Characterization of *B. animalis* subsp. *lactis* strains from variable environments. By V. Bunesova[1], J. Killer[1, 2], M. Geigerova[1], E. Vlkova[1], B. Javurkova[3], *[1] Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Kamýcka 129, Prague 6, 165 21, [2] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague 4, 14 200, Department of Biochemistry and Microbiology, [3] Institute of Chemical Technology, Technická 5, Prague 6, 166 28, Czech Republic*

Introduction

Bifidobacteria are ecologically distributed in six ecologically niches such as the human intestine, oral cavity, insect and animal intestine, sewage, blood and food. All these niches are directly or indirectly linked to the human/animal intestinal environment (1). One of the bifidobacterial species widely used as a probiotic is *Bifidobacterium animalis* subsp. *lactis*. These strains are extensively exploited by the food industry as health-promoting bacteria, although the genetic variability of members belonging to this taxon has so far not received much scientific attention (2). The strain *B. animalis* subsp. *lactis* (UR1; DSM 10140) was first described in 1997 as a unique species of the genus *Bifidobacterium* and was identified as an oxygen tolerant isolate from yogurt (3). However, the natural habit of the taxon is the intestine of chickens and rabbits (4), dogs (5) and pigs (6). It is generally known, that repeated passaging and culturing decreases bacterial genome size and compromises critical aspects of microbial function. Members of the *B. animalis* subsp. *lactis* taxon have so far been shown to contain the smallest chromosome among the sequenced bifidobacteria. It should be noted that strains belonging to *B. animalis* subsp. *lactis*, such as BB12 and BLC1, are commonly exploited as probiotics and, as such, are produced by cultivation in synthetic media for an extended number of generations. This procedure may be responsible for a genome evolution process known as genome decay, which may result in loss of chromosomal regions that apparently are superfluous in an environment different from the original ecological niche (1, 7). The aim of this study was to characterized genotypic and phenotypic variability of *B. animalis* subsp. *lactis* strains from different niches.

Materials and Methods

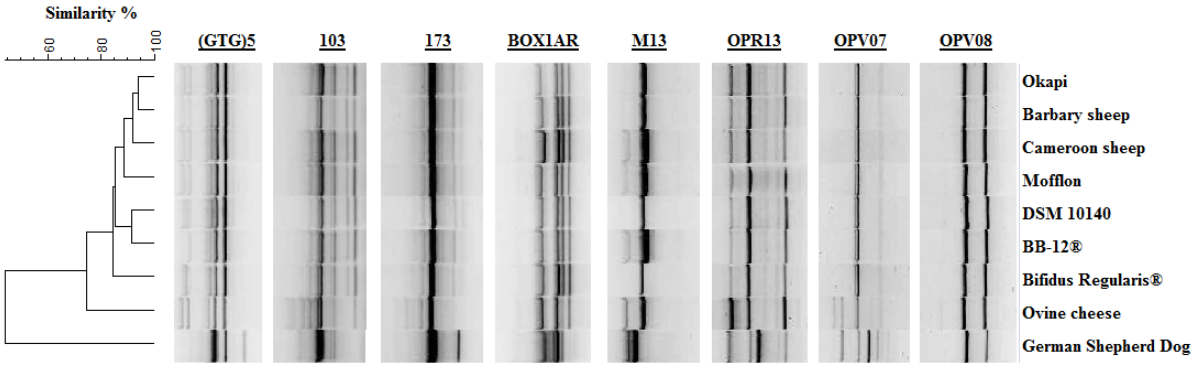
Nine different strains of *B. animalis* subsp. *lactis* were collected for genotypic and phenotypic characterization. The animal origin strains were isolated from faeces of Cameroon sheep, Barbary sheep, Okapi, Mofflon (new unpublished strains), and German Shepherd Dog (5). One strain was isolated from ovine cheese (8). Also commercial strains (BB-12®; Bifidus Regularis®) and collection strain DSM 10140 were used in this study. For isolation and cultivation of the strains was used Wilkins-Chalgren agar (WSPmup) supplemented with soya peptone (5 g/L, Oxoid), L-cysteine (0.5 g/L, Sigma), Tween 80 (1 mL/L, Sigma), mupirocin (100 mg/L, Merck), and glacial acetic acid (1 mL/L). The isolates were identified as *B. animalis* subsp. *lactis* using various methods such as PCR with subspecies-specific primers Bflac2/Bflac5 and Lw-A/Lw-B, as described Mayer et al. (6). Also matrix-assisted laser desorption/ionization time-of-flight analysis (ethanol-formic acid extraction procedure, MALDI TOF MS) and sequencing 16S rRNA (9) was used. Fermentation and enzymatic characteristics and of the isolates were obtained using API 50CHL and Rapid ID32A kits

(BioMérieux). The strains were clustered according to their fingerprinting profiles. RAPD method was performed using the primers 173 and 103 (10), OPR13, OPV07 and OPV 08 (6), and M13 as described Torriani et al. (11). For REP-PCR were used the primers $(GTG)_5$ according to Gevers et al. (12) and BOXA1R (13). The similarity between strains was calculated using UPGMA (average from experiments – used primers).

Results and Discussion

All tested bifidobacterial strains were identified using a combination of several independent methods such as subspecies-species PCRs, MALDI TOF MS and sequencing as *B. animalis* ssp. *lactis*. The 16S rRNA genome sequences of tested strains were 100% identical to collection strain *B. animalis* ssp. *lactis* DSM 10140. Only the strain *B. animalis* subsp. *lactis* (P2N1) isolated from faeces of German Shepherd Dog was different in one nucleotide of the complete sequence of the 16S rRNA gene. Similar results were obtained by fingerprinting profiles (Fig. 1). The profile of the German Shepherd Dog strain was different from the other strains with the seven used primers. Nevertheless, the primer OPV08 did not give a clear resolution between tested strain profiles. A slightly different profile showed also strain isolated from ovine cheese.

Fig. 1 Dendogram of tested *B. animalis* subsp. *lactis* strains based on RAPD-PCR and REP-PCR



The strains also differed in their ability to utilise substrates contained in API kit. These results are shown in Table 1. The *B. animalis* subsp. *lactis* strain isolated from faeces of moufflon did not utilize glucose. This strain also showed very weak ability to utilize lactose as opposed to other tested strains. Generally, in bifidobacteria have been described ability to ferment glucose, galactose and fructose (14). However, some authors reported about glucose non-fermenting *Bifidobacterium* strains (15-17). Moreover, the biggest differences in fermentation profile were observed in dog origin isolate compared to other tested strains. This isolate was not able to utilize lactose and amygdaline. Nevertheless, dog isolate compared to other strains was able to utilize L-arabinose, D-turanose and D-galactose. D-galactose was weakly utilized also by the strain originating from the faeces of moufflon. According Gueimonde et al. (18) the fermentation profile of wild-type strains is often varied, because they have multiple sources of carbon compounds unlike strains isolated from yogurt.

Table 1 Fermentation characteristic of *B. animalis* subsp. *lactis* strains of different origin

Substrate	DSM 10140	BB-12®	Bifidus Regularis®	Ovine cheese	Cameroon sheep	Barbary sheep	Okapi	Moufflon	Dog
L-Arabinose	-	-	-	-	-	-	-	-	+
D-Ribose	+	+	+	+/-	+	+	+	+/-	+
D-Xylose	+	+/-	-	+/-	+	-	-	-	+
D-Galactose	-	-	-	-	+/-	-	-	-	+
D-Glucose	+	+	+	+	+	+	+	-	+
Amygdaline	+	+/-	+	+	+	+	+	-	-
Esculine	+	+	+	+	+	+	+	+	-
D-Maltose	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+	+/-	-
D-Melibiose	+	+	+	+	+	+	+	+	+
D-Saccharose	+	+	+	+	+	+	+	+	+
D-Rafinose	+	+	+	+	+	+	+	+	+
D turanose	-	-	-	-	-	-	-	-	+

(+) positive reaction; (-) negative reaction; (+/-) weak reaction

Conclusion

B. animalis subsp. *lactis* have been isolated from different sources and belong to non-host specific bifidobacterial species. The phenotypic and genotypic differences described in present study showed the variability between strains depending on their origin. Future analyses such as protein-coding housekeeping gene and whole genome sequencing are important in order to provide information about the adaptation of this group of microorganisms to the different ecological niches.

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