Analysis of *Borrelia* species associated with Lyme disease by rRNA gene restriction fragment length polymorphism

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We investigated the usefulness of rRNA gene restriction fragment length polymorphism (RFLP) for grouping of the *Borrelia* isolates associated with Lyme disease or from ixodid ticks. Genomic DNA was digested with a restriction enzyme, blotted and hybridized with an *rrl* (23S rRNA) gene probe. The sizes of the restriction bands showed a good correlation with the genotypes recently proposed, and *Borrelia* isolates of diverse geographic origin formed four distinct DNA groups. Group I included all of the USA isolates and some European isolates; group II contained European isolates and Asian isolates; group III comprised European and Asian isolates; group IV included Japanese isolates and an eastern Russian isolate. Groups I, II and III corresponded to *Borrelia burgdorferi*, *B. garinii* and group VS461, respectively. The RFLPs of Japanese isolates were rather divergent and some of the isolates were quite distinct from the USA and European isolates. RFLP analysis of the rRNA genes and flanking regions, using *rrl* gene probes as we reported here, may be useful in the taxonomic study of *Borrelia*.

Introduction

Since the first isolation of *Borrelia burgdorferi* (Burgdorfer *et al.*, 1982; Johnson *et al.*, 1984), a large number of *B. burgdorferi* isolates have been obtained from patients, animal reservoirs and tick vectors from various geographic areas of the world (Hughes & Johnson, 1990; Hughes *et al.*, 1992; Nakao *et al.*, 1992; Postic *et al.*, 1990). These organisms have been characterized from the immunological, biochemical and molecular biological aspects (Barbour & Hayes, 1986; Barbour & Garon, 1987; Bergström *et al.*, 1989; Howe *et al.*, 1985; Hyde & Johnson, 1984), and now the species *B. burgdorferi* is regarded as a heterogeneous one which encompasses diverse isolates from geographically distinct areas. Recently, on the basis of DNA homology, specific rRNA gene restriction pattern and protein electrophoresis patterns, three genospecies for the strains of *B. burgdorferi* have been proposed. Two of them are named *B. burgdorferi* and *B. garinii*, and the third genospecies is referred to as 'group VS461' (Baranton *et al.*, 1992).

A number of reports have appeared on the characterization of a variety of genera by using restriction fragment

length polymorphism (RFLP) of the rRNA operons (Grimont & Grimont, 1986; Luccini & Altwegg, 1992; Smith & Callihan, 1992; Stull *et al.*, 1988). Typically, these RFLPs are identified by genomic Southern hybridization with radiolabelled probe(s), and these procedures are most successful for characterizing the bacterial species and are also useful in identifying geographical isolates and for epidemiological studies.

We are studying the organization of the genes coding for the rRNAs of spirochaetes. Organization and transcription of genes for rRNA have been highly conserved among prokaryotes because the synthesis of rRNA is an essential process in the growth of any organism (Nomura & Morgan, 1977; Nomura *et al.*, 1984). The genes for the rRNAs are, in general, organized into operons with the order of *rrs-rrl-rrf*; the operon is transcribed as a single precursor RNA, and is processed to yield the mature rRNA molecules (Srivastava & Schlessinger, 1990). In contrast, *B. burgdorferi* has a unique organization of rRNA genes. The *rrs* gene is physically separated from the *rrl/rrf* gene cluster and specifically a 3.2 kb direct tandem duplication of *rrl/rrf* has been detected (Davidson *et al.*, 1992; Fukunaga *et al.*, 1992; Fukunaga & Sohnaka, 1992; Schwartz *et al.*, 1992). On the basis of these findings, preliminary observations of RFLP associated with the *rrl/rrf* tandem gene cluster have been reported (Schwartz *et al.*, 1992). An RFLP analysis using tandem repetition could be

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Abbreviations: *rrs*, *rrl*, *rrf*, 16S, 23S and 5S rRNA genes, respectively; RFLP, for restriction fragment length polymorphism.

Table 1. Bacterial strains, origins and genotypes.

Strain or Isolate	Origin	Genospecies*	Geographic location	Provided by:†	RFLP pattern
B31	<i>I. dammini</i>	<i>B. burgdorferi</i>	USA	ATCC 35210	I
297	Patient	<i>B. burgdorferi</i>	USA	Y. Yanagihara	I
CT-1	<i>I. dammini</i>	<i>B. burgdorferi</i>	USA	R. C. Johnson	I
TXGW	Patient		USA	R. C. Johnson	I
PAL	Patient		USA	R. C. Johnson	I
ALA	<i>I. pacificus</i>		USA	R. C. Johnson	I
IPS	<i>I. pacificus</i>		USA	R. C. Johnson	I
MMT1	<i>I. dammini</i>		USA	R. C. Johnson	I
MM1	<i>I. dammini</i>		USA	R. C. Johnson	I
NCH-1	Patient		USA	R. C. Johnson	I
20006	<i>I. ricinus</i>	<i>B. burgdorferi</i>	France	Y. Yanagihara	I
212	<i>I. ricinus</i>	<i>B. burgdorferi</i>	France	G. Baranton	I
IP1	Patient	<i>B. burgdorferi</i>	France	G. Baranton	I
IP2	Patient	<i>B. burgdorferi</i>	France	Y. Yanagihara	I
IP3	Patient	<i>B. burgdorferi</i>	France	G. Baranton	I
SV1	<i>I. ricinus</i>	<i>B. burgdorferi</i>	France	G. Baranton	I
SV3	<i>I. ricinus</i>	<i>B. burgdorferi</i>	France	G. Baranton	I
GeHo	Patient		Germany	G. Baranton	I
P/Bi	Patient	<i>B. garinii</i>	Germany	Y. Yanagihara	II-1
N34	<i>I. ricinus</i>	<i>B. garinii</i>	Germany	Y. Yanagihara	II-1
A	Tick		Germany	G. Baranton	II-1
K48	<i>I. ricinus</i>		Germany	R. C. Johnson	II-1
G25	<i>I. ricinus</i>	<i>B. garinii</i>	Sweden	Y. Yanagihara	II-1
SV2	<i>I. ricinus</i>		France	G. Baranton	II-1
PD89	Patient		China	R. C. Johnson	II-1
Ir210	<i>I. ricinus</i>		Russia	R. C. Johnson	II-1
153	<i>I. ricinus</i>	<i>B. garinii</i>	France	G. Baranton	II-2
2/B45	<i>I. ricinus</i>		Germany	Y. Yanagihara	II-3
HP1	<i>I. persulcatus</i>		Japan	N. Sato	II-4
HP3	<i>I. persulcatus</i>	<i>B. garinii</i>	Japan	N. Sato	II-4
Sika2	<i>I. persulcatus</i>		Japan	E. Isogai	II-4
HT18	<i>I. persulcatus</i>		Japan	M. Nakao	II-4
NT23	<i>I. persulcatus</i>		Japan	M. Nakao	II-4
HP13	<i>I. persulcatus</i>		Japan	N. Sato	II-5
NP5	<i>I. persulcatus</i>		Japan	Y. Yanagihara	II-6
NP10	<i>I. persulcatus</i>		Japan	Y. Yanagihara	II-6
Ip90	<i>I. persulcatus</i>	<i>B. garinii</i>	Russia	G. Baranton	II-7
BO23	Patient	VS461	Germany	G. Baranton	III-1
M7	<i>I. persulcatus</i>		China	R. C. Johnson	III-1
ASA-1	Rodent		Japan	M. Nakao	III-1
HP6	<i>I. persulcatus</i>		Japan	Y. Yanagihara	III-1
IpF	<i>I. persulcatus</i>		Japan	M. Nakao	III-2
Iper3(Ip3)	<i>I. ricinus</i>	VS461	Russia	G. Baranton	III-3
UMO1	Patient	VS461	Sweden	G. Baranton	III-3
SMS1	Patient		Sweden	Y. Yanagihara	III-3
PGau	Patient	VS461	Germany	G. Baranton	III-3
NT28	<i>I. persulcatus</i>		Japan	M. Nakao	III-3
Ip89	<i>I. persulcatus</i>		Russia	R. C. Johnson	IV-1
NT24	<i>I. persulcatus</i>		Japan	M. Nakao	IV-1
NT29	<i>I. persulcatus</i>		Japan	M. Nakao	IV-1
HT67	<i>I. persulcatus</i>		Japan	M. Nakao	IV-2

* Genospecies proposed by Baranton *et al.* (1992)

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useful in identifying closely related isolates of the same species.

We report here a grouping of *Borrelia* strains isolated from Europe, the USA and Asia. The results of this

analysis of about 50 isolates indicate that the European and USA isolates can be divided into three distinct RFLP groups. RFLP patterns of the Japanese isolates are more divergent than the USA or European isolates.

