Distinctive Electrophoretic Patterns of Esterases from *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes* and *E. gergoviae*

By PH. GOULLET†

Laboratoire de Microbiologie, Faculté de Médecine Xavier-Bichat,
Université Paris VII, Institut Biomédical des Cordeliers, 21 rue de l'Ecole
de Médecine, 75020 Paris Cedex 06, France

(Received 5 October 1979)

Esterases of 14 strains of *Klebsiella pneumoniae*, 14 strains of *K. oxytoca*, 16 strains of *Enterobacter aerogenes* and 16 strains of *E. gergoviae* were analysed by horizontal electrophoresis in polyacrylamide-agarose gel. Four principal esterase bands (designated E₁ to E₄) and nine minor bands differing in their activity towards synthetic substrates and in their sensitivity to heat and to di-isofluoropropyl phosphate were defined. The comparative distribution of bands showed that the four species analysed were characterized by distinct electrophoretic patterns of their esterases. Band E₁ was found in all four species, bands E₂ and E₃ only in *K. oxytoca* and band E₄ only in some strains of *E. gergoviae*. The apparent molecular weights of esterases E₂ and E₃, determined by electrophoresis in a 4 to 30% polyacrylamide gradient gel, were 58000 (±1000) and 72000 (±1800), respectively.

INTRODUCTION

Klebsiella and *Enterobacter* species, which are now frequently isolated from nosocomial and opportunistic infections following the widespread use of antibiotics, differ only in a few common biochemical tests. Indole-producing, gelatin-liquefying *Klebsiella* (*K. oxytoca* or *Bacterium oxytoca*) are not given species status in *Bergey's Manual of Determinative Bacteriology* (Orskov, 1974), but DNA reassociation studies have shown that *K. pneumoniae* and *K. oxytoca* are different species (Brenner *et al.*, 1977; Jain *et al.*, 1974). The recently described species *Enterobacter gergoviae* (Richard, 1979; Richard *et al.*, 1976) can be distinguished from *E. aerogenes* by its biochemical reactions. This new species (*E. gergoviae*) is not listed in *Bergey's Manual* (Buchanan & Gibbons, 1974).

To improve the phenotypic characterization of these organisms at the molecular level, their esterases were studied by polyacrylamide-agarose gel and polyacrylamide gradient gel electrophoresis.

METHODS

Bacterial strains. The names and sources of the strains are given in Table 1.

Growth conditions. These were as described previously (Goullet, 1975). Bacteria were harvested during the exponential or stationary phases of growth.

Preparation of extracts. After centrifugation, cells were washed twice with 200 vol. 0.06 M-Tris/glycine buffer pH 8.7, resuspended in an equal volume of the same buffer and disrupted by intermittent sonic oscillation (Sonifier cell disruptor B30, Branson Sonic Power Co., Danbury, Conn., U.S.A.) for 6 min at

† Present address: Laboratoire de Microbiologie, Faculté de Médecine Xavier-Bichat, 16 rue H. Huchard, 75018 Paris, France.

