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NUMBER AND HETEROGENEITY OF rRNA OPERONS IN RUMEN BACTERIAL MEMBERS FROM THE GENUS *Prevotella*

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A B S T R A C T

The number of rRNA operons and internal spacer region heterogeneities in members of the rumen genus *Prevotella* were analysed. Chromosomal DNA from 20 strains belonging to *P.ruminicola*, *P.albensis*, *P.bryanti* and *P.brevis* was isolated, digested by five restriction enzymes, and hybridized with 16S and 23S rDNA probes. The resulting hybridization patterns indicate that *P. albensis* strains possess 4 or 5 rRNA operons, strains belonging to *P. brevis* possess 4 rRNA operons and *P. bryantii* strains 6 or 7 rRNA operons. Strains belonging to *P. ruminicola* probably possess 4 rRNA operons, with one exception having 6 or 7 rRNA operons. 16S-23S spacer region was also PCR amplified. The results suggest that there are at least three size variable 16S-23S spacer regions in *P. albensis* and *P. brevis*, two in *P. ruminicola*, and only one spacer region in *P. bryantii*.

Key words: microbiology / molecular genetic / bacteria / *Prevotella* / rRNA operon / internal spacer region / rumen

ŠTEVILO IN HETEROGENOST rRNK OPERONOV PRI VAMPNIH BAKTERIJSKIH VRSTAH IZ RODU *Prevotella*

I Z V L E Č E K

Analizirali smo število operonov ribosomskih RNK in heterogenost internih medgenskih regij pri vampnih bakterijskih vrstah iz rodu *Prevotella*. Izolirali smo genomsko DNK dvajsetih sevov iz vrst *P.ruminicola*, *P.albensis*, *P.bryanti* in *P.brevis* in jo razrezali s petimi restrikcijskimi endonukleazami. Hibridizacijo smo izvedli s 16S in 23S rDNK sondami. Glede na število hibridizacijskih signalov lahko sklepamo, da imajo sevi vrste *P. albensis* 4 ali 5 rRNK operonov, sevi vrste *P. brevis* 4 rRNK operone, sevi vrste P. *bryantii* 6 ali 7 rRNK operonov. Vrsta *P. ruminicola* ima 4 rRNK operone, z izjemo enega seva, ki ima 6 ali 7 rRNK operonov. Namnoževanje regije med genoma za 16S in 23S rRNK je pokazalo, da so pri vrstah *P. albensis* in *P. brevis* vsaj tri različno dolge interne medgenske regije, pri vrsti *P. ruminicola* dve in pri vrsti *P. bryantii* samo ena.

Ključne besede: mikrobiologija / molekularna genetika / bakterije / *Prevotella* / rRNK operon / interne medgenske regije / vamp

INTRODUCTION

Molecular taxonomy enables classification of micro-organisms into an evolutionary based system (Woese, 1987). The most widely used tool of molecular taxonomy is a comparison between 16S rRNA genes sequences of different organisms. The genes encoding 16S rRNA are organised in operons together with 23S rRNA and 5S rRNA genes. In bacterial operons rRNA genes are usually linked in 16S-23S-5S order with inteRNAl spacer regions (ISR) between them. This order and linkage of rRNA genes is common, though not universal feature of bacterial rRNA organisation. Ribosomal RNA operons also differ with regard to the presence or absence and identity of tRNA genes in the spacer region (Krawiec and Riley, 1990). The number of rRNA gene copies in different bacterial species varies greatly; from 1(*Mycoplasma*) to 12 (*Paenibacillus*) rRNA operons per genome. Although 16S rRNA analysis is increasingly used in microbial taxonomy and ecology, until recently, presence of multiple copies of 16S rRNA genes, and possible sequence differences between these copies were generally ignored (Clayton *et al*., 1995).

The aim of our work was to determine the number of rRNA operons and inteRNAl spacer region heterogeneities in strains of recently re-classified *Prevotella ruminicola*, *Prevotella albensis*, *Prevotella bryantii* and *Prevotella brevis* (Avguštin *et al*., 1997), which are anaerobic bacterial species and represent important microbial part of the rumen ecosystem.

MATERIAL AND METHODS

Bacterial strains, media and culture conditions

The bacterial strains belonging to *P. ruminicola, P. albensis, P. bryantii and P. brevis* used in this study have been described previously (Avguštin *et al*., 1994; Avguštin *et al*., 1997). Strains stored in semi-solid (with 0,7 % agar) M2 medium (Hobson, 1969) were cultured according to Bryant modification of Hungate technique for cultivation of anaerobic micro-organisms (Bryant, 1972).

DNA extraction

Total DNA was extracted from bacterial strains by modified method from "Current Protocols in Molecular Biology" (Ausubel *et al*., 1987). Bacterial cultures were grown overnight in liquid M2 medium. The cells were harvested, supeRNAtant was discarded, and cells were resuspended in TE buffer (pH 8,0). A 0,5 ml of 10% SDS (pH 7,2) and 50 µl proteinase K (20 mg/ml) were added and suspension was incubated for 20 minutes at 37^{o}C. After that 1,8 ml of 5 M NaCl and 1,5 ml of CTAB/ NaCl (10% hexadecyltrimethyl ammonium bromide in 0,7 M NaCl) were added, mixed and incubated for 20 minutes at 65^{o}C. The mixture was then extracted with an equal volume of chloroform-isoamyl alcohol (24:1). Following the centrifugation at 6000 g and room temperature for 10 minutes, the aqueous phase was transferred to a new vessel. DNA was recovered by spooling, after precipitation with 0,6 volume of isopropanol, and redissolved in 4 ml of TE buffer. Chromosomal DNA was further purified by CsCl – ethidium bromide density gradient centrifugation. The ethidium bromide was removed by sequential extraction with water-saturated *n*-butanol, samples were dialysed against TE buffer at 4^{o}C, and the DNA was concentrated by ethanol precipitation.

Hybridization probes

The amplification of 16S rDNA with polymerase chain reaction (PCR) was carried out with universally conserved 16S bacterial primer FD1 (AGAGTTTGATCCTGGCTCAG-3'; 8-26 *E. coli* numbering) (Weisburg *et al*., 1991) and universally conserved bacterial reverse 16S primer (aagcttgcggccgcACGGGCGGTGTGTRC - 3', R=A/G; 1392-1406 *E. coli* numbering) (Olsen *et al*., 1986) as described previously (Peterka *et al*., 1997). Amplification of 23S rDNA with PCR was carried out with forward primer (GGAACTGAAACATCTAAGTA-3'; 188-207 *E. coli* numbering) (Van Camp *et al*., 1993) and reverse primer (ACCCGACAAGGAATTT CGC-3'; 1933-1951 *E. coli* numbering) (Amann *et al*., 1995). Subsequently, 16S rDNA and 23S rDNA were labelled with digoxigenin according to manufacturer instructions (DIG DNA Labelling and Detection Kit, Boehringer Mannheim).

Hybridization analysis

Chromosomal DNA was digested with five restriction enzymes (*BstE*II, *Bgl*II, *Xho*I, *Sca*I, *Sac*I, Boehringer Mannheim), separated through 0,8% agarose gel (2,5 V/cm) and transferred to nylon membrane by Southern blot. Hybridization with DIG - labelled 16S rDNA and 23S rDNA probes was carried out at 68^{o}C.

16S – 23S spacer region amplification

16S-23S spacer regions were amplified by PCR using forward 16S rRNA primer P1 (TGGGGTGAAGTCGTAACAAGGTA-3'; 1484-1506 *E. coli* numbering) (Rijpens *et al*., 1996) and reverse 23S rRNA primer (TACTTAGATGTTTCAGTTCC3'; 188-207 *E. coli* numbering) (Van Camp *et al*., 1993).

RESULTS AND DISCUSSION

Hybridization of digested genomic DNA and DIG - labelled 16S rDNA and 23S rDNA probes was used to determine the number of rRNA genes in bacterial strains from the genus *Prevotella*. All of the used restriction enzymes were tested for activity inside of the 16S and 23S probes too, and observations were considered at results interpretation. The number of hybridization bands with 16S rDNA and 23S rDNA probes for all restriction enzymes are shown in Table 1.

Table 1. The number of detected rDNA targets (bands) in digested genomic DNA of analysed species.
Preglednica 1. Število s hibridizacijo odkritih rDNK tarč v razrezanih genomskih DNK pri preiskanih vrstah.

Restriction enzyme	*BstE*II		*Sca*I		*Bgl*II		*Sac*I		*Xho*I	
Probe / Species	**16S**	**23S**	**16S**	**23S**	**16S**	**23S**	**16S**	**23S**	**16S**	**23S**
P.ruminicola	4	4*	4	4*	-	-	-	-	4	4
P. albensis	4/5	4*	5	8/9*	-	4	4	-	5*	5/6
P. bryanti	6	6	7	13*	7	6/7	6	6	6*	6/7
P. brevis	4	4	-	3	4	4	3	3	2	2

*- enzyme cut inside of the probe

Results indicate that *P. ruminicola* possesses 4 rRNA operons with exception of one strain that possesses 6 or 7 rRNA operons. Strains belonging to *P. brevis* possess 4 rRNA operons, *P. albensis* strains possess 4 or 5 rRNA operons and strains belonging to *P. bryantii* 6 or 7 rRNA operons. To determine the exact number and structure of rRNA operons, further hybridization analysis should be done.

Amplification of 16S – 23S rDNA spacer regions, using conserved 16S and 23S rRNA primers, produced three bands in *P. albensis* (530 bp, 630 bp, 670 bp) and *P. brevis* (700 bp, 800 bp, 850 bp), two bands in *P. ruminicola* (720 bp, 840 bp) and only one band in *P. bryantii* (770 bp) (Figure 1). According to primers positions and size of the amplified fragments, the sizes of ISR in *P. albensis* are 270 bp, 370 bp and 410 bp. The sizes of ISR in *P. brevis* are 440 bp, 540 bp and 590 bp. *P. ruminicola* has two ISR, which are 460 bp and 580 bp long. Although *P. bryantii* possesses 6 or 7 rRNA operons (most of all analysed species), it has a single ISR of 510 bp.

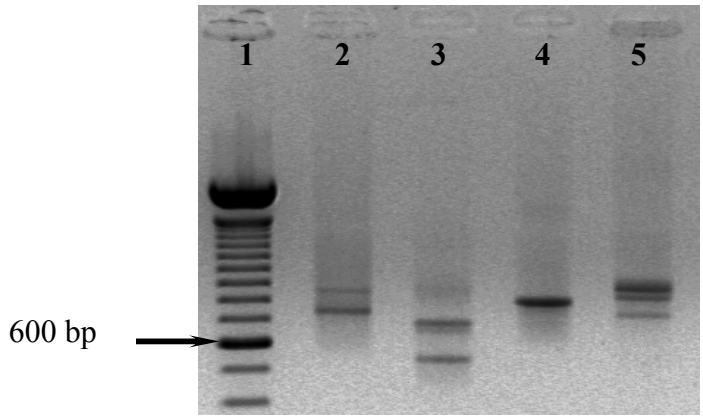

Figure 1. Amplification of 16S – 23S rDNA spacer region. Lane 1=100 bp standard (Gibco, BRL); 2=*P. ruminicola* 23T; 3=*P. albensis* M384; 4=*P. bryantii* B14; 5=*P. brevis* GA33

Slika 1. Namnoževanje regije med 16S rDNK in 23S rDNK. Linija 1=velikostni standard 100 bp (Gibco, BRL); 2=*P. ruminicola* 23T; 3=*P. albensis* M384; 4=*P. bryantii* B14; 5=*P. brevis* GA33

POVZETEK

Molekularna taksonomija omogoča razvrščanje mikroorganizmov v evolucijsko utemeljeni sistem. V veliki meri temelji na primerjavah sekvenc genov za manjšo ribosomsko podenoto RNK t.j. 16S rRNK. Vpliv različnega števila genov za rRNK v genomu na filogenetske in ekološke študije je skoraj popolnoma neraziskan. V našem delu smo analizirali število operonov ribosomskih RNK in heterogenost internih medgenskih regij pri vampnih bakterijskih vrstah iz rodu *Prevotella*. Izolirali smo genomsko DNK dvajsetih sevov iz vrst *P.ruminicola*, *P.albensis*, *P.bryanti* in *P.brevis* in jo razrezali s petimi restrikcijskimi endonukleazami. Hibridizacijo smo izvedli s 16S in 23S rDNK sondami. Glede na število hibridizacijskih signalov lahko sklepamo, da imajo sevi vrste *P. albensis* 4 ali 5 rRNK operonov, sevi vrste *P. brevis* 4 rRNK operone, sevi vrste P. *bryantii* 6 ali 7 rRNK operonov. Vrsta *P. ruminicola* ima 4 rRNK operone, z izjemo enega seva, ki ima 6 ali 7 rRNK operonov. Namnoževanje regije med genoma za 16S in 23S rRNK je pokazalo, da so pri vrstah *P. albensis* in *P. brevis* vsaj tri različno dolge vmesne regije, pri vrsti *P. ruminicola* dve in pri vrsti *P. bryantii* samo ene.

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