Some of the Japanese isolates are classified into groups II and III, but the other isolates do not belong to these groups.

Methods

Bacterial strains and medium. Strain designations and origins of the *Borrelia* isolates used in the present study are given in Table 1. All organisms were kept at -80°C and were routinely grown in BSKII medium (Barbour, 1984). Cells were collected, washed by centrifugation and stored at -80°C until further use. *Escherichia coli* HB101 was used as the recipient of the recombinant plasmid DNA.

Preparation of DNA and genomic hybridization. Total cellular DNA was extracted by the method of Fukunaga & Mifuchi (1989a). DNAs were digested with the enzymes indicated and electrophoresed in agarose gels. Genomic DNA fragments in an agarose gel were stained with ethidium bromide, photographed and transferred to a nylon membrane (Fukunaga & Mifuchi, 1989a). Southern hybridization was performed as described previously (Fukunaga *et al.*, 1992).

Probes used in this study. We used two types of probe in this study as indicated in Fig. 1. The plasmid containing the *NheI*–*PstI* fragment (about 200 bp of the 5' part of gene *rriI*) or the *StyI* fragment (about 300 bp of the 3' part of *rriI*) was made by appropriate restriction enzyme digestion, blunting and a self-ligation of deletion kit. The plasmid DNA was extracted and purified as previously described (Fukunaga & Mifuchi, 1989b) and subjected to polymerase chain reaction (PCR) amplification in 50 μl of a solution containing 10 mmol Tris/HCl (pH 8.3), 1.5 mmol MgCl_2 , 50 mmol KCl, 0.1% Triton X-100, 200 μmol each of dATP, dGTP, dCTP and dTTP, 2.5 units of *Taq* polymerase (Takara Shuzo Co., Kyoto, Japan), 0.4 μmol each of universal primers (M4 and RV) and 1 ng of plasmid DNA. The reaction was carried out for 30 cycles in a DNA thermal cycler (ATTO Zymoreactor, ATTO Corp., Tokyo, Japan), with a profile of denaturation at 94°C for 30 s, annealing at 45°C for 20 s, and extension at 72°C for 90 s. Each PCR product (5 μl sample of the reaction mixture) was labelled by using deoxycytidine 5'-[α - ^{32}P]triphosphate ([α - ^{32}P]dCTP) [6000 Ci mmol^{-1} (222 TBq mmol^{-1}); Amersham] and a random primer labelling kit (Takara Shuzo), and used as a genomic hybridization probe.

Results

Genomic Southern hybridization

Fifty-one *Borrelia* isolates were obtained from ticks of the the family *Ixodidae*, rodents and patients from various geographic locations. Genomic DNA was

isolated from these isolates and digested with *EcoRV*, *HincII* or *PstI*. These enzymes were chosen on the basis of our previous studies on ribosomal RNA gene organization of *B. burgdorferi* (Fukunaga & Sohnaka, 1992; Fukunaga *et al.*, 1992). DNA digests were separated in an agarose gel, blotted and hybridized with the probe. *EcoRV* cleaves inside the *rriI* sequence at a single site near the 3' end, *HincII* cleaves at the middle, and *PstI* cleaves near the 5' end (Fig. 1). Genomic Southern hybridization yielded patterns, usually sharing a common 3.2 kb fragment. As shown in Fig. 1, a 3.2 kb band represents a fragment which begins at the enzyme cleavage site in the first *rriI* sequence, includes the first *rriI* sequence, and ends at the cleavage site in the second copy of the *rriI* sequence. A second band which comprises the remainder of the second *rriI* sequence, the second *rriI* sequence, and the downstream region until the next restriction site was detected by using probe 'Sty'. Using probe 'NP' revealed a second band consisting of the remainder of the first *rriI* sequence and the upstream region until the next enzyme cleavage site.

The results of one such experiment using the 'NP' probe are shown in Fig. 2. The results presented in Fig. 2(a) reveal two bands, the 3.2 kb band being common (except in strain BO23). Five isolates (B31, MMT1, MM1, NCH-1 and IP3) gave a 5.1 kb second band which contains the 5' half of the first *rriI* sequence and the upstream region until the next *HincII* site. Iper3(Ip3) and SMS1 yielded a 3.7 kb second band but BO23 gave a single 5.0 kb band, which indicates that there is no *HincII* restriction site of the *rriI* sequence for BO23. The genomic Southern hybridization of *PstI* digests is shown in Fig. 2(b,c). By using probe 'NP', four Japanese isolates (HT18, Sika2, HP1 and HP3) and two German isolates (P/Bi and N34) presented contrasting profiles. The Japanese isolates have a *PstI* site near the 5' region of the *rriI* sequence but the *PstI* site in the German isolates is distant from the *rriI* genes. Two other isolates (Ip90 and 2/B45) have no *PstI* site in the *rriI* sequence.

Schematic representation of RFLP for *Borrelia* isolates

The results obtained with the 'NP' and 'Sty' probes are summarized and presented schematically in Fig. 3, and the RFLP patterns exhibited by the 51 *Borrelia* isolates are indicated in Table 1. Four major RFLP groups were found as shown in Table 1. All of the isolates classified into group I, including the *B. burgdorferi* B31 type strain, produced an identical RFLP pattern, and the other groups had minor differences in the sizes of some fragments.

EcoRV or *HincII* digests of the isolates of group II revealed a high degree of similarity among these isolates of diverse geographic origin, while the *PstI* digests

Fig. 1. Partial restriction map of the *rriI/rriI* gene cluster for *B. burgdorferi* strain B31. The location of the genes and probes ('NP', 'Sty') used in this study are indicated.

Fig. 2. Genomic Southern hybridization of *Borrelia* isolates. Genomic DNA from isolates as indicated in the figure was digested with *HincII* (a) or *PstI* (b, c), electrophoresed, blotted and hybridized with the 'NP' probe. All other experimental conditions are described in Methods.

Fig. 3(a)

Fig. 3(b)

Fig. 3. Schematic representation of the hybridization patterns with the probe. (a) Genomic DNA was digested with *EcoRV*, *HincII* and *PstI* and hybridized with the 'NP' probe. (b) Genomic digests were probed with 'Sty'. All results are given as averages of size estimates obtained from at least two independent Southern blots. The isolates whose fragment sizes were close together were electrophoresed side by side and the exact size was measured. The thick lines represent doublet bands. The names of strains included in each RFLP type are shown in Table 1.

showed different polymorphic bands (Fig. 3*a,b*). Of the 10 isolates belonging to group III, four lacked a *HincII* cleavage site in the *rrl* sequence while all isolates displayed an identical band with *EcoRV* or *PstI* digestion. The isolates of group IV shared a common pattern with enzyme digestion by *EcoRV* and *HincII* but not *PstI*. In the case of *EcoRV* digestion, both probes showed the same upstream *EcoRV* cleavage site. All the isolates share a common 3.2 kb band with *EcoRV* digestion, and the sizes of the second band fell into three different groups (6.5 kb, 6.3 kb and 5.2 kb). Similar results were obtained with the *EcoRV* or *HincII* digests of the isolates of group I and IV. The most consistent difference between groups I and IV was the lack of the *PstI* site in all isolates from group IV. The group III isolates and some isolates of group II also lack the *PstI* site in the *rrl* sequence. The isolates of group I and II were clearly differentiated by *EcoRV* digestion.

Discussion

A number of *Borrelia* isolates have been obtained from diverse geographic locations. Many *Borrelia* isolates have been examined by using monoclonal antibodies (Adam *et al.*, 1991; Barbour & Hayes, 1986; Barbour *et al.*, 1985), plasmid profile analysis (Barbour & Garon, 1987; Howe *et al.*, 1985; Hughes *et al.*, 1992; Schwan *et al.*, 1988) and DNA restriction pattern analysis (Hughes *et al.*, 1992; LeFebvre *et al.*, 1989). RFLP analysis of rRNA genes has several advantages because of their stability, while proteins may not be stably expressed. In fact, determination of rRNA gene RFLP (ribotyping) was previously used successfully in molecular epidemiology to differentiate various *Borrelia* isolates (Baranton *et al.*, 1992; Postic *et al.*, 1990; Schwartz *et al.*, 1992). The ribotyping has been used to differentiate *Borrelia* isolates at the level of genetic species. Baranton *et al.*, (1992) proposed three genospecies: I, *B. burgdorferi sensu stricto*; II, *B. garinii* sp. nov.; and III, group VS461.

We have reported direct tandem repeat of *rrl/rrf* sequences in *B. burgdorferi* strain B31 (Fukunaga & Sohnaka, 1992). It was of interest to determine whether there is unique rRNA gene organization in other *Borrelia* isolates, which could be useful in classifying closely related isolates. Genomic DNA was extracted from 51 different *Borrelia* isolates, digested with *EcoRV*, *HincII* or *PstI*, electrophoresed, blotted, and probed with two specific types of *rrl* sequence, 'NP' and 'Sty'. The results revealed that all *Borrelia* isolates contain two sets of *rrl/rrf* sequence, and four different RFLP groups were present. Group I comprised all the USA isolates and many European isolates. Group II and group III consisted of isolates obtained from Europe and Asia.

Group IV contained Japanese isolates and a Russian Asia isolate. Groups I, II and III corresponded to *B. burgdorferi*, *B. garinii* and group VS461, respectively, as stated by Baranton *et al.* (1992).

In contrast to group I, the isolates belonging to group II showed diverse RFLP patterns with *PstI* or *HincII* digestion. By using probe 'NP', all the Japanese isolates (group II-4, 5, and 6) showed a pattern distinct from that of the isolates from other geographic locations. It seemed possible, therefore, that this group would form a distinct subset of the species. With *HincII* digests of group III isolates, quite characteristic patterns were obtained. Group III (group VS461) might be divided into at least two subgroups [group III-1 and group III-3, strain IPF: group III-2 exhibiting both fragments characteristic of group III-1 (5.0 kb) and III-3 (3.2 kb)]. The isolates that belonged to group IV, including a Russian isolate (Ip89, isolated from *I. persulcatus* ticks in Khabarovsk territory), showed an RFLP pattern distinct from that of any other groups.

Our studies indicate that RFLP analysis for tandem duplication of rRNA genes and flanking regions in *Borrelia* species by using *rrl* gene probes ('NP' and 'Sty') provides a simple and useful tool for determination of genetic relatedness between different *Borrelia* isolates. We are now applying this technique in an epidemiological study of Japanese *Borrelia* isolates from various sources.

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References

- ADAM, T., GASSMAN, G. S., RASIAH, C. & GÖBEL, U. B. (1991). Phenotypic and genotypic analysis of *B. burgdorferi* isolates from various sources. *Infection and Immunity* **59**, 2579–2585.
- BARANTON, G., POSTIC, D., SAINT GIRONS, I., BOERLIN, P., PIFFARETTI, J. C., ASSOUS, M. & GRIMONT, P. A. D. (1992). Delineation of *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *International Journal of Systematic Bacteriology* **42**, 378–383.
- BARBOUR, A. G. (1984). Isolation and cultivation of Lyme disease spirochetes. *Yale Journal of Biology and Medicine* **57**, 521–525.
- BARBOUR, A. G. & GARON, C. F. (1987). Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. *Science* **237**, 409–411.
- BARBOUR, A. G. & HAYES, S. F. (1986). Biology of *Borrelia* species. *Microbiological Reviews* **50**, 381–400.
- BARBOUR, A. G., HEILAND, R. A. & HOWE, T. R. (1985). Heterogeneity of major proteins in Lyme disease *Borrelia*: a molecular analysis of North American and European isolates. *Journal of Infectious Diseases* **152**, 478–484.
- BERGSTRÖM, S., BUNDOC, V. G. & BARBOUR, A. G. (1989). Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochete *Borrelia burgdorferi*. *Molecular Microbiology* **3**, 478–486.
- BURGDORFER, W., BARBOUR, A. G., HAYES, S. F., BENACH, J. L., GRUNWALDT, E. & DAVIS, J. P. (1982). Lyme disease – a tick borne spirochetosis? *Sciences* **216**, 1317–1319.

- DAVIDSON, B. E., MACDOUGALL, J. & SAINT GIRONS, I. (1992). Physical maps of the linear chromosome of the bacterium *Borrelia burgdorferi* 212, a causative agent of Lyme disease, and localization of rRNA genes. *Journal of Bacteriology* **174**, 3766–3774.
- FUKUNAGA, M. & MIFUCHI, I. (1989*a*). The number of large ribosomal RNA genes in *Leptospira interrogans* and *Leptospira biflexa*. *Microbiology and Immunology* **33**, 459–466.
- FUKUNAGA, M. & MIFUCHI, I. (1989*b*). Unique organization of *Leptospira interrogans* rRNA genes. *Journal of Bacteriology* **171**, 5763–5767.
- FUKUNAGA, M. & SOHNAKA, M. (1992). Tandem repeat of the 23S and 5S ribosomal RNA genes in *Borrelia burgdorferi*, the etiological agent of Lyme disease. *Biochemical and Biophysical Research Communications* **183**, 952–957.
- FUKUNAGA, M., YANAGIHARA, Y. & SOHNAKA, M. (1992). The 23S/5S ribosomal RNA genes (*rrl/rrf*) are separate from the 16S ribosomal RNA gene (*rrs*) in *Borrelia burgdorferi*, the aetiological agent of Lyme disease. *Journal of General Microbiology* **138**, 871–877.
- GRIMONT, F. & GRIMONT, P. A. D. (1986). Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Annales de l'Institut Pasteur/Microbiology* **137B**, 165–175.
- HOWE, T. R., MAYER, L. W. & BARBOUR, A. G. (1985). A single recombination plasmid expressing two major surface proteins of the Lyme disease spirochete. *Science* **227**, 645–646.
- HUGHES, C. A. N. & JOHNSON, R. C. (1990). Methylated DNA in *Borrelia* species. *Journal of Bacteriology* **172**, 6602–6604.
- HUGHES, C. A. N., KODNER, C. B. & JOHNSON, R. C. (1992). DNA analysis of *Borrelia burgdorferi* NCH-1, the first northcentral U. S. human Lyme disease isolate. *Journal of Clinical Microbiology* **30**, 698–703.
- HYDE, F. W. & JOHNSON, R. C. (1984). Genetic relationship of Lyme disease spirochetes to *Borrelia*, *Treponema* and *Leptospira*. *Journal of Clinical Microbiology* **20**, 151–154.
- JOHNSON, R. C., HYDE, F. W., SCHMIDT, G. P. & BRENNER, D. J. (1984). *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. *International Journal of Systematic Bacteriology* **34**, 496–497.
- LEFEBVRE, R. B., PERNG, G. C. & JOHNSON, R. C. (1989). Characterization of *Borrelia burgdorferi* isolates by restriction endonuclease analysis and DNA hybridization. *Journal of Clinical Microbiology* **27**, 636–639.
- LUCCINI, G. M. & ALTWEGG, M. (1992). rRNA gene restriction patterns as taxonomic tools for the genus *Aeromonas*. *International Journal of Systematic Bacteriology* **42**, 384–389.
- NAKAO, M., MIYAMOTO, K., UCHIKAWA, K. & FUJITA, H. (1992). Characterization of *Borrelia burgdorferi* isolated from *Ixodes persulcatus* and *Ixodes ovatus* ticks in Japan. *American Journal of Tropical Medicine and Hygiene* **47**, 505–511.
- NOMURA, M., GOURSE, R. & BAUGHMAN, G. (1984). Regulation of the synthesis of ribosomes and ribosomal components. *Annual Review of Biochemistry* **53**, 75–117.
- NOMURA, M. & MORGAN, E. A. (1977). Genetics of bacterial ribosomes. *Annual Review of Genetics* **11**, 297–347.
- POSTIC, D., EDLINGER, C., RICHAUD, C., GRIMONT, F., DUFRESNE, Y., PEROLAT, P., BARANTON, G. & GRIMONT, P. A. D. (1990). Two genomic species in *Borrelia burgdorferi*. *Research in Microbiology* **141**, 465–475.
- SCHWAN, T. G., BURGDORFER, W., SCHRUMPF, M. E. & KARSTENS, R. H. (1988). The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). *Journal of Clinical Microbiology* **26**, 893–895.
- SCHWARTZ, J. J., GAZUMYAN, A. & SCHWARTZ, I. (1992). rRNA gene organization in the Lyme disease spirochete, *Borrelia burgdorferi*. *Journal of Bacteriology* **174**, 3757–3765.
- SMITH, C. J. & CALLIHAN, D. R. (1992). Analysis of rRNA restriction fragment length polymorphism from *Bacteroides* spp. and *Bacteroides fragilis* isolates associated with diarrhea in humans and animals. *Journal of Clinical Microbiology* **30**, 806–812.
- SRIVASTAVA, A. K. & SCHLESSINGER, D. (1990). Mechanism and regulation of bacterial ribosomal RNA processing. *Annual Review of Microbiology* **44**, 105–129.
- STULL, T. L., LIPUMA, J. J. & EDLIND, T. D. (1988). A broad spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *Journal of Infectious Diseases* **157**, 280–286.