia Microbiol.* **57** 291–293

Lamendella R, Santo Domingo JW, Kelty C and Oerther DB 2008 Bifidobacteria in feces and environmental waters. *Appl. Environ. Microbiol.* **74** 575–584

Mayer HK, Amtmann E, Phlilippi E, Steinegger G, Mayrhofer S and Kneifel W 2007 Molecular discrimination of new isolates of *Bifidobacterium animalis* subsp. *lactis* from reference strains and commercial probiotic strains. *Int. Dairy J.* **17** 565–573

Orban JI and Patterson JA 2001 Modification of the phosphoketolase assay for rapid identification of bifidobacteria. *J. Microbiol. Meth.* **40** 221–224

Rada V and Petr J 2000 A new selective medium for the isolation of glucose non-fermenting bifidobacteria from hen caeca. *J. Microbiol. Meth.* **43** 127–132

Reuter G 1963 Vergleichende Untersuchungen über die Bifidus-Flora des Säuglings- und Erwachsenenstuhl. *Zentralbl. Bakteriol. Parasitenkd. Orig. Abt. I.* **191** 486–507

Roy D and Sirois S 2000 Molecular differentiation of *Bifidobacterium* species with amplified ribosomal DNA restriction analysis and alignment of short regions of the *ldh* gene. *FEMS Microbiol. Lett.* **191** 17–24

Russell DA, Ross RP, Fitzgerald GF and Stanton C 2011 Metabolic activities and probiotic potential of bifidobacteria. *Int. J. Food Microbiol.* **146** 88–105

Sakata S, Kitahara M, Sakamoto M, Hayashi H, Fukuyama M and Benno Y 2002 Unification of *Bifidobacterium infantis* and *Bifidobacterium suis* as *Bifidobacterium longum*. *Int. J. Syst. Evol. Microbiol.* **52** 1945–1951

Scardovi V, Sgorbati V and Zani G 1979 Multiple electrophoresis forms of transaldolase and 6-phosphogluconic dehydrogenase and their relationships to the taxonomy and ecology of bifidobacteria. *Int. J. Syst. Bacteriol.* **29** 312–327

Turroni F, Van Sinderen D and Ventura M 2011 Genomics and ecological overview of the genus *Bifidobacterium*. *Int. J. Food Microbiol.* **149** 37–44

Walter J 2008 Ecological role of lactobacilli in the gastrointestinal tract: implication or fundamental and biomedical research. *Appl. Environ. Microbiol.* **74** 4985–4996

MS received 19 October 2012; accepted 28 March 2013

Corresponding editor: Anand Kumar Bachhawat