EPIDEMIOLOGICAL TYPING

Identification and typing of pyogenic streptococci by enzyme electrophoretic polymorphism

F. BERT*, B. PICARD, N. LAMBERT-ZECHOVSKY and P. GOULLET

Laboratoire de Microbiologie, Faculté de Médecine Xavier-Bichat (Université Paris VII), 16 rue Henri Huchard, 75018 Paris, France

Summary. Polyacrylamide-agarose gel electrophoresis was used to study polymorphism of lactate dehydrogenase (LDH), nucleoside phosphorylase (NSP), phosphoglucose isomerase (PGI), hydroxybutyrate dehydrogenase (HBD), adenylate kinase (ADK) and esterases of 44 strains of *Streptococcus pyogenes*, 25 group G streptococcal strains, 11 “*S. equisimilis*” strains, seven *S. dysgalactiae* strains, four *S. canis* strains, three *S. equi* strains and seven *S. zooepidemicus* strains. Analysis of LDH, NSP, PGI, HBD and ADK provided valuable interspecies differentiation, by showing that four groups of strains corresponded to the four known DNA homology groups. Esterases showed greater intraspecies variation than the other enzymes. The combined analysis of the six enzymes indicated 31 zymotypes among *S. pyogenes*, 14 in group G streptococci and 11 in “*S. equisimilis*” strains. This was shown to be an effective technique for typing pyogenic streptococci.

Introduction

Since the description of group-specific antigens by Lancefield¹ the classification of streptococci has mainly relied on serological grouping; serological groups were often regarded as distinct species. Although this technique is a useful and simple tool for distinguishing pathogenic β -haemolytic streptococci, its taxonomic value is limited because it provides no information on the genetic relationships among species. Moreover, many strains of streptococci cannot be typed by this technique. Other classifications have been based on physiological and biochemical characters² and numerical taxonomic studies.³ DNA-DNA and DNA-rRNA hybridisation studies indicate that the term pyogenic streptococci should be confined to strains of serological groups A, C, G (large colony type) and L, and includes five species, *Streptococcus pyogenes*, *S. dysgalactiae*, *S. canis*, *S. equi* and *S. iniae*.^{4–6} According to these studies, strains designated “*S. equisimilis*” and streptococci of serological groups G and L belong to the species *S. dysgalactiae*. Strains designated *S. zooepidemicus* are genetically similar to *S. equi* and should be reclassified as *S. equi* subsp. *zooepidemicus*, thereby automatically creating *S. equi*

subsp. *equi*. The findings of these studies are inconsistent with the serological classification since they bring together strains of distinct serological groups and separate strains belonging to the same group. They also conflict with the results of a numerical study that included “*S. equisimilis*” and *S. zooepidemicus* in a single phenon.³ Such discrepancies emphasise the continuing confusion over the taxonomic relationships among and within these species.

In the late 1980s, there was an upsurge in the worldwide incidence of severe infections with *S. pyogenes*, including toxic-shock-like syndrome and acute rheumatic fever.^{7,8} This emphasises the need for increased epidemiological and basic laboratory research in this field. Most epidemiological studies on group A streptococcal infections have been based on serological typing of the M protein, often combined with that of the T protein and serum opacity factor antigen.⁹ The detection of T protein antigens is also a useful method for serotyping streptococci of groups C and G,¹⁰ but the cost and difficulty of preparing antisera mean that these techniques are restricted to a few reference laboratories. Furthermore, many *S. pyogenes* strains are M-non-typable, so more refined typing methods are required for further epidemiological studies. Ten biotypes of group A streptococci have been described and shown to be closely associated with serotypes.¹¹ DNA analysis has been used for *S. pyogenes* typing, particularly restriction fragment length polymorphism (RFLP) analysis of total

Received 12 Dec. 1994; accepted 15 Dec. 1994.

* Address for correspondence: Hôpital Beaujon, Service de Microbiologie, 100 Bd du Général Leclerc, 92118 Clichy Cedex, France.

Table 1. Features of the streptococcal strains examined

DNA homology group ^a	Species (Lancefield group)	Strain code	Origin	Zymotype
1	<i>S. pyogenes</i> (group A)	A4	Scarlet fever	1
		5502	Throat	2
		5750	Pus	3
		CIP 5649	Scarlet fever	4
		CIP 5650	Throat	4
		CIP 5678	Unknown	5
		CIP 5660	Unknown	6
		5800	Ear	6
		5723	Pus	7
		CIP 5679	Unknown	8
		CIP 5662	Throat	9
		5403	Abscess	10
		CIP 54.5	Unknown	11
		CIP 56.44	Unknown	11
		CIP 70.3	Pleural fluid	11
		CIP 56.52	Throat	12
		5837	Wound	13
		5538	Abscess	14
		5780	Unknown	15
		A21	Throat (acute rheumatic fever)	16
		CIP 56.45	Unknown	17
		5241	Unknown	18
		5402	Pus	19
		5524	Abscess	19
		5840	Erysipelas	19
		5778	Wound	20
		5358	Wound	21
		5542	Wound	22
		5726	Abscess	23
		A30	Unknown	24
		CIP 56.61	Acute rheumatic fever	25
		CIP 56.65	Throat	25
		CIP 56.1	Puerperal fever	26
		CIP 56.42	Scarlet fever	26
		CIP 56.54	Unknown	26
		CIP 56.56	Throat (acute rheumatic fever)	26
		CIP 56.59	Scarlet fever	26
		CIP 56.64	Scarlet fever	26
		CIP 56.53	Unknown	27
		CIP 56.57	Throat (acute rheumatic fever)	28
		CIP 56.47	Throat	29
		CIP 56.66	Unknown	29
		5742	Pus	30
		CIP 56-63	Throat (acute rheumatic fever)	31
2	<i>Streptococcus</i> sp. (group G)	5404	Pus	34
		5712	Throat	35
		4002	Blood	36
		G6	Unknown	37
		G14	Unknown	38
		2987	Blood	38
		5362	Throat	38
		5544	Wound	38
		5706	Ear	38
		5990	Pus	38
		5942	Pleural fluid	39
		5578	Pus	40
		5748	Trachea	40
		5356	Fistula	41
		5357	Pus	41
		5500	Pus	41
		2329	Blood	42
		5539	Wound	42
		5622	Trachea	42
		5991	Cerebrospinal fluid	42
		5686	Throat	43
		G4	Unknown	44
		5679	Unknown	45

Table I (cont.)

DNA homology group ⁶	Species (Lancefield group)	Strain code	Origin	Zymotype
3	" <i>S. equisimilis</i> " (group C)	5728	Trachea	47
		5031	Blood	51
		6069	Unknown	32
		6818	Wound	46
		5953	Blood	48
		5283	Trachea	51
		5753	Trachea	52
		6851	Pus	53
		6850	Pus	54
		ES2	Veterinary	55
		ES1	Veterinary	56
		ES4	Veterinary	59
		ES3	Veterinary	60
	<i>S. dysgalactiae</i> (group C)*	CIP 55.123	Unknown	33
		CIP 55.119	Unknown	49
		CIP 55.120	Unknown	50
		C90	Unknown	57
		C89	Unknown	58
		C91	Unknown	61
		CIP 102914T	Unknown	61
	<i>S. canis</i> (group G)	CAN1	Veterinary	62
		CIP 103223	Bovine mastitis	63
		CAN2	Veterinary	63
		CAN3	Veterinary	63
4	<i>S. equi</i> (group C)	EQ1	Veterinary	64
		EQ2	Veterinary	64
		CIP 102910T	Unknown	65
	<i>S. zooepidemicus</i> (group C)	Z4	Veterinary	66
		5374	Blood	67
		Z3	Veterinary	68
		Z1	Veterinary	69
		CIP 103228T	Bovine mastitis	70
		Z2	Veterinary	71
		A7	Hen	72

* With the exception of strains CIP 55.120 (group G) and CIP 55.123 (group L).

DNA^{12,13} and ribosomal DNA regions¹² and DNA RFLP analysis with rarely cutting restriction endonucleases coupled with pulsed-field gel electrophoresis.¹⁴ These techniques can differentiate strains of different M serotypes, and also epidemiologically distinct strains within a serotype. Random amplified polymorphic DNA analysis has been evaluated for typing group A streptococci.¹⁵

Enzyme electrophoresis is a powerful tool for differentiating bacterial species and distinguishing strains of the same species on the basis of their genetic relationships¹⁶⁻²⁴ and was used recently to evaluate the genetic relationships among the oral streptococci,¹⁶ to study clonal diversity of *S. pyogenes* causing toxic-shock-like syndrome⁷ and to describe the *S. pyogenes* strains responsible for recurrent pharyngitis.²⁵ The present work examined the usefulness of enzyme electrophoresis for both interspecies and intraspecies differentiation of pyogenic streptococci belonging to Lancefield groups A, C and G.

Materials and methods

Bacterial strains

The 101 strains tested, designated according to

Farrow and Collins,⁴ were distributed as follows: 44 strains of *S. pyogenes*, 25 of group G streptococci, 11 of "*S. equisimilis*", seven of *S. dysgalactiae*, four of *S. canis*, three of *S. equi* subsp. *equi* (*S. equi*) and seven of *S. equi* subsp. *zooepidemicus* (*S. zooepidemicus*) (table I). Forty-one were reference strains and 60 were clinical isolates of human or animal origin, epidemiologically unrelated and originating from three French hospitals. For esterase analysis, 31 additional isolates of *S. pyogenes* were studied. The strains were identified on the basis of serological grouping (Streptococcal Grouping Kit, Oxoid) and biochemical tests (API 20 STREP, bioMérieux).

Preparation of bacterial extracts

Bacteria were grown for 18 h at 37°C with constant agitation in a buffered broth containing tryptone 0.5%, yeast extract 0.25%, 20 mM DL-threonine, 0.6 mM MgSO₄, 0.03 mM MnSO₄, 0.08 mM NaCl, 25 mM NH₄HCO₃, 1.0 M L-cysteine hydrochloride and glucose 0.4% in 40 mM potassium phosphate buffer.¹⁶ The bacterial growth was collected by centrifugation and washed with 60 mM Tris-glycine buffer, pH 8.7, resuspended in the same buffer and the cells were

disrupted by intermittent sonication for 4 min at 4°C. The crude extracts were stored at -20°C until used.

Electrophoresis

Horizontal slab gel electrophoresis was performed by the method of Uriel²⁶ in a composite polyacrylamide-agarose gel with a discontinuous Tris-glycine buffer, pH 8.7, at constant voltage (7 V/cm) until the bromophenol blue marker had run 13 cm. The gel contained polyacrylamide 5% for analysis of nucleoside phosphorylase (NSP) and phosphoglucose isomerase (PGI) and polyacrylamide 7% for analysis of lactate dehydrogenase (LDH), hydroxy-butyrate dehydrogenase (HBD) and adenylate kinase (ADK). The relative mobility (M_F value) is the distance moved by the enzyme band as a percentage of the distance moved by the dye front. M_F values were compared by running the bacterial extracts side by side in the same gel.

Specific staining procedures

The six enzymes studied, LDH, NSP, PGI, HBD, ADK and esterases, were revealed on the gel by specific staining. LDH activity was detected by the method of Siciliano and Shaw.²⁷ The staining methods for NSP, PGI, ADK and HBD were as described by Selander *et al.*²³ For NSP, PGI and ADK, the stain solution was applied to the gel in an agar overlay. Esterases were stained according to Uriel²⁸ with the following specific substrates: α - and β -naphthyl acetates, α - and β -naphthyl propionates, α - and β -naphthyl butyrates and indoxyl acetate.

Genetic diversity

Genetic diversity at a locus among isolates was calculated as $h = 1 - \sum x_i^2 / [n(n-1)]$, where x_i is the frequency of the i th allele and n is the number of isolates.²³ Mean genetic diversity per locus (H) is the arithmetic average of h values for all loci.

Statistical analysis

The data were summarised in a two-way table of 101 rows, one for each strain, and 28 columns corresponding to the number of M_F variants detected in all strains for five enzymes tested (LDH, NSP, PGI, HBD and ADK), in addition to a column for absence of detection of each enzyme. For each column, each strain was coded as a binary code: present = 1 or absent = 0. This table was used for a factorial analysis of correspondence (FAC)²⁹ and a dissimilarity matrix was constructed for hierarchical clustering.

Results

Detection of enzyme bands

LDH gave a single, well-stained band with 73 strains. The remaining 28 strains, including all the *S. zooepidemicus* strains and two of the three *S. equi*

Table II. Number of alleles and genetic diversity (h) at five enzyme loci among the 101 pyogenic streptococcal strains

Enzyme locus	Number of alleles	h
LDH	4	0.650
NSP	4	0.273
PGI	5	0.268
HBD	8	0.755
ADK	10	0.740

strains, showed no detectable LDH band, although the LDH activity of these bacterial extracts measured biochemically was not significantly different from that of the other strains and are thus regarded as allozymes that are undetectable by electrophoresis. A single HBD band was detected in most strains, with the exception of *S. equi* and *S. zooepidemicus* which had two bands. Thirty-one strains, including all *S. canis* and "*S. equisimilis*" strains, had no HBD activity. Bands from *S. pyogenes* were well stained, but those from *S. equi* and *S. zooepidemicus* were less focused. The activity with group G streptococci was very weak and detection required overnight staining. NSP, PGI and ADK each gave a single well-stained band, with the exception of one strain of "*S. equisimilis*" for which no PGI was detected. Esterases were detected in all strains, and the number of bands varied from one to four. Most of the strains had a main band that stained intensely, with one or several faint bands. Since they all hydrolysed each of the seven synthetic substrates, the strains could not be characterised in terms of esterase type by their spectrum of activity towards these substrates.^{17,20}

Genetic heterogeneity and zymotypes

LDH, NSP, PGI, HBD and ADK were polymorphic. The genetic diversity of these enzymes ranged from 0.268 for PGI to 0.755 for HBD (table II), with a mean genetic diversity of 0.537. HBD gave a total of eight zymotypes and ADK produced 10, but the other enzymes were less variable. Differences in number and mobility of esterase bands led to the identification of 49 esterase electrophoretic patterns (EEPs) in the 101 isolates (fig. 1). Several EEPs were encountered more frequently, particularly EEP 1, 2 and 3 which were found in six, nine and 10 *S. pyogenes* strains, respectively. Reference strains and clinical isolates could not be separated by the electrophoretic pattern of their esterases. EEP20 and EEP22 were common to group G streptococci and "*S. equisimilis*", and EEP31 to *S. dysgalactiae* and "*S. equisimilis*". None of the EEPs found in *S. pyogenes* was found in the other species. Overall, the electrophoretic variations of the six enzymes gave a total of 72 distinct zymotypes in the 101 strains (table III).

Species identification

Projections of the strains on the plane F1, F2 (fig. 2) of the FAC accounted for 47.5% of the total variance

Fig. 1. Diagram of the esterase electrophoretic patterns (EEP) of 101 strains of pyogenic streptococci. *S. pyogenes*, EEP 1–15; group G streptococci, EEP 16–28; *S. canis*, EEP 29–30; *S. dysgalactiae*, EEP 31–35; “*S. equisimilis*”, EEP 20, 22, 31, 36–42; *S. equi*, EEP 43–44; *S. zooepidemicus*, EEP 45–49.

and indicated that the *S. pyogenes* strains were distinguished from group G streptococci, *S. dysgalactiae* and “*S. equisimilis*” strains by the first axis, whereas the *S. equi* and *S. zooepidemicus* strains were differentiated by the positive values of the second axis. Projections of the electrophoretic variants on the plane indicated the principal features providing genetic species identification of pyogenic streptococci by enzyme polymorphism. Thus, the electrophoretic

variants, no. 4 of ADK (ADK4), no. 3 of LDH (LDH3) and no. 3 of HBD (HBD3) were projected with the *S. pyogenes* strains. The dissimilarity between strains was used to construct a dendrogram (fig. 3), this indicated the correct classification of all strains within each of the four species distinguished by DNA-DNA hybridisation.¹⁸ Within the DNA homology group 4, *S. equi* and *S. zooepidemicus* were separated by their enzyme polymorphism, whereas in the DNA

Table III. Zymotypes detected in 101 strains of pyogenic streptococci

DNA homology group ⁶	Species	Number of strains	Allele at the indicated enzyme locus*					EEP†	Zymotype
			LDH	NSP	PGI	HBD	ADK		
1	<i>S. pyogenes</i>	1	0‡	2	2	3	5	7	1
		1	0	2	2	3	4	5	2
		1	0	2	2	3	4	2	3
		2	0	2	2	3	4	3	4
		1	0	2	2	3	2	14	5
		2	0	2	2	3	2	2	6
		1	0	2	2	3	6	11	7
		1	0	2	2	3	1	2	8
		1	0	4	2	0	2	13	9
		1	0	2	2	0	2	3	10
		3	0	2	2	0	4	3	11
		1	0	2	2	0	4	9	12
		1	0	2	2	0	4	11	13
		1	3	2	2	0	4	8	14
		1	3	2	2	0	5	12	15
		1	3	2	2	3	5	3	16
		1	3	2	2	3	4	5	17
		1	3	2	2	3	4	2	18
		3	3	2	2	3	4	3	19
		1	3	2	2	3	4	11	20
		1	3	2	2	1	4	3	21
		1	3	2	2	1	4	8	22
		1	3	2	2	5	4	11	23
		1	3	2	2	3	2	6	24
		2	3	2	2	3	2	2	25
		6	3	2	2	3	2	1	26
		1	3	2	2	3	2	9	27
		1	3	2	2	0	2	4	28
		2	3	2	2	0	2	2	29
		1	3	2	2	0	7	10	30
		1	3	2	2	3	7	15	31
2	<i>S. equisimilis</i>	1	2	2	2	0	6	40	32
		1	2	2	2	0	6	31	33
	<i>S. dysgalactiae</i>	1	2	2	2	6	5	17	34
		1	2	2	2	6	5	18	35
		1	2	2	2	6	5	21	36
		1	2	2	2	6	5	23	37
		6	2	2	2	6	5	22	38
		1	2	2	2	6	5	24	39
		2	2	2	2	6	5	26	40
		3	2	2	2	6	5	27	41
		4	2	2	2	6	5	19	42
		1	2	2	2	6	5	28	43
		1	2	2	2	6	5	25	44
		1	0	2	2	6	5	16	45
		1	2	2	2	0	5	22	46
		1	2	2	2	0	5	24	47
		1	2	2	2	0	5	26	48
	<i>S. dysgalactiae</i>	1	2	2	2	0	5	34	49
		1	2	2	2	0	5	35	50
	<i>S. equisimilis</i>	2	2	2	2	0	5	20	51
		1	2	2	2	0	5	38	52
	<i>S. equisimilis</i>	1	2	2	2	0	5	42	53
		1	2	4	2	0	5	41	54
		1	2	2	1	0	5	22	55
		1	2	2	0	0	5	31	56
		1	2	2	2	1	5	31	57
	<i>S. dysgalactiae</i>	1	2	2	2	1	5	32	58
		1	2	2	3	2	5	37	59
	<i>S. equisimilis</i>	1	2	2	3	2	5	36	60
		2	2	2	3	3	5	33	61
3	<i>S. canis</i>	1	0	3	2	0	3	29	62
		3	2	3	2	0	8	30	63
4	<i>S. equi</i>	2	0	3	3	7	10	44	64
		1	1	3	3	7	10	43	65

Table III (cont.)

DNA homology group ⁶	Species	Number of strains	Allele at the indicated enzyme locus*					EEP†	Zymotype
			LDH	NSP	PGI	HBD	ADK		
	<i>S. zooepidemicus</i>	1	0	4	3	7	8	47	66
		1	0	4	3	7	8	45	67
		1	0	4	3	7	8	46	68
		1	0	4	3	7	10	46	69
		1	0	4	3	7	10	48	70
		1	0	1	3	7	9	47	71
		1	0	4	4	4	10	49	72

* Alleles are numbered consecutively in order of decreasing anodal mobility.

† Esterase electrophoretic pattern.

‡ No detectable enzyme activity.

Fig. 2. Correspondence analysis of 101 pyogenic streptococci strains performed on the enzyme data. Projection of strains of *S. pyogenes* (■), group G streptococci (●), *S. dysgalactiae* (△), "*S. equisimilis*" (▲), *S. canis* (○), *S. equi* (▽) and *S. zooepidemicus* (▼) and electrophoretic variants of lactate dehydrogenase (e.g., variant no. 3 = LDH 3), nucleoside phosphorylase (NSP), phosphoglucose isomerase (PGI), hydroxybutyrate dehydrogenase (HBD) and adenylate kinase (ADK) on plane F1, F2. For clarity, the dimensions of the symbols vary with the number of strains projected on the same point of the plane, (e.g., for *S. pyogenes* ■, 1 strain; ■, 2–10 strains; ■, > 10 strains).

homology group 2, group G streptococci, "*S. equisimilis*" and *S. dysgalactiae* were not clearly separated.

Strain typing

LDH, NSP, PGI, HBD and ADK analysis led to the identification of 18 zymotypes for *S. pyogenes*, three for group G streptococci and six for "*S. equisimilis*". Fifteen EEPs were delineated among the 44 *S. pyogenes* strains. Eight other EEPs were found among the 31 additional isolates for which esterases alone were studied, providing a total of 23 EEPs in the 75 *S.*

pyogenes strains (data not shown). Thirteen EEPs were delineated among group G streptococci strains and 10 among "*S. equisimilis*" strains. Thirty-one zymotypes were found for *S. pyogenes*, 14 for group G streptococci and 11 for "*S. equisimilis*" (table IV) when the analysis of esterases and other enzymes was combined.

Discussion

Analysis of enzyme electrophoretic polymorphism has been shown to be of taxonomic value in dealing with gram-negative bacilli and oral streptococci, for

Fig. 3. Dendrogram prepared from the dissimilarity matrix distance for enzyme electrophoretic data for pyogenic streptococci. The number of strains is indicated for each branch.

Table IV. Number of zymotypes detected among 101 strains of pyogenic streptococci

Species (number of strains)	LDH	NSP	PGI	HBD	ADK	Esterase	Enzyme*
<i>S. pyogenes</i> (44)	2	2	1	4	6	15	31
Group G streptococci (25)	2	1	1	2	1	13	14
" <i>S. equisimilis</i> " (11)	1	2	4	2	2	10	11
<i>S. dysgalactiae</i> (7)	1	1	2	3	3	5	6
<i>S. canis</i> (4)	2	1	1	1	2	2	2
<i>S. equi</i> (3)	2	1	1	1	1	2	2
<i>S. zooepidemicus</i> (7)	1	2	2	3	3	5	7
All strains (101)	4	4	5	8	10	49	72

* Number of zymotypes resulting from the combined analysis of six enzymes.

which there is a good correlation with DNA analysis.^{16, 18, 19, 23, 24} Similarly, the results of the present study on pyogenic streptococci are consistent with those obtained by DNA hybridisation. Thus, enzyme electrophoretic polymorphism showed that *S. dysgalactiae*, "*S. equisimilis*" and group G streptococci were closely related, and also *S. equi* and *S. zooepidemicus*, whereas strains of *S. pyogenes* were clearly distinguished from the other strains. The results shown as a FAC (fig. 2) illustrate the differentiation of these groups and indicate the main electrophoretic variants of each group. Four major branches are identified (fig. 3), each of which includes all the strains belonging to the same DNA homology group; the genetic relationships between those groups are similar to those indicated by the dendrogram based on DNA-DNA hybridisation.⁶ Statistical analysis was performed with electrophoretic polymorphism data for five of the six enzymes studied (LDH, NSP, PGI, HBD and ADK). Esterases were not included in this because of their high degree of intraspecies polymorphism and the absence of biochemical differentiation among these enzymes. This limited their taxonomic value for pyogenic streptococci.

The present work establishes the efficacy of enzyme electrophoretic polymorphism analysis for typing pyogenic streptococci. The high degree of polymorphism of esterases allowed the identification of 15 zymotypes among 44 strains of *S. pyogenes*, 13 in 25 group strains of G streptococci and 10 in 11 strains of "*S. equisimilis*". The other enzymes were less polymorphic and the smaller variation in their mobility within each species limits their usefulness as epidemiological markers. However, the combination of esterase analysis with that of the other enzymes increased the number of zymotypes detected among 44 strains of *S. pyogenes* from 15 to 31 (table IV). The results were less impressive for group G streptococci, for which esterase

typing efficiency was little improved when combined with analysis of other enzymes. However, all strains of "*S. equisimilis*" and *S. zooepidemicus* were differentiated by this technique.

Enzyme electrophoretic polymorphism analysis has been used by Musser *et al.* to estimate genetic diversity among *S. pyogenes* strains responsible for toxic-shock-like syndrome.⁷ They studied 108 isolates and 12 enzymes, including the six enzymes used in the current work and found approximately the same number of zymotypes. This result can be explained by the presence of two related clones that accounted for nearly half of the strains. The number of patterns differentiated by random amplified polymorphic DNA analysis¹⁵ among 160 unrelated isolates of *S. pyogenes* was identical to the number of zymotypes found in this study. However, only one primer was selected, and the discriminatory power of this method could be improved by the use of a combination of primers.

The present study shows that enzyme electrophoresis is a valuable method for the differentiation of species of pyogenic streptococci and the estimation of the genetic relationships between them. It supports the taxonomic results obtained by DNA hybridisation and confirms the poor species specificity of the Lancefield serological classification. However, enzyme electrophoresis, which surveys the products of individual gene loci, provides additional information on the genetic diversity within species, whereas DNA hybridisation is usually performed with only a small number of reference strains. Thus the analysis of the polymorphism of six enzymes, esterases in particular, is an effective technique for typing strains of pyogenic streptococci.

We thank Drs C. Bizet and T. Horaud for providing reference strains, Mrs C. Gaillard and N. Hautier for technical assistance, Mrs C. Gillet for secretarial assistance and Dr O. Parkes for editorial help.

References

1. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. *J Exp Med* 1933; **57**: 571-595.
2. Jones D. Composition and differentiation of the genus *Streptococcus*. In: Skinner FA, Quesnel LB (eds) *Streptococci*. Society for Applied Bacteriology Symposium series no. 7. London, Academic Press Inc. 1978: 1-49.
3. Bridge PD, Sneath PHA. Numerical taxonomy of *Streptococcus*. *J Gen Microbiol* 1983; **129**: 565-597.
4. Farrow JAE, Collins MD. Taxonomic studies on streptococci of serological groups C, G and L and possibly related taxa. *Syst Appl Microbiol* 1984; **5**: 483-493.

5. Kilpper-Bälz R, Schleifer KH. Nucleic acid hybridization and cell wall composition studies of pyogenic streptococci. *FEMS Microbiol Lett* 1984; **24**: 355–364.
6. Schleifer KH, Kilpper-Bälz R. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. *Syst Appl Microbiol* 1987; **10**: 1–19.
7. Musser JM, Hauser AR, Kim MH, Schlievert PM, Nelson K, Selander RK. *Streptococcus pyogenes* causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. *Proc Natl Acad Sci USA* 1991; **88**: 2668–2672.
8. Schwartz B, Facklam RR, Breiman RF. Changing epidemiology of group A streptococcal infection in the USA. *Lancet* 1990; **336**: 1167–1171.
9. Swift HF, Wilson AT, Lancefield RC. Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes. *J Exp Med* 1943; **78**: 127–133.
10. Efstratiou A. Outbreaks of human infection caused by pyogenic streptococci of Lancefield groups C and G. *J Med Microbiol* 1989; **29**: 207–219.
11. Bouvet A, Geslin P, Kriz-Kuzemenska P, Blanc V, Devine C, Grimont F. Restricted association between biotypes and serotypes within group A streptococci. *J Clin Microbiol* 1994; **32**: 1312–1317.
12. Bingen E, Denamur E, Lambert-Zechovsky N *et al.* Mother-to-infant vertical transmission and cross-colonization of *Streptococcus pyogenes* confirmed by DNA restriction fragment length polymorphism analysis. *J Infect Dis* 1992; **165**: 147–150.
13. Cleary PP, Kaplan FL, Livdahl C, Skjold S. DNA fingerprints of *Streptococcus pyogenes* are M type specific. *J Infect Dis* 1988; **158**: 1317–1323.
14. Single LA, Martin DR. Clonal differences within M-types of the group A *Streptococcus* revealed by pulsed field gel electrophoresis. *FEMS Microbiol Lett* 1992; **91**: 85–90.
15. Seppälä H, He Q, Österblad M, Huovinen P. Typing of group A streptococci by random amplified polymorphic DNA analysis. *J Clin Microbiol* 1994; **32**: 1945–1948.
16. Gilmour MN, Whittam TS, Killian M, Selander RK. Genetic relationships among the oral streptococci. *J Bacteriol* 1987; **169**: 5247–5257.
17. Goullet P. An esterase zymogram of *Escherichia coli*. *J Gen Microbiol* 1973; **77**: 27–35.
18. Goullet P, Picard B. Characterization of *Enterobacter cloacae* and *E. sakazakii* by electrophoretic polymorphism of acid phosphatase, esterases, and glutamate, lactate and malate dehydrogenases. *J Gen Microbiol* 1986; **132**: 3105–3112.
19. Goullet P, Picard B. 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. *J Gen Microbiol* 1988; **134**: 317–325.
20. Goullet P, Picard B. The electrophoretic polymorphism of bacterial esterases. *FEMS Microbiol Rev* 1995; **16**: 7–31.
21. Picard B, Goullet P. Epidemiological complexity of hospital aeromonas infections revealed by electrophoretic typing of esterases. *Epidemiol Infect* 1987; **98**: 5–14.
22. Picard B, Goullet P. Epidemiological typing of *Acinetobacter* strains by esterase electrophoresis. *FEMS Microbiol Lett* 1990; **72**: 229–234.
23. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Appl Environ Microbiol* 1986; **51**: 873–884.
24. Selander RK, Musser JM, Caugant DA, Gilmour MN, Whittam TS. Population genetics of pathogenic bacteria. *Micro Pathog* 1987; **3**: 1–7.
25. Musser JM, Gray BM, Schlievert PM, Pichichero ME. *Streptococcus pyogenes* pharyngitis: characterization of strains by multilocus enzyme genotype, M and T protein serotype, and pyrogenic exotoxin gene probing. *J Clin Microbiol* 1992; **30**: 600–603.
26. Uriel J. Méthode d'électrophorèse dans des gels d'acrylamide-agarose. *Bull Soc Chim Biol* 1966; **48**: 969–982.
27. Siciliano M, Shaw CR. Separation and visualization of enzymes on gels. In: Smith I (ed) *Chromatographic and electrophoretic techniques*, 4th edn. Chicago, Yearbook Medical Publishers. 1976: 185–209.
28. Uriel J. Caractérisation des cholinestérases et d'autres estérases carboxyliques après électrophorèse et immuno-électrophorèse en gélose (application à l'étude des estérases du sérum humain normal). *Ann Inst Pasteur* 1961; **101**: 104–119.
29. Lebart L, Morineau A, Warwick KM. Multivariate descriptive analysis: correspondence analysis and related techniques for large matrices. New York, Wiley-Interscience. 1984: 231.