Table 1. *Strains of Klebsiella and Enterobacter examined*

Code	Organism	Strain*	Bio-type†	Capsular antigen†	Source‡	Growth condition(s)§ (37 °C)
1	<i>K. pneumoniae</i>	413.76	a	NC	H. blood, Toulouse	S
2	<i>K. pneumoniae</i>	LOU-1.75	b	3	H. sputum, Louvain	S
3	<i>K. pneumoniae</i>	LOU-9.75	b	3	H. sputum, Louvain	S
4	<i>K. pneumoniae</i>	645.B.71	b	3	H. pus, Tangier	S
5	<i>K. pneumoniae</i>	502.77	d	11	H. blood, Paris	S
6	<i>K. pneumoniae</i>	H112.73	Dc	3	H. urine, Paris	S
7	<i>K. pneumoniae</i>	1413.70			H. sputum, Tangier	E, S
8	<i>K. pneumoniae</i>	1332.70			H. urine, Tangier	S
9	<i>K. pneumoniae</i>	1H.73			H. blood, Paris	S
10	<i>K. pneumoniae</i>	19B.74			H. blood, Brussels	S
11	<i>K. pneumoniae</i>	39B.74			H. blood, Brussels	S
12	<i>K. pneumoniae</i>	17L.74			H. drain, Louvain	S
13	<i>K. pneumoniae</i>	11.76			H. faeces, Strasbourg	S
14	<i>K. pneumoniae</i>	397.76			H. trachea, Paris	
15	<i>K. oxytoca</i>	432.76	Dc	17(31)	H. transplant, Paris	S
16	<i>K. oxytoca</i>	456.77	Dc	NC	H. blood, Paris	S
17	<i>K. oxytoca</i>	457.77	Dc	13	H. blood, Paris	S
18	<i>K. oxytoca</i>	488.77	Dc	47	H. blood, Paris	S
19	<i>K. oxytoca</i>	493.77	Dc	NC	Environmental origin	
20	<i>K. oxytoca</i>	443.77			Excipient, Paris	S
21	<i>K. oxytoca</i>	477.71			H. rhinopharynx, Tangier	S
22	<i>K. oxytoca</i>	1021.71			H. urine, Tangier	E, S
23	<i>K. oxytoca</i>	1204.71			H. sputum, Tangier	S
24	<i>K. oxytoca</i>	92.73			H. urine, Barcelona	S
25	<i>K. oxytoca</i>	102.73			H. blood, Barcelona	S
26	<i>K. oxytoca</i>	124.73			H. trachea, Paris	S
27	<i>K. oxytoca</i>	48B.74			H. blood, Brussels	S
28	<i>K. oxytoca</i>	10.77			H. urine, Lille	S
29	<i>E. aerogenes</i>	1.69	a	26	H. faeces, India	S
30	<i>E. aerogenes</i>	4.76	a	68	H. sputum, U.S.A., ATCC 13048	S
31	<i>E. aerogenes</i>	10.76	a	68	H. urine, Strasbourg	S
32	<i>E. aerogenes</i>	1.77	a	68	H. urine, Tangier	S
33	<i>E. aerogenes</i>	1.73	a	NC	H. pus, Paris	E, S
34	<i>E. aerogenes</i>	2.70	b	26	H. nasal sample, Tangier	E, S
35	<i>E. aerogenes</i>	9.76	b	42	H. faeces, Gisors	S
36	<i>E. aerogenes</i>	5.75	b	59	H. faeces, Château-Gontier	E, S
37	<i>E. aerogenes</i>	4.70	b	68	H. sputum, Tangier	E, S
38	<i>E. aerogenes</i>	38.72	b	68	H. faeces, Baghdad	E, S
39	<i>E. aerogenes</i>	2.75	b	68	H. sputum, Paris	E, S
40	<i>E. aerogenes</i>	1.76	b	68	H. blood, Paris	E, S
41	<i>E. aerogenes</i>	1.70	b	NC	H. urine, Tangier	E, S
42	<i>E. aerogenes</i>	1.72	Da	11	H. faeces, Toulon	E, S
43	<i>E. aerogenes</i>	6.75	Db	4	H. urine, Canada	E, S
44	<i>E. aerogenes</i>	11.76	Db	59	H. sputum, Strasbourg	S
45	<i>E. gergoviae</i>	63.72			H. blood, Africa	S
46	<i>E. gergoviae</i>	1.73			H. throat, Paris	E, S
47	<i>E. gergoviae</i>	22.73			H. faeces, Paris	S
48	<i>E. gergoviae</i>	37.73			H. gastric sample, Paris	S
49	<i>E. gergoviae</i>	6.74			H. pus, Paris	S
50	<i>E. gergoviae</i>	16.74			H. urine, Clermont-Ferrand (CIP 76.01)	S
51	<i>E. gergoviae</i>	20.75			H. urine, Clermont-Ferrand	S
52	<i>E. gergoviae</i>	25.75			H. urine, Clermont-Ferrand (CIP 76.02)	E, S
53	<i>E. gergoviae</i>	11.76			Environmental origin, Paris	S
54	<i>E. gergoviae</i>	12.76			H. pus, Versailles	E, S
55	<i>E. gergoviae</i>	1.77			Cosmetic, Paris	E, S
56	<i>E. gergoviae</i>	2.77			H. pus, CDC Atlanta	S
57	<i>E. gergoviae</i>	3.77			H. blood, CDC Atlanta	S
58	<i>E. gergoviae</i>	5.77			H. pus abdominal wound, CