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Factors influencing morphological variability of rumen ciliates from the genus *Ophryoscolex*[*]

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ABSTRACT

The population of the rumen ciliates *Ophryoscolex caudatus* developed from three typical individuals was maintained *in vitro*. During the long term cultivation we observed the appearance of individuals resembling *Ophryoscolex purkynjei* and *Ophryoscoex spinous* while the numbers of particular forms in the culture depended on the growth conditions. Neither amplified ribosomal DNA restriction analysis (ARDRA) nor comparison of nucleotide sequences in the 18S rDNA showed genetic diversity of the morphologically different ciliates.

KEY WORDS: rumen ciliates, cell morphology, 18S rDNA, restricting analysis

INTRODUCTION

According to Williams and Coleman (1992) eight species of rumen protozoa represent the genus *Ophryoscolex*. This number is, however, in disagreement with the earlier opinion of Latteur (1966) who believed that only *Ophryoscolex purkynjei* and *Ophryoscolex caudatus* are distinct species whereas the others have to be regarded as morphs (morphological variants) of the two species. On the other hand we observed the appearance of individuals resembling *Ophryoscolex purkynjei* and *Ophryoscolex spinosus* in population of *Ophryoscolex caudatus* that was cultured *in vitro*. The aim of this investigation was to analyse the morphological variability of the cultured protozoa and to confirm their identity by an analysis of their ribosomal genes.

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MATERIAL AND METHODS

Three typical individuals *Ophryoscolex caudatus* f. *tricoronatus* (Dogiel, 1927) were isolated from the rumen fluid of sheep and cultured *in vitro* in "*caudatum*" type medium (Coleman et al., 1972) composed of g/l: K_2HPO_4-6.3; KH_2PO_4-5.0; $MgSO_4 \times 7H_2O$-0.09; NaCl-0.65; $CaCl_2 \times 6H_20$-0.09; CH_3COONa-0.75. The ciliates were fed a diet consisting of %: powdered hay 60, wheat gluten 16, crystalline cellulose 12 and barley flour 12. The protozoa were maintained by the bath and continuous culture methods (Michałowski, 1979; Michałowski et al., 1985) for over one year and were counted two times a week. To compare the ribosomal genes 10 individuals from each morphotype were isolated by hand. The total DNA was extracted using the Chelex method and the18S rDNA was amplified by PCR using eukaryotic primers. The amplified DNA was digested with the restricting enzymes BstUI, HaeII and Mbo followed by separation of the digestion product on an 2% agarose (Regensbogenová et al., 2004). In addition the 18S rDNA was sequenced and analysed using ClustalW and GeneDoc software.

RESULTS AND DISCUSSION

The inoculum consisted of three individuals exhibiting the typical features of *Ophryoscolex caudatus* f. *tricoronatus* (Figure 1A). However, after one month of cultivation appeared ciliates with a short and thick caudal spine (Figure 1B). Such a spine is the taxonomical feature identifying *Ophryoscolex purkynjei* (Dogiel, 1927). Further reduction in length up to complete disappearance (Figure 1C) was observed with increasing cultivation time. This morphological change was accompanied by a simplification of the ring forming spines. These individuals resembled *Ophryoscolex spinosus* (Kofoid and MacLennan, 1933). Similar variations of the spine morphology had been observed earlier by Coleman and Reynolds (1982).

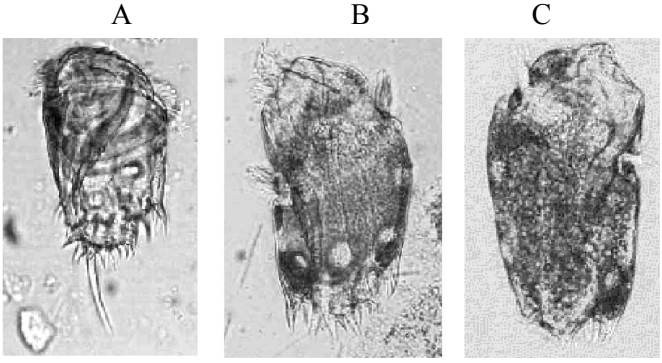

Figure 1.Three morphological forms (morphs) present in the *in vitro* culture of *Ophryoscolex caudatus*. A - typical form of the species, B - "*purkynjei*" form, C - "*spinosus"* form

The number of the various forms was influenced by the growth conditions. The appearance of "*spinosus*" forms could be stimulated by the replacement of CO_2 by a mixture consisting of N_2 (95%) and CO_2 (5%) which were used to saturate the cultivation medium. Also the change of the cultivation conditions from the bath method to continuous culture (Michałowski, 1979) resulted in an increase of the typical "*caudatus*" forms from about 21 to 78% of the total number of ciliates. The reverse change of the cultivation methos caused an increase of the was followed by an increment in the "*spinosus*" forms (from 6 to 47%) and a decrease of the "*caudatus*" morphs to 35%. The third morphological form i.e. "*purkynjei*" contributed to 16-18% of the total count of ciliates. Notably, all the different morphs exhibited an identical restriction pattern after ARDRA (amplified ribosomal DNA restriction analysis) regardless whether BstUI, HaeII or Mbol restriction enzymes were used (Figure 2). On the other hand comparison of the 18S rDNA sequences of the type strain *O. caudatus* (www.ercule.com) and *O. purkynjei* (U57768) revealed that they differ by 12 nucleotides only.

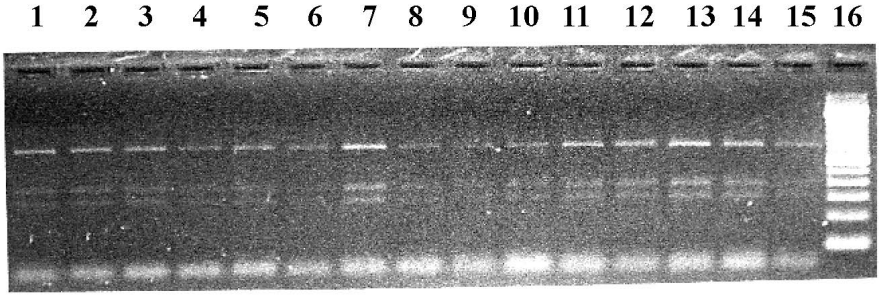

Figure 2. Comparison of ARDRA profiles following digestion with BstUI restriction endonuclease. Lanes 1-6 *O. caudatus* f. *caudatus*, lanes 6-10 *O. c.* f. *purkynjei* lanes 11-15 *O. c.* f. *spinosus*. Lane 16 - marker of molecular weight

CONCLUSIONS

The observed variations in morphology of the *Ophryoscolex caudatus* f. *tricoronatus* individuals cultured *in vitro* appeared to belong to the normal spectrum of morphs of one and the same species, since DNA analysis revealed no differences between the morphological variants. This observation, however does not argue against of different *Ophryoscolex* species in the rumen, since the DNA analysis of the type strains could reveal significant differences between the different isolates. Additional studies using ruminal isolates and *in vitro* cultivation are required to solve this problem.

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