Correlation between ribosomal DNA polymorphism and electrophoretic enzyme polymorphism in *Yersinia*

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Ribosomal DNA (rDNA) polymorphism was compared with electrophoretic enzyme polymorphism for the intra- and interspecies differentiation of *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Y. intermedia*, *Y. aldovae*, *Y. frederiksenii* and *Y. kristensenii*. DNA from 90 strains previously classified into six zymotypes (*Y. enterocolitica* and *Y. frederiksenii*) and into distinct enzyme electrophoretic patterns (the four other species) was digested with *EcoRI* or *HindIII* and analysed by Southern blotting. The six species were clearly differentiated from each other. In *Y. enterocolitica*, the subclassification of biotype 1 into zymotypes 1A and 1B was also reflected in the rDNA and the four other bio-zymotypes gave four different classes of restriction pattern. In *Y. frederiksenii*, both *EcoRI* and *HindIII* gave five distinct riboclasses which correlated with the zymotypes. In the four other species, the phenotype polymorphism appeared to be better correlated with the restriction fragment length polymorphism data in some enzymes than others. The data demonstrate that the inter- and intraspecies classification by rDNA polymorphism using two restriction enzymes is similar to that based on electrophoretic enzyme polymorphism. The analysis could be refined for taxonomic and epidemiological purposes by using other restriction enzymes.

Introduction

The genetic diversity of bacterial species can be studied either by electrophoretic polymorphism of enzymes or directly by analysing chromosomal DNA polymorphism. Differential melting profiles of total DNA hybridized to a reference DNA have given information on the overall genetic relatedness of bacterial species, but this approach is inappropriate for establishing genetic relationships among closely related organisms. The restriction patterns of DNA, visualized by ethidium bromide staining, have been used as taxonomic or epidemiological markers for *Leptospira* (Marshall *et al.*, 1984), *Chlamydia* (McClenaghan *et al.*, 1984), *Campylobacter* (Kakoyianis *et al.*, 1984), *Neisseria meningitidis* (Kristiansen *et al.*, 1984) and *Branhamella catharralis* (Patterson *et al.*, 1988). However, the complexity of the large number of

fragments obtained with commonly used restriction enzymes made a rigorous side-by-side comparison of the stained patterns mandatory in order to understand the DNA relationships. Some authors (Allardet-Servent *et al.*, 1988; Schwartz & Cantor, 1984) used low-cleavage frequency restriction enzymes to circumvent this problem, but the resulting detectable DNA polymorphism is naturally low and makes the technique less useful for studies of intraspecies differences.

The optimal situation would be to use the common restriction enzymes combined with selective visualization of a reasonable number of polymorphic fragments. Grimont & Grimont (1986) and others (Bercovier *et al.*, 1986; Magee *et al.*, 1987; Irino *et al.*, 1988; Kuijper *et al.*, 1989; Stull *et al.*, 1988; Yogeve *et al.*, 1988) have demonstrated that the restriction fragment length polymorphism (RFLP) of ribosomal DNA (rDNA) may be a useful genetic marker for species and subspecies classification.

In previous papers (Goullet & Picard, 1984, 1988), we have analysed the enzyme polymorphism of *Yersinia*

Abbreviations: GDH, glutamate dehydrogenase; MDH, malate dehydrogenase; RBR, relative binding ratio; RFLP, restriction fragment length polymorphism; M_F , electrophoretic mobility.

enterocolitica, *Y. pseudotuberculosis*, *Y. intermedia*, *Y. aldovae*, *Y. frederiksenii* and *Y. kristensenii* and demonstrated that the 192 strains studied may be identified at the species level by their enzymic electrophoretograms. Within *Y. enterocolitica*, biotype 1 was divided into two zymotypes (1A and 1B), while the four other biotypes corresponded to four different zymotypes. In the absence of an intraspecies classification by biotype, we have delineated six zymotypes in *Y. frederiksenii*. The sub-grouping of the electrophoretic patterns into zymotype appeared to be less convincing for the four other species.

The aim of the present work was to evaluate the potential of ribotyping technology for the inter- and intraspecies classification of *Yersinia* and to compare the results with those obtained by enzyme electrophoretic polymorphism. To this end, we studied 90 *Yersinia* strains belonging to the above six species. The strains were chosen for their distinct enzyme electrophoretic pattern.

Methods

Bacterial strains. The 90 *Yersinia* strains tested had been previously analysed for their enzyme polymorphism (Goulet & Picard, 1984, 1988) (Table 1).

RFLP of rDNA. The original method described by Grimont & Grimont (1986) was modified to streamline the entire procedure

(Picard-Pasquier *et al.*, 1989). For preparation of DNA, bacteria from a 10 ml overnight culture at 28 °C were harvested by centrifugation at 4000 g for 5 min at 4 °C and washed with 300 µl 50 mM-Tris/HCl, pH 8, 1 mM-EDTA. The washed pellet was resuspended in 300 µl, 50 mM-Tris/HCl, pH 8, 25% (w/v) sucrose, to which were added 200 µl lysozyme (300 µg ml⁻¹) and 120 µl 0.5 M-EDTA, pH 8. The suspension was mixed and allowed to stand on ice for 5 min. The cells were lysed by adding 1.4 ml 6 M-guanidium chloride followed by 100 µl 7.5 M-ammonium acetate and incubating at room temperature with agitation for 1 h. Proteins were digested with 15 µl proteinase K (10 mg ml⁻¹) plus 200 µl 10% (w/v) Sarkosyl for 60 min at 60 °C. High molecular mass DNA was precipitated with 2 vols 100% ice-cold ethanol and recovered by spooling. The DNA was resuspended in 6 mM-Tris/HCl, pH 7.5, 6 mM-NaCl, 0.1 mM-EDTA. DNA (1–5 µg) was digested with *EcoRI* or *HindIII*, size-separated by electrophoresis on submarine agarose gels (0.8%) for 3 h at 1.5 V cm⁻¹ and transferred to nylon membranes (GeneScreen Plus) by the procedure of Southern (1975) for 18 h. rRNA (250 ng of 16S and 23S RNA from *E. coli*, Boehringer) was labelled to high specific activity (6 × 10⁷ d.p.m.) by a random oligopriming technique using a mixture of hexanucleotides (Pharmacia) and cloned reverse transcriptase from M-MLV (BRL) according to the manufacturer's instructions in the presence of [³²P]dCTP [800 Ci mmol⁻¹ (29.6 TBq mmol⁻¹) Amersham]. Cold template RNA was destroyed by boiling with 2 M-NaOH, 10% (w/v) SDS for 10 min and the labelled rDNA fragments were purified by column chromatography on Sephadex G-50. The DNA was hybridized according to the membrane manufacturer's instructions (final composition of the hybridization solution 50 mM-Tris/HCl, pH 7.5, 1 M-NaCl, 1%, w/v, SDS, 10%, w/v, dextran sulphate) for 3 h at 65 °C and the filters were washed twice with 0.15 M-NaCl, 0.015 M-sodium citrate (SSC), pH 7.3, containing 0.1% SDS for 15 min at room temperature. The filters were autoradiographed by exposure to Hyper-film MP (Amersham) for 30 min at room temperature.

Table 1. *Strains examined*

Laboratory code	Strain designation*	Biotype†	Zymotype‡	Main enzyme electrophoretic results of the intraspecies characterization correlating with the RFLP data
<i>Yersinia enterocolitica</i>				
1	CNY 96	1	1A	Esterase E ₂ at M _F 65 Esterase E ₃ at M _F 55 Esterase E ₄ at M _F 48 Esterase E ₅ at M _F 20 Esterase E ₆ at M _F -7
2	CNY 846	1	1A	
3	CNY 161	1	1A	
4	CNY 3419	1	1A	
5	CNY 10389	1	1A	
6	CNY 9023	1	1B	Esterase E ₃ at M _F 59 Esterase E ₅ at M _F 22 Acid phosphatase at pI 8.5
7	CNY 3865	1	1B	
8	CNY 106	1	1B	
9	CNY 373	2	2	Esterase E ₂ at M _F 63 Esterase E ₇ at M _F 16 Esterase E _{aa} undetected
10	CNY 336	2	2	
11	CNY 211	2	2	
12	CNY 39	3	3	Esterase E _{aa} at M _F 69 Esterase E _{ab} at M _F 69 Esterase E ₆ at M _F -21
13	CNY 8928	3	3	
14	CNY 9018	4	4	
15	CNY 374	4	4	GDH at M _F 16 Esterase E ₆ at M _F -21 and M _F -5 GDH at M _F 17
16	CNY 1	5	5	
17	CNY 3662	5	5	

Table 1—continued

Laboratory code	Strain designation*	Biotype†	Zymotype‡	Main enzyme electrophoretic results of the intraspecies characterization correlating with the RFLP data
<i>Yersinia pseudotuberculosis</i>				
1	CNY 2555			Esterase P ₄ undetected
2	CNY 2549			Esterase P _{αα} undetected
3	CNY 1830			Esterase P _{αα} undetected
4	CNY 2463			Esterase P ₄ undetected
5	CNY 2550			—
6	CNY 2556			—
7	CNY 2553			Esterase P ₂ at M _F 65
8	CNY 1553			Esterase P ₁ at M _F 82
9	CNY 1554			Esterase P ₁ at M _F 82
<i>Yersinia intermedia</i>				
1	CNY 6030			Esterase E _{αα} at M _F 75
2	CNY 6151			Esterase E _{αα} at M _F 75
3	CNY 8877			Esterase E _{αα} at M _F 76
4	CNY 8301			Esterase E _{αα} undetected
5	CNY 8771			Esterase E _{αα} at M _F 77
6	CNY 5630			Esterase E _{αα} at M _F 77
7	CNY 6300			Esterase E _{αα} at M _F 77
8	CNY 6194			Esterase E _{αα} at M _F 77
9	CNY 6237			Esterase E _{αα} undetected
10	CNY 5983			Acid phosphatase at pI 8.8
<i>Yersinia aldovae</i>				
1	CNY 7927			Esterase E ₁ at M _F 78
2	CNY 7928			Esterase E ₁ at M _F 79
3	CNY 6005			Esterase E _{αα} undetected
4	CNY 8286			Esterase E _{αα} at M _F 78
5	CNY 7090			Esterase E _{αα} at M _F 78
6	CNY 8285			Esterase E _{αα} at M _F 78
7	CNY 7109			Esterase E _{αα} at M _F 78
8	CNY 8290			Esterase E _{αα} at M _F 78
9	CNY 7112			Esterase E _{αα} at M _F 78
10	CNY 8287			Esterase E _{αα} at M _F 78
11	CNY 7970			Esterase E _{αα} at M _F > 78
12	CNY 7618			Esterase E _{αα} at M _F > 78
13	CNY 7622			Esterase E _{αα} at M _F > 78
14	CNY 7971			Esterase E _{αα} at M _F > 78
15	CNY 7632			Esterase E _{αα} at M _F > 78
16	CNY 7898			Esterase E _{αα} at M _F > 78
<i>Yersinia frederiksenii</i>				
1	CNY 5975	F ₁	}	Presence of esterase E _{βR} Presence of esterase E _{αc} GDH at M _F 17 Acid phosphatase at pI 9.5 or pI 9.45
2	CNY 5960	F ₁		
3	CNY 8721	F ₁		
4	CNY 8684	F ₁		
5	CNY 5966	F ₁		
6	CNY 5981	F ₁		
7	CNY 5982	F ₁		
8	CNY 6334	F ₁		
9	CNY 6150	F ₁		
10	CNY 8718	F ₁	}	Esterase E ₁ at M _F 87 GDH at M _F 16 Acid phosphatase at pI 8.8 Acid phosphatase at pI 9.35 GDH at M _F 14
11	CNY 6175	F ₂		
12	CNY 8801	F ₂		
13	CNY 8797	F ₂		
14	CNY 5976	F ₃	}	Presence of esterase E _{AI} GDH at M _F 19
15	CNY 867	F ₄		
16	CNY 8824	F ₅	}	Presence of esterase E _{AB} GDH at M _F 18
17	CNY 8826	F ₅		
18	CNY 8849	F ₅		
19	CNY 8786	F ₆		
20	CNY 8787	F ₆		

Table 1—continued

Laboratory code	Strain designation*	Biotype†	Zymotype‡	Main enzyme electrophoretic results of the intraspecies characterization correlating with the RFLP data
<i>Yersinia kristensenii</i>				
1	CNY 103			Esterase E ₂ ' at M _F 63
2	CNY 490			Esterase E ₂ ' at M _F 70, Esterase E _{aa} at M _F 79
3	CNY 8680			Esterase E ₂ ' at M _F 70, Esterase E _{aa} at M _F 79
4	CNY 8346			Esterase E ₂ ' at M _F 68, Esterase E _{aa} at M _F 79
5	CNY 6048			Esterase E ₂ ' at M _F 68, Esterase E _{aa} at M _F 79
6	CNY 8782			Esterase E ₂ ' at M _F 68
7	CNY 3605			Esterase E ₂ ' at M _F 70
8	CNY 5553			Esterase E ₂ ' at M _F 68, Esterase E _{AB}
9	CNY 5520			Esterase E _{aa} ' at M _F 68, Esterase E _{AB}
10	CNY 8711			Esterase E _{aa} ' at M _F 68, Esterase E _{AB}
11	CNY 8347			Esterase E _{aa} at M _F 68
12	CNY 5894			Esterase E _{aa} at M _F 77
13	CNY 1475			—
14	CNY 5874			Esterase E _{aa} at M _F 77
15	CNY 5878			Esterase E _{aa} at M _F 77
16	CNY 105			MDH at M _F 36
17	CNY 1474			MDH at M _F 36
18	CNY 5591			MDH at M _F 36

* CNY, Collection du Centre National et Centre Collaborateur OMS pour les *Yersinia* (Professor H. H. Mollaret), Institut Pasteur, Paris, France.

† According to the biotyping scheme of Bercovier *et al.* (1980).

‡ Zymotype as in Goullet & Picard (1984, 1988).

Results and Discussion

A representative RFLP pattern obtained for different species of *Yersinia* is shown in Fig. 1.

Y. enterocolitica

Seventeen strains belonging to biotypes 1 to 5 were analysed for their rDNA polymorphism by the RFLP pattern for *Hind*III (Fig. 2a) and *Eco*RI (Fig. 2b) restriction enzymes. Two distinct riboclasses encompassing the same strains were identified with each enzyme. The strains falling within riboclass 1 belong to biotype 1; all the others of riboclass 2 belong to biotypes 2 to 5. Thus, the RFLP data demonstrate unequivocally that the *Y. enterocolitica* species can be divided into two major genetic groups: the strains of riboclass 1 corresponding to biotype 1 and a distinct group of riboclass 2 strains encompassing all the genetically related biotypes 2 to 5.

The zymotype study supported a similar conclusion: it distinguished the strains of biotype 1 from those of biotypes 2 to 5 both by the presence of esterase bands E₃ and E₅ and by the distinct mobility of esterases E₂, E₄ and E₆ (Table 1) (Goullet & Picard, 1984). Further differences in the RFLP patterns of both restriction enzymes within riboclass 1 (essentially with fragments of high molecular size) allow its subdivision into ribosubclasses 1a and 1b. These ribosubclasses correspond to the subdivision of biotype 1 strains into zymotypes 1A and 1B (Table 1), which was based on the electrophoretic polymorphism of acid phosphatase and esterase bands E₃ and E₅. Within zymotypes 1A and 1B, strains CNY 10389 and CNY 106 (lanes 5 and 8, respectively) are distinguished from the other strains by their characteristic esterase E₁ polymorphism (Goullet & Picard, 1984), as they are here by their particular RFLP pattern. The differences in the *Eco*RI RFLP patterns of the strains in riboclass 2 (biotypes 2 to 5) indicates that

Fig. 1. A representative RFLP pattern obtained for different species of *Yersinia*: *Y. frederiksenii* strain CNY 5981 (lane 1); *Y. enterocolitica* strain CNY 161 (lane 2); *Y. pseudotuberculosis* CNY 2555 (lane 3); *Y. aldovae* strain CNY 8287 (lane 4); *Y. intermedia* CNY 8877 (lane 5); and *Y. kristensenii* strain CNY 8782 (lane 6). A fragment of 2.3 kb is common to all of the *Yersinia* species except *Y. intermedia* (see text for discussion).

they fall into two subclasses, 2a and 2b, although the *Hind*III data indicate no such subdivision. The strains of zymotype 2 (biotype 2) are of class 2a and those of zymotypes 4 and 5 (biotypes 4 and 5) are class of 2b. One of the two zymotype 3 (biotype 3) strains (lane 12) fell within riboclass 2a while the other (lane 13) was included in riboclass 2b. A retrospective examination of the enzyme electrophoretic data indicates that the former strain resembled the zymotype 2 strains as judged by the mobility of esterase E_2 and the latter resembled zymotypes 4 and 5 in possessing esterase E_{ab} . The strains of zymotype 5 are distinguished from zymotype 4 only by the absence of a 3.2 kb *Eco*RI fragment and a 6 kb *Hind*III fragment; all the other fragments were identical. Zymotypes 4 and 5 were differentiated only on the basis of mobility of glutamate dehydrogenase (GDH). Thus the RFLP data suggest that the strains of biotypes 4 and 5 are closely related, as previously indicated by the similarity of their esterase electrophoretic patterns (Goullet & Picard, 1984). In addition, two fragments of 2.3 kb and 1.4 kb were common to all the *Eco*RI digests of *Y. enterocolitica* (Fig. 1).

Y. pseudotuberculosis

Five ribotypes of the nine strains studied were distinguished by their *Hind*III patterns (Fig. 3a) and four were distinguished by their *Eco*RI patterns (Fig. 3b). Most of these patterns correlated with the previously described esterase electrophoretic types. The exception was strain CNY 2556 (lane 6), which was indistinguishable from strain CNY 2550 (lane 5) by esterase electrophoretic data. The converse was found for strain CNY 1830 (lane 3) and strain CNY 2463 (lane 4) which have distinct esterase electrophoretic patterns. Strains CNY 2553 (lane 7), CNY 1553 (lane 8) and CNY 1554 (lane 9), which were differentiated from the other *Y. pseudotuberculosis* strains by the mobilities of esterases P_2 for strain CNY 2553 and P_1 for strains CNY 1553 and CNY 1554, were clearly distinguished by the two restriction enzymes.

Y. pseudotuberculosis strains gave a 2.3 kb fragment in *Eco*RI digests as did the *Y. enterocolitica* strains, but could be distinguished from all other *Yersinia* strains by the presence of a 1.1 kb fragment (Fig. 1).

Y. intermedia

All but one of the 10 strains examined fell in the same riboclass with both *Eco*RI and *Hind*III (Fig. 4). The exception, strain CNY 5983 (lane 10), showed considerable differences. It was previously distinguished from the others by the pI of its acid phosphatase (Table 1). RFLP data suggest that the species *Y. intermedia* is homogeneous. Similar conclusions were reached from a comparison of enzyme electrophoretic data (genetic diversity) with the DNA melting profiles at 60 °C (Goullet & Picard, 1988). After *Eco*RI digestion all strains gave three low molecular size fragments; the 2.3 kb fragment seen in the other *Yersinia* species was not detected in *Y. intermedia*, but strains of the latter had a 1.4 kb fragment in common with *Y. enterocolitica* (Fig. 1).

Y. aldovae

The *Hind*III RFLP patterns placed all the strains in a single major riboclass (Fig. 5a), which agrees perfectly with the monomorphism of GDH and malate dehydrogenase (MDH). However three minor classes of patterns can be distinguished, and such a division appears to correlate with the different esterase electrophoretic types observed (Table 1). For example, strains CNY 7927 and CNY 7928 (lanes 1 and 2), which have an esterase E_1 of M_F 78 and M_F 79 constitute a particular ribotype (minor class 1), whereas strains CNY 7970, CNY 7618, CNY 7622, CNY 7971, CNY 7632 and CNY 7898

Fig. 2. RFLP rDNA patterns of *Y. enterocolitica* strains. (a) Schematic representation of *Hind*III restriction pattern and (b) *Eco*RI restriction pattern of DNA from strains (numbered as in Table 1) after hybridizing to ^{32}P -labelled *E. coli* rDNA probe.

Fig. 3. RFLP rDNA patterns of *Y. pseudotuberculosis* strains. (a), (b), As Fig. 2.

Fig. 4. RFLP rDNA patterns of *Y. intermedia* strains. (a), (b), As Fig. 2.

Fig. 5. RFLP rDNA patterns of *Y. aldovae* strains. (a), (b), As Fig. 2.

Fig. 6. RFLP rDNA patterns of *Y. frederiksenii* strains. (a), (b), As Fig. 2.

(lanes 11 to 16), whose esterases E_{aa} have an $M_F > 78$, constitute a different minor riboclass (minor class 3). The remaining *Y. aldovae* strains have an esterase E_{aa} of M_F 78 (or no E_{aa} was detected) and constitute another group

(minor class 2). Only two minor classes were distinguished by *EcoRI* digestion (Fig. 5b). Minor class 2 corresponds to the minor classes 2 and 3 defined by *HindIII*. All strains gave three low molecular size

Fig. 7. RFLP rDNA patterns of *Y. kristensenii* strains. (a), (b), As Fig. 2.

fragments (Fig. 1). A 1.4 kb fragment and a 2.3 kb fragment were common with *Y. enterocolitica* (except for the strains of the minor class 1 which did not exhibit the 2.3 kb fragment).

Y. frederiksenii

The rDNA polymorphism revealed by *Hind*III digestion allowed the strains to be divided into five riboclasses

(Fig. 6a). An identical subspecies classification was obtained when the *EcoRI* RFLP was studied (Fig. 6b). The strains of zymotype F₁ fell within the riboclass 1 group, whereas the RFLP pattern of strains of zymotypes F₂ and F₃ were indistinguishable using either *EcoRI* or *HindIII*. The strains of zymotypes 4, 5 and 6 gave clearly different hybridization patterns with both restriction enzymes. The results unequivocally demonstrate that the classification of *Y. frederiksenii* strains by RFLP corresponds to that of the zymotypes (Table 1) (Goullet & Picard, 1988). The only discrepancy was that the F₂ and F₃ zymotypes were indistinguishable in terms of their RFLP and hence can be considered to be closely related. A 2.3 kb fragment was present in the *EcoRI* digests of all the strains, except for strain CNY 867 (lane 15, F₄ zymotype). This particular strain has a relative binding ratio (RBR) at 60 °C of only 64% with the reference strain (CNY 6175) belonging to zymotype F₂ (Brenner *et al.*, 1980; Ursing *et al.*, 1980). Data on the RBR of the strains of zymotypes F₅ and F₆, which were clearly distinct from those of zymotypes F₁ to F₃ in this RFLP study, are not available. The RFLP and enzyme electrophoresis data suggest that the zymotype F₅ and F₆ strains, like strain CNY 867, constitute a distinct group within the species. Similar comparisons were made by Denny *et al.* (1988) for two pathovars of *Pseudomonas syringae* using selected cosmid clones of *P. syringae* pathovar *tomato* DNA as an anonymous probe in the RFLP study.

Y. kristensenii

The strains could be divided into four riboclasses on the basis of the *HindIII* RFLP data (Fig. 7a). The correlation with the previous enzyme electrophoretic typing (Table 1) indicates that the strains of riboclass 1 (lanes 1 to 7) have esterase E₂' M_F values from 63 to 70 and esterase E_{aa} M_F values of 79. The strains of riboclass 2 (lanes 8 to 11) were characterized by an E_{aa}' esterase M_F 68 and/or the presence of an esterase band similar to esterase E_{AB}. The strains of riboclass 4 (lanes 16 to 18), which exhibit a very distinct RFLP pattern, were phenotypically distinguished by a MDH M_F value of 36. Thus, as for the strains of zymotypes F₅ and F₆ of *Y. frederiksenii*, the electrophoretic variation of the MDH, which exhibits a little polymorphism, corresponds to a major intraspecies differentiation. Three riboclasses of RFLP were identified by *EcoRI* digestion (Fig. 7b). Riboclasses 1 and 3 were identical to *HindIII* riboclasses 1 and 4 whereas riboclass 2 corresponded to the strains of class 2 and 3 defined by *HindIII* digestion. This suggests that the strains of *HindIII*-defined classes 2 and 3 are closely related. All *Y. kristensenii* strains exhibited two common fragments of 2.3 kb and 1.4 kb with *EcoRI*

digestion, which were also observed in most strains belonging to *Y. enterocolitica*, *Y. aldovae* and *Y. frederiksenii* (Fig. 1).

Conclusions

An explanation of the correlation of enzyme electrophoretic polymorphism with rDNA polymorphism observed in the strains of the six *Yersinia* species could be based on the fact that rDNA sequences exist in several copies which are interspersed among single copy sequences of the bacterial DNA. Consequently, we hypothesize that the polymorphism of the rDNA sequences reflects the evolution of the single copy sequences.

References

- ALLARDET-SERVENT, A., BOURG, G., RAMUZ, M., PAGES, M., BELLIS, M. & ROIZES, G. (1988). DNA polymorphism in strains of the genus *Brucella*. *Journal of Bacteriology* **170**, 4603–4607.
- BERCOVIER, H., KAFRI, O. & SELA, S. (1986). Mycobacteria possess a surprisingly small number of ribosomal RNA genes in relation to the size of their genome. *Biochemical and Biophysical Research Communications* **136**, 1136–1141.
- BRENNER, D. J., URSING, J., BERCOVIER, H., STEIGERWALT, A. G., FANNING, G. R., CARTER, G. P. & MOLLARET, H. H. (1980). Deoxyribonucleic acid relatedness in *Yersinia enterocolitica* and *Yersinia enterocolitica*-like organisms. *Current Microbiology* **4**, 195–200.
- DENNY, T. P., GILMOUR, M. N. & SELANDER, R. K. (1988). Genetic diversity and relationships of two pathovars of *Pseudomonas syringae*. *Journal of General Microbiology* **134**, 1949–1960.
- GOULLET, PH. & PICARD, B. (1984). Distinctive electrophoretic and isoelectric focusing patterns of esterases from *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Journal of General Microbiology* **130**, 1471–1480.
- GOULLET, PH. & PICARD, B. (1988). Characterization of *Yersinia enterocolitica*, *Y. intermedia*, *Y. aldovae*, *Y. frederiksenii*, *Y. kristensenii* and *Y. pseudotuberculosis* by electrophoretic polymorphism of acid phosphatase, esterases, and glutamate and malate dehydrogenases. *Journal of General Microbiology* **134**, 317–325.
- GRIMONT, F. & GRIMONT, P. A. D. (1986). Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Annales de l'Institut Pasteur/Microbiologie* **137B**, 165–175.
- IRINO, K., GRIMONT, F., CASAN, I., GRIMONT, P. A. D. & THE BRAZILIAN PURPURIC FEVER STUDY GROUP (1988). rRNA gene restriction patterns of *Haemophilus influenzae* biogroup *aegyptius* strains associated with Brazilian purpuric fever. *Journal of Clinical Microbiology* **26**, 1535–1538.
- KAKOYIANNIS, C. K., WINTER, P. J. & MARSHALL, R. B. (1984). Identification of *Campylobacter coli* isolates from animals and humans by bacterial restriction endonuclease DNA analysis. *Applied and Environmental Microbiology* **48**, 545–549.
- KRISTIANSEN, B. E., SORESENSEN, B., SPANNE, O. & BJØRVATN, B. (1985). Typing of *Aeromonas* strains by DNA restriction endonuclease analysis and polyacrylamide gel electrophoresis of cell envelopes. *Journal of Clinical Microbiology* **27**, 1280–1285.
- MCCLENAGHAN, M., HERRING, A. J. & AITKEN, I. D. (1984). Comparison of *Chlamydia psittaci* isolates by DNA restriction endonuclease analysis. *Infection and Immunity* **45**, 384–389.

- MAGEE, B. B., D'SOUZA, T. M. & MAGEE, P. T. (1987). Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. *Journal of Bacteriology* **169**, 1639–1643.
- MARSHALL, R. B., WINTER, P. J. & YANAGAWA, Y. (1984). Restriction endonuclease DNA analysis of *Leptospira interrogans* serovars *icterohaemorrhagiae* and *hebdomadis*. *Journal of Clinical Microbiology* **20**, 808–810.
- PATTERSON, T. F., PATTERSON, J. E., MASECAR, B. L., BARDEN, G. E., HIERHOLZER, W. J. & ZERVOS, M. J. (1988). A nosocomial outbreak of *Branhamella catarrhalis* confirmed by restriction endonuclease analysis. *Journal of Infectious Diseases* **157**, 996–1001.
- PICARD-PASQUIER, N., OUAGHED, M., PICARD, B., GOULLET, PH. & KRISHNAMOORTHY, R. (1989). A simple, sensitive method of analyzing bacterial ribosomal DNA polymorphism. *Electrophoresis* **10**, 186–189.
- SCHWARTZ, D. C. & CANTOR, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**, 67–75.
- SOUTHERN, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.
- 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.
- URSING, J., BRENNER, D. J., BERCOVIER, H., FANNING, R., STEIGERWALT, A. G., BRAULT, J. & MOLLARET, H. H. (1980). *Yersinia frederiksenii*: a new species of Enterobacteriaceae composed of rhamnose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Current Microbiology* **4**, 213–217.
- YOGEV, D., HALACHMI, D., KENNY, G. E. & RAZIN, S. (1988). Distinction of species and strains of mycoplasmas (mollicutes) by genomic DNA fingerprints with an rRNA gene probe. *Journal of Clinical Microbiology* **26**, 1198–1201.