Different strategies used by ruminal lactate-utilizing bacteria to overcome bacteriophage infections

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INTRODUCTION

Restriction-modification (R-M) systems are generally believed to protect bacterial cells against invading foreign DNA molecules, particularly bacteriophages. The most abundant are type II R-M systems, which comprise two separate sequence-specific enzymes: a restriction endonuclease and a modification methyltransferase. Restriction endonucleases are the main defense mechanism against bacteriophage infection. Mitsuokella multiacida (previously Bacteroides multiacidus), Megabphaera elsdenii and ecologically closely related species Selenomonas ruminantium use very similar nutritional strategy which is based on utilization of lactate and soluble carbohydrates released from initial hydrolys of complex polysaccharides by other ruminal microorganisms. In addition to that, all these species are closely phylogenetically related and they are placed into Acidaminococcaceae family of Firmicutes phylum (low G+C contents Gram-positive bacteria).

The main purpose of this study was to compare R-M system contents in three closely related ruminal species S. ruminantium, M. elsdenii and M. multiacida.

MATERIAL AND METHODS

M. multiacida and M. elsdenii strains were analysed for restriction endonuclease content using Heparin-Sepharose chromatography as described previously. The recognition sequence of purified endonucleases was identified by a series of single and double digestions of non-methylated pBR322, pLITMUS38 and λ-DNA substrates and by comparison of the resulting restriction patterns with those theoretically generated by the known restriction endonucleases using Gene Tool software. Total (genomic) DNA was isolated by sodium dodecylsulphate lysis and subsequent phenol extractions and analysed for site specific DNA modification by restriction endonucleases cleavage.

RESULTS

Heparin-Sepharose chromatography of cell extracts was used for purification of restriction endonucleases from seven rumen strains of M. elsdenii and two strains of M. multiacida. The purified enzymes from M. elsdenii were designated MeIOI, MeSD1, MeLTI, MeETTI, MeJLI, MeETLI and MmuS1 resp. MmuP2I from M. multiacida strains. They were used to digest various DNA substrates. Cleavage of non-methylated λ-DNA resulted in multiple fragments in range from 2.500 bp to less than 100 bp (Fig. 1A). Methylation of λ-DNA by the restriction enzyme of M. elsdenii and M. multiacida resulted in distinct banding patterns highly characteristic for each restriction system investigated (Fig. 1B).

Table 1 Comparison of restriction and modification activities in rumen lactate-utilizing bacteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Modification activity</th>
<th>Restriction activity</th>
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<tbody>
<tr>
<td></td>
<td>GA^TC</td>
<td>GATC</td>
</tr>
<tr>
<td>M. elsdenii</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M. multiacida</td>
<td>+</td>
<td>+</td>
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<tr>
<td>S. ruminantium</td>
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Comparison of obtained banding patterns with those theoretically generated by the known restriction endonucleases indicated that all strains of M. elsdenii and M. multiacida possess the isoschizomer of MboI endonuclease, recognizing GATC sequence. While all M. multiacida strains were resistant to the action of Sau3AI thereby suggesting cytosine methylation within GATC sequences and the presence of complete Sau3AI isospecific R-M systems, Megabphaera elsdenii strains were resistant to the action of Sau3AI thereby suggesting cytosine methylation within GATC sequences and the presence of complete Sau3AI isospecific R-M systems. Interestingly, total DNA were found to be resistant to MboI and sensitive to DpnI cleavage indicating adenine methylation of GATC sequence, as well.

Screening of closely related S. ruminantium species led to the detection of extremely variable population of R-M systems, when at least 8 different restriction phenotypes (endonucleases) and 13 types of modification phenotypes were detected. Quite different situation was observed in phylogenetically and ecologically close rumen species M. elsdenii and M. multiacida. While the frequency of type II R-M systems in these species is relatively high, all strains were found to possess GATC specific R-M systems only (Fig. 2A).

The lack of similarity between R-M contents of rumen lactate-utilizing bacteria is quite unexpected since R-M systems seem to be very frequently exchanged between related species. This transfer is so frequent that more than 50 different R-M system specificities among natural strains of Escherichia coli have already been found. Similarly, in other enterobacteria, which are ecologically and/or evolutionarily close, identical (in the sense of specificity) restriction endonucleases were detected (Fig. 2B).

The intra-species variety of R-M systems would be a natural consequence of selection for variability. However, possession of an identical R-M system in all strains tested does not provide efficient bacteriophage protection. Phages have a non-negligible chance of escaping restriction, becoming methylated and thereby capable of infecting all other strains. While possession of multiple type II R-M systems is probably the main mechanism for bacteriophage protection in S. ruminantium, M. elsdenii and M. multiacida use probably the different strategies.

CONCLUSIONS

Analysis of restriction and modification activities in Megabphaera elsdenii and Mitsuokella multiacida species revealed the presence of GATC-specific restriction-modification (R-M) systems in all strains tested. While restriction endonucleases isolated from M. elsdenii strains were found to be sensitive to Dam methylation, enzymes from M. multiacida cleaved DNA irrespective of Dam methylation. The comparison of R-M system specificities in closely related lactate-utilizing ruminal bacteria indicated complete lack of restriction and/or modification enzymes previously characterized from Selenomonas ruminantium. It could be assumed that M. elsdenii and M. multiacida use the different strategy for bacteriophage protection compared to S. ruminantium.

ACKNOWLEDGMENT

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