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TECHNIQUES AND STRATEGIES

Denaturing gradient gel electrophoresis analysis of faecal bacterial communities of marmot (*Marmota marmota latirostris*) in the Tatra mountains and the fragmentation of marmot population

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Abstract. Fecal samples of Alpine Marmot were collected from 8 localities in High, Low and West Tatras. DGGE analysis revealed several bacterial species involved in bowel fermentation. There dominated *Selenomonas ruminantium*, *Ruminococcus albus*, *Butyrivibrio sp.*, *Megasphaera elsdenii* and *Escherichia coli*. Bifidobacteria estimated by separate DGGE were represented mainly by *Bifidobacterium bifidum, B. longum a B. breve*. Dendrogram homology of the whole bacterial population was separating samples from different mountain ranges.

Key words: Marmot, DGGE, Tatras, community, population

Introduction

Alpine Marmots live high up in the Alps and Carpathians, in Alpine meadows where they dig their holes at an altitude ranging from 1,000 to 3,200 m. In Slovakia, they live over the forest zone. The species life style and geographical separation of colony localities limits contacts of individuals from different colonies. This leads to fragmentation of the whole population to subpopulations.

We have estimated the composition of bacterial population in marmot faeces from different locations (Table 1) and described the fragmentation of their population in North Slovakia. Faecal bacteria in marmots were studied e.g. by Pagano *et al.* (1985).

Material and Methods

Faecal samples of Alpine Marmots were collected in 8 locations in High, Low and West Tatras from 27 May 2003 to 2 October 2003. For analysis were selected fresh samples, rapidly transported and frozen to –20 °C. Bacterial DNA from the samples was isolated with QIAamp DNA Stool Mini Kit (Qiagen, USA). For PCR reaction were used DGGE primers specific for bacterial 16S DNA: FP-338GC a RP-534 (Donskey *et al.* 2003). Total bacterial populations were analyzed on DGGE gels (gradient from 40 to 60%) using a protocol based on the method of Temmerman *et al.* (2003). The electrophoresis was performed at 60 °C, at 70V for 14 hours.

Nested-PCR-denaturing gradient gel electrophoresis (nested-DGGE) was used for the detection and identification of different bifidobacteria species. The method begins with PCR amplification of 16S rRNA fragments with bifidobacteria-specific PCR primers Lm26-f (GAT TCT GGC TCA GGA TGA ACG) and Lm3-r (CGG GTG CTI CCC ACT TTC ATG) (Kaufmann *et al.* 1997, Temmerman *et al.* 2003). The product was amplified with general primers for bacteria with a clamp: FP338cl (5'- CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCG CCG CCG CAC TCC TAC GGG AGG CAG CAG - 3') and reverse primer RP534 (5'- ATT ACC GCG GCT GCT GG - 3') according to Muyzer *et al.* (1993).

Results and Discussion

There were estimated main bacterial species in the marmot faeces. Calibration of the DGGE was done with facultative and obligate anaerobic bacteria from the digestive tract of different animals. In the marmot samples dominated *Selenomonas ruminantium*, *Ruminococcus albus*, *Butyrivibrio sp.*, *Megasphaera elsdenii* and *Escherichia coli*.

Mountain range	Location	Number of sample collections
High Tatras	Malá Studená dolina (MSD)	3
	Veľká Studená dolina (VSD)	1
Western Tatras	Tichá dolina (TD)	1
	Račková dolina (RD)	2
	Žiarska dolina (ZD)	3
Low Tatras	Kráľova hoľa (KH)	3
	Chopok (CH)	1
	Chabenec (CHb)	2

Table 1. Localities of sample collections of mammrot faeces.

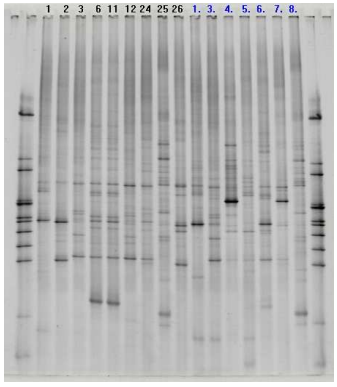

Fig. 1. DGGE profiles of bacterial species in marmot faecal samples.

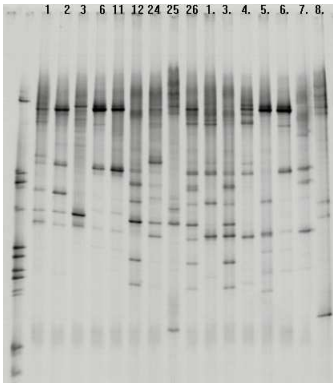

Fig. 3. DGGE profiles of bifidobacteria in marmot faecal samples.

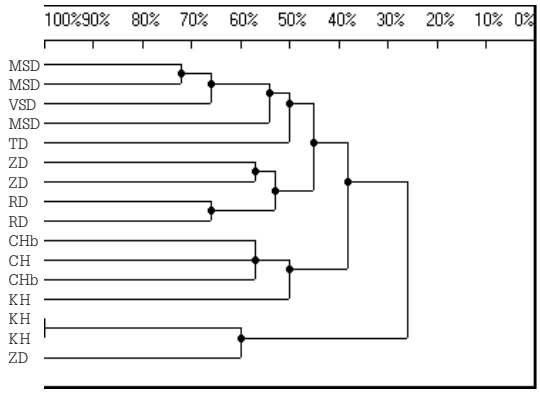

Fig. 2. Dendrogram of species homology of anaerobic bacteria in samples from different locations in Northern Slovakia. For abbreviations see Table 1.

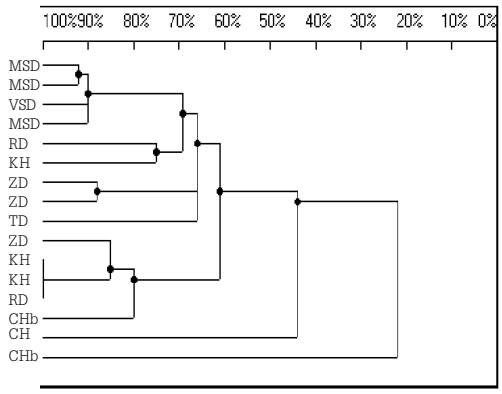

Fig. 4. Dendrogram of species homology of bifidobacteria in samples from different locations in Northern Slovakia. For abbreviations see Table 1.

Bifidobacteria estimated by separate DGGE were represented mainly by *Bifidobacterium bifidum*, *B. longum* and *B. breve*.

Dendrogram homology (Figs. 1 and 2) of the whole bacterial population from the marmot faeces separates samples from different locations – High, Low and Western Tatras. Samples from continual mountain chain - High and Western Tatras - were more similar than the samples from different mountains - the Low Tatras which are separated from th West and High Tatras by large Liptov and Spiš valleys. The genetic fingerprinting of bacterial species in faeces of marmots probably reflects the fragmentation of alpine marmot populations living in High, Low and Western Tatras.

When the species of genus *Bifidobacterium* in faecal samples were tested, the differences were not so significant. Dendrogram homology separated samples from the High Tatras only (Figs. 3 and 4).

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