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DEOXYRIBONUCLEASE ACTIVITIES OF RUMEN BACTERIA FROM THE GENUS *PREVOTELLA*

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ABSTRACT

Twenty-nine strains belonging to four species of the anaerobic bacterial genus *Prevotella* were tested for deoxyribonuclease activity against the nonmethylated λ phage DNA and circular plasmid pBR322 DNA. The rate of DNA degradation when comparing different species and even strains within the tested species showed large variation. The strains of the species *P. brevis* and *P.bryantii* (with exception of the strain TC1-1) all exhibit high activity and completely degraded λ DNA after 30 seconds. No DNase activity could however be detected with 14 of the 29 tested strains. The survey of the DNase activities confirmed the validity of the recent reclassification of rumen bacteria from the genus *Prevotella.* A simple method allowing the screening of DNase activities of anaerobic rumen microorganisms on agar plates is described too.

Key words: microbiology / bacteria / *Prevotella* / rumen / enzymes / deoxyribonuclease

DEOKSIRIBONUKLEAZNE AKTIVNOSTI VAMPNIH BAKTERIJ IZ RODU *PREVOTELLA*

IZVLEČEK

Pri 29 sevih iz anaerobnega bakterijskega rodu *Prevotella* smo preverili deoksiribonukleazno aktivnost proti nemetilirani DNK faga λ in krožni plazmidni DNK pBR322. Opazili smo velike razlike med stopnjo aktivnosti preiskovanih sevov. Sevi vrste *P. brevis* in *P.bryantii* (z izjemo seva TC1-1) so vsi zelo aktivni in popolnoma razkrojijo λ DNK že po 30 sekundah. Kar pri 14 od 29 preiskanih sevov nismo odkrili DNazne aktivnosti. Pregled DNaznih aktivnosti je potrdil nedavno predlagano reklasifikacijo vampnih bakterij iz rodu *Prevotella*. Opisali smo tudi enostavno metodo, ki omogoča pregledovanje DNaznih aktivnosti vampnih anaerobnih mikroorganizmov na agarskih ploščah.

Ključne besede: mikrobiologija / bakterije / *Prevotella* / vamp / encimi / deoksiribonukleaze

INTRODUCTION

The deoxyribonucleases represent an enzymatic protection system against the unwanted acquisition of the heterologuous DNA by bacterial cells. Their activities can also present major problems when gene transfer system development is concerned, especially in bacterial groups where our knowledge is limited. Rumen bacteria from the genus *Prevotella*, all gram negative and strictly anaerobic, received a fair share of the genetic research among rumen microorganisms in recent years (Flint, 1994). Species from this genus were assumed to play an important role in a

number of processes going on in the rumen, such as breakdown of the structural polysaccharides of the plant cell wall like hemicelluloses and pectin and breakdown of starch, proteins and peptides (Avguštin *et al.*, 1994). Gene transfer has recently been investigated in a smaller number of predominant rumen bacteria among others in *P.ruminicola* and some to a certain extent successful vector systems were elaborated (Thomson and Flint, 1989, Flint and Thomson 1991, Bechet *et. al.*, 1993). The DNase activity of a limited number of strains belonging to different species of this genus was also tested and a considerable variety was noted (Flint in Thomson, 1990).

Species belonging to the genus *Prevotella* have recently undergone major taxonomic changes as a result of increased use of molecular taxonomy approach. Shah and Collins (1990) transferred the species *Bacteroides ruminicola* from the genus *Bacteroides* into a newly constituted genus *Prevotella.* A low level of phylogenetic homogeneity was shown to exist within the species (Mannarelli *et al.*, 1991, Avguštin *et al.*, 1994). The findings started to bring down the established view of the role of this particular species in the ruminal ecosystem and have also given a new insight into the problems connected to the gene transfer system development. Recently a reclassification of the *P.ruminicola* was proposed and the establishment of three new species was suggested (Avguštin *et al.*, 1997).

We have tested a wider selection of strains shown to belong to one of the four ruminal species from the genus *Prevotella* i.e. *P.ruminicola*, *P.bryantii*, *P.brevis* and *P.albensis* for deoxyribonuclease activity. The assays should provide new physiological data that would expectedly confirm the phylogenetically based proposals for reclassification. At the same time this approach should tell us more about the enzyme protection systems of mentioned bacteria and possibly explain some of the problems connected with the usage of developed plasmid vectors.

MATERIALS AND METHODS

Bacterial strains and their origins are shown in Table 1. M2 medium (Hobson, 1969) was prepared anaerobically under O_2-free CO_2 according to the Bryant's technique (Bryant, 1972).

Rapid nuclease assay

The cultures were grown until stationary growth phase at 38^o C, centrifuged for 15 min at 2000 g and 0.5 ml of the supernatant was transferred to a sterile 1.5 ml microcentrifuge tube. The samples were centrifuged at 13400 g for 3 min. and 70 µl of the supernatant was transferred to a fresh microcentrifuge tube. DNA was added to a final concentration 5 µg ml^{-1}. Non methylated λ phage DNA (λ C1857 Sam7) (host *dam*- dcm- *E.coli* GM119) (Sigma, D-3654) and circular plasmid pBR322 DNA (host *E.coli* HB101, Pharmacia) was used. The microcentrifuge tubes (with supernatants and DNA) were incubated in a water bath at 38^{0}C. 30 µl of a sample was transferred into a fresh microcentrifuge tube and mixed with 30 µl of a (1:1) Tris equilibrated phenol (pH 7.4) and chloroform. Samples were centrifuged for 3 min at 13400 g and 10 µl of supernatant was loaded on a 0.6 % (w/v) agarose gel and electrophoresed (Flint and Thomson, 1990).

Nuclease plate assay

Bacterial strains were grown in an anaerobic chamber (Scholzen Technik) at 38° C on a solid M2 medium containing 100 µg ml^{-1} salmon spearm DNA (Fluka) and 10 mM $MgCl_2$. When visible growth was observed the bacteria was scraped off the plates with a sterile glass spreader. The plates were washed three times with 0.9 % NaCl and stained for two hours with 10 ml of

1 μg ml^{-1} ethidium bromide in 10mM Tris-HCl buffer (pH=7.4). Nuclease activity was revealed as an absence of fluorescence under UV-light (Muro-pastor *et al.,* 1992).

RESULTS AND DISCUSSION

Rapid nuclease assay

Twenty-nine (29) pure culture strains (Table 1) were tested for deoxyribonuclease activities. Cultures were grown to stationary phase and the supernatants were tested by incubation with linear λ DNA and circular plasmid pBR322 DNA. Nonmethylated λ DNA was tested only, since it was previously shown that there is no significant variation between nonmethylated and methylated λ DNA degradation by rumen *Prevotella* strains (Flint and Thomson, 1990). DNase activity and the degradation of the substrate were assessed by agarose electrophoresis (Fig.1.)

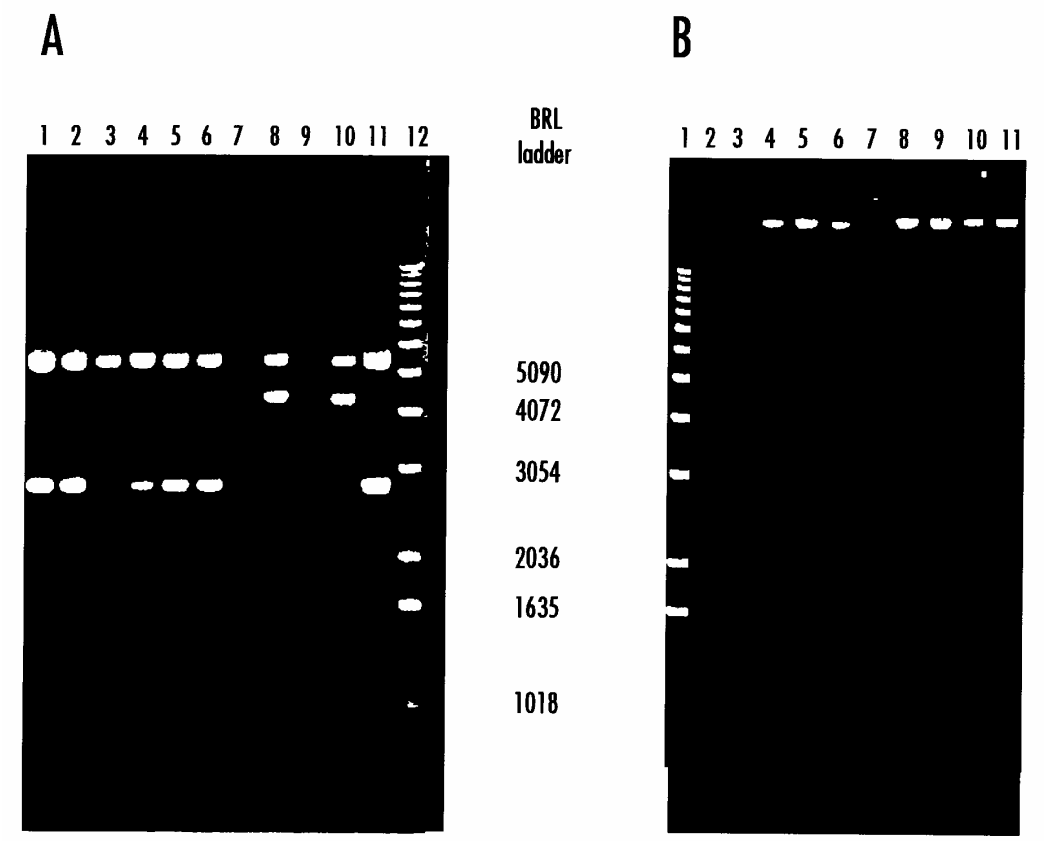

Fig. 1. DNase activity of rumen *Prevotella* strains. A: degradation of circular plasmid pBR322 DNA. $^{30''}$ = 30 seconds incubation time; $^{10'}$ = 10 minutes incubation time. = Lane 1 = TC27$^{10'}$, 2 = TC27$^{30''}$, 3 = TC20$^{10'}$, 4 = TC20$^{30''}$, 5 = TC18$^{10'}$, 6 = TC18$^{30''}$, 7 = FC2$^{10'}$, 8 = FC2$^{30''}$, 9 = 9958/78$^{10'}$, 10 = 9958/78$^{30''}$, 11 = pBR322, 12 = 1 kbp ladder (BRL). B: degradation of nomethylated λ bacteriophage DNA. Lane 1 == 1 kbp ladder (BRL), 2 = FC2$^{30''}$, 3 = FC2$^{10'}$, 4 = TC18$^{30''}$, 5 = TC18$^{10'}$, 6 = TC20$^{30''}$, 7 = TC20$^{10'}$, 8 = TC27$^{30''}$, 9 = TC27$^{10'}$, 10 = TC35$^{30''}$, 11 = TC35$^{10'}$.

Culture supernatants were tested for DNase activity only since it was shown previously (Flint and Thomson, 1990) in a smaller number of strains that strains exhibit similar level of the activity when comparing the culture supernatants and broken cell preparations.

Table 1. DNase activities of rumen *Prevotella* strains and the origin of strains.

Species	Strain	Origin of strains	λ DNA (48 kbp)	PBR322 (4,3 kbp)
P.ruminicola	23^T	1	-	-
	118B	1	-	-
	TC18	2	-	-
	TC27	2	-	-
	TC35	2	-	-
	TC44	2	-	-
	TC2-3	2	-	-
	TS1-2	2	-	-
	TS4-6	2	-	-
	TF1-5	2	++	++
	TF1-10	2	-	-
P.ruminicola – like	TC20	2	+	+-
	TC2-18	2	+-	+-
	TC2-24	2	-	-
	TC2-28	2	-	-
	TS2-7	2	++	+-
	223/M2/7A	3	-	+-
P.brevis	$GA33^T$	5	++	+
	FC2	6	++	+
	FC4	6	++	++
	FC6	6	++	+
P.brevis – like	9958/78	4	+-	+
	TF2-5	2	+	-
P.bryantii	$B_1 4$	8	++	++
	TF1-3	2	++	++
	TC1-1	2	-	-
	TS1-5	2	++	++
P.albensis	M384	6	-	+-
Prevotella spp.	2202	7	-	-

Legend: linear λ bakteriofhage DNA (48 kilobase pairs = kbp) and circular plasmid pBR322 DNA (4.3 kbp) degradation extent was assessed by agarose gel electrophoresis after 30 sec or 10 minutes incubation with culture supernatants. ++ : complete degradation after 30 sec.; + : complete degradation after 10 minutes; +- : partial degradation after 10 minutes; - : no detectable degradation. Origins of strains were as follows: 1. M.Cotta, U.S. Department of Agriculture, Peoria, Illinois, USA (Bryant *et al.*, 1958); 2. N.O. VanGylswyk, Uppsala, Sweden (VanGylswyk, 1990); 3. Rowett Research Institute, Aberdeen, UK (Flint and Stewart, 1987); 4. H.N.Shah, London Hospital Medical College, London, UK; 5. American Type Culture Collection, Rockville, Md., USA; 6. R.J.Wallace, Rowett Research Institute, Aberdeen, UK (Wallace and Brammall, 1985, McKain *et al.*, 1992); 7. National Collection of Food Bacteria, Shinfield, UK; 8. J.B.Russell, Cornel University, Ithaca, N.Y., USA (Bryant *et al.*, 1958).

The results of the screening support the proposed reclassification of the *P.ruminicola* into *P.ruminicola sensu stricto* and three new species (Avguštin *et al.*, 1994, Avguštin *et al.*, 1997). All of the *P.brevis* strains as well as the strains designated *P.brevis* – like (Avguštin *et al.*, 1997) exhibit strong DNase activity whereas all but one (TF1-5) strain of *P.ruminicola sensu stricto*

show no activity. Four of six tested strains designated *P.ruminicola* – like showed some activity which agrees with the established genetic differences between *P.ruminicola sensu stricto* strains and *P.ruminicola* – like strains. Three of four tested *P.bryantii* strains show high activity which we found particularly interesting since the strain TC1-1 showed remarkable similarities in all other analysed characteristics apart from the DNase activity (Avguštin, 1992, Avguštin *et al.*, 1997).

Nuclease plate assay

Two *P.bryantii* strains, $B_1$4 and TC1-1 were used for the nuclease plate assay. They were chosen as model strains for presence and absence of the DNase activity. It can be seen from Fig.2 however, that both possess nuclease activity albeit it is much weaker in the strain TC1-1. Both strains were grown to the same cell density as judged by optical density measurement (not shown). The difference in activities can be easily established. It was surprising however, that some activity can be seen with the strain TC1-1 too.

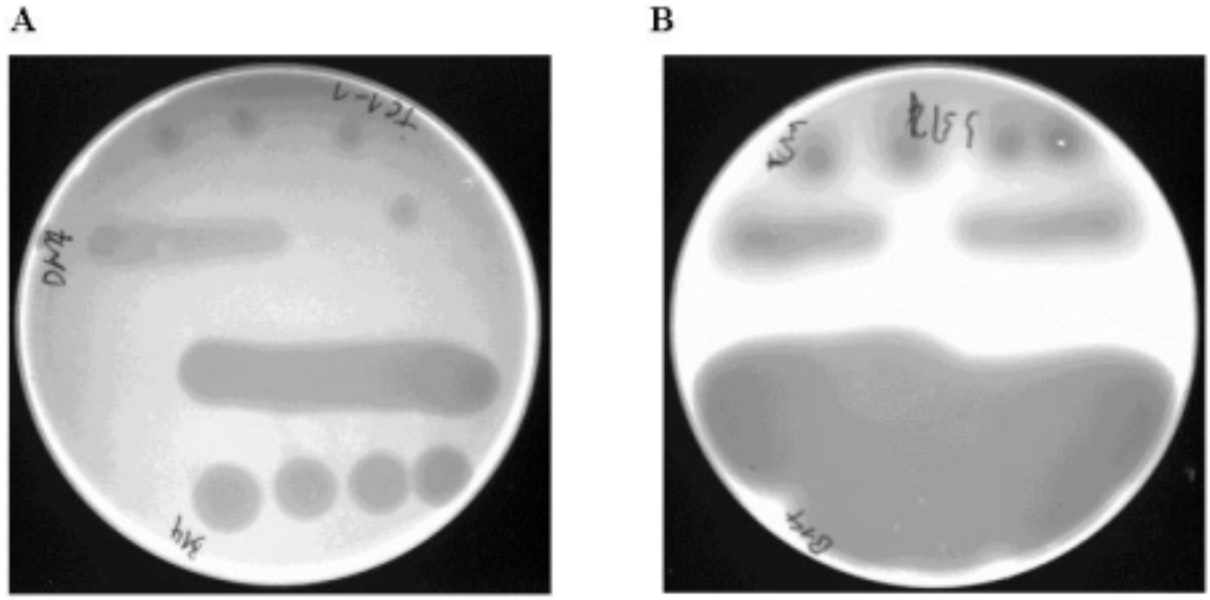

Fig. 2. $B_1$4 and TC1-1 were streaked and toothpicked on the plates in roughly the same pattern. A: 20 h incubation. Top TC1-1, bottom $B_1$4. B: 70 h incubation. Top TC1-1, bottom $B_1$4.

We can conclude that the DNase activity screening provided further information about the phenotypical and taxonomic differences between the recently proposed ruminal species of the genus *Prevotella*. The described method for screening of the DNase activities on agar plates showed however that we need to be careful if when comparing or even combining phenotypic and genotypic characteristics in the taxonomic analysis. The TC1-1 strain obviously exhibit DNase activity too, albeit at the lower level. It must therefore posses the genetic bases which codes for the activity too. The background of this phenomenon is the object of our future work.

POVZETEK

Preverili smo DNazno aktivnost 29 sevov iz štirih vrst vampnega bakterijskega rodu *Prevotella*. Vsi sevi uvrščeni v vrsto *P.brevis*, in tudi tisti, ki so tej vrsti najbolj podobni in jih opisujemo kot *P.brevis* – podobni, izražajo močno DNazno aktivnost in razkrojijo DNK prej kot

v 30 sekundah. Podobno velja za seve vrste *P.bryantii*, kjer le pri sevu TC1-1 aktivnosti nismo opazili. Obratno velja za seve vrste *P.ruminicola*, kjer smo opazili aktivnost le pri enem od preverjenih 11 sevov. Večjo raznolikost smo opazili pri sevih opisanih kot *P.ruminicola* – podobni, kar pa se sklada z že prej ugotovljenimi fenotipskimi in genotipskimi razlikami med tema skupinama sevov. Opisali smo tudi enostavno metodo za preverjanje DNazne aktivnosti anaerobnih bakterij na agarskih ploščah in kot modelna organizma uporabili DNaza pozitiven sev *P.bryantii* $B_1$4 in DNaza negativen sev *P.bryantii* TC1-1. Razliko med sevoma smo z lahkoto opazili, vendar smo na naše presenečenje odkrili tudi šibko aktivnost seva TC1-1. Rezultati podpirajo predlagane taksonomske spremembe v tem rodu, opozarjajo pa na previdnost pri primerjanju taksonomskih značilnosti fenotipskega in genotipskega izvora.

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