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Novel rumen bacterial isolates from reindeer (*Rangifer tarandus tarandus)*[*]

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ABSTRACT

Reindeer in northern Norway experience large seasonal variations in access and quality of food. Pastures are often blocked by ice and snow during winter and reindeer are consequently exposed to periods of starvation. Supplementary feeding has become increasingly common during winter in the traditional reindeer husbandry in Norway. Transitional feeding may cause digestive problems that could be reduced by use of probiotics. This paper presents novel bacterial isolates from reindeer rumen contents, as characterized in a search for a probiotic for reindeer.

KEY WORDS: rumen, bacteria, reindeer, probiotic, 16S rDNA sequences

INTRODUCTION

Reindeer (*Rangifer tarandus tarandus*) in northern Norway live in a sub-Arctic environment with large seasonal variations in access and quality of forage. Pastures are occasionally covered with snow and ice during winter making them inaccessible and exposing reindeer to periods of starvation. Supplementary feeding has become increasingly common in the reindeer husbandry during the last 15 years. Transitional feeding of reindeer affects the rumen bacterial

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population and may be associated with digestive problems like bloat and acidosis. Treating reindeer with probiotic during phases of transitional feeding is likely to reduce such digestive problems. Studies indicate that the rumen microbial flora of reindeer is unique as a result of an evolution with adaptation to lichens and vascular plants in the region (Mackie et al., 2003; Sundset et al., 2004) and it is not desirable to introduce new bacterial strains to this complex system of which we still know very little. Rather a probiotic should be based on the bacterial strains that occur naturally in the reindeer rumen. The purpose of the current project is to isolate and characterize rumen bacteria from healthy reindeer in a search for strains that could be used as probiotics for reindeer. Probiotics for reindeer are likely to have a positive impact on animal health as well as economical benefits for reindeer herders.

MATERIAL AND METHODS

Bacterial isolates and cultural conditions

Rumen bacteria were isolated from rumen fluid (10^7 dilution) of reindeer rumen content (Olsen et al., 1997). They were cultured in a modified M8V medium in which rumen fluid from sheep was replaced by a mixture of fatty acids (Dr. J. Kopečný, personal communication; Olsen et al., 1997). One isolate (R2-25) did not grow well on the modified M8V medium and was grown in DSM medium no. 330 (Kopečný et al., 2003).

Cell morphology and motility

Bacterial morphology was determined in liquid media (M8V or M330) using light microscopy and transition electron microscopy (Olsen et al., 2002). Motility was also determined in liquid media, and Gram properties according to Huckner's staining method.

Genetic identification

Complete 16S rDNA sequence analyses were carried out on all isolates. Cell pellets were prepared from each isolate and shipped in a frozen condition to BCCM™/LGM (Belgium) for further analysis. Total DNA was prepared according to the protocol of Niemann et al. (1997). 16S rDNA genes were amplified by PCR using the primers 16F27 and 16R1522 and amplified 16S rDNA were purified using the NucleoFast® 96 PCR Clean-up Kit (Macherey-Nagel, Germany). Sequencing reactions were performed using BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and purified using Montage™ SEQ_{96} Sequencing Reaction Cleanup Kit (Milipore, MA, USA). Sequencing was performed using an ABI Prism® 3100 Genetic Analyser (Applied Biosystems, CA, USA). Sequence assembly was performed using the program AutoAssembler™

(Applied Biosystems, CA, USA) and phylogenetic analysis utilizing the software package BioNumerics (Applied Maths, Belgium).

RESULTS

Data on morphology and motility are presented in Table 1 and Figure 1. Phylogenetic analyses based on an almost complete 16S rDNA sequence indicate that isolate R6-1 is a new species in the genus *Actinomyces*. Its closest relatives are *Actinomyces oricola* and *Actinomyces slackii* with respectively 94.4 and 92.5% 16S rDNA sequence identity. Strain R2-25 is probably a new taxon in cluster XIVa (Collins et al., 1994) of the *Clostridium* subphylum of the Gram-positive bacteria. Its closest relative is *Eubacterium ventriosum* with a 94.2% 16S rDNA sequence identity. Isolate R15-13 is in the genus *Butyrivibrio*. The closest relatives are *Butyrivibrio hungatei* (96.0% identity) and *Butyrivibrio fibrisolvens* (94.2% identity). 16S rDNA sequence similarities of less than 97% indicate that the isolate represents a new and separate species in the genus *Butyrivibrio.* For isolate R8-9 taxonomical analyses reveal a great degree of similarity in 16S rDNA sequence to *Pseudobutyrivibrio ruminis* (99.0%) and *Pseudobutyrivibrio xylanivorans* (97.5%). Thus, isolate R8-9 is phylogenetically positioned in the genus *Pseudobutyrivibrio* and may belong to one of the above-mentioned species or may represent new and separate *Pseudobutyrivibrio* species.

DISCUSSION

Among the bacteria isolated and characterized by us are novel strains, as confirmed by 16S rDNA analyses. We are now screening more isolates as well as doing further investigations to determine physiological properties such as fermentation patterns. Based on new knowledge of novel and formerly described strains we will produce a bacterial probiotic cocktail for reindeer. The probiotic will include fibrolytic strains in order to increase cellulose degradation and lactate fermenting strains to reduce acidosis related to transitional feeding.

Table 1. Morphology and motility of bacterial isolates from reindeer on different diets[1]

Isolate	Morphology	Gram reaction	Motility
R2-25	Coccoid cells	Negative	+
R6-1	Short rod	Negative	+
R8-9	Long rod	Positive	-
R15-13	Long rod	Negative	+

[1] natural summer pastures (isolate R2-25 and R15-13), first cut timothy silage (R6-1), regrowth timothy silage (R8-9)

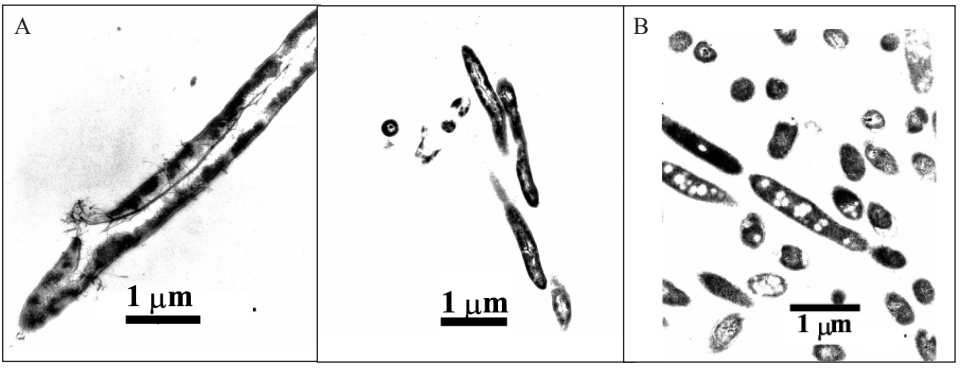

Figure 1. Transmission electron micrographs of rumen bacteria from reindeer documenting two different isolates of rod shaped cells: (A) isolate R8-9 (ca. 3 μm) and (B) isolate R15-13 with a large number of vacuoles (ca. 2 μm). Scale bars = 1 μm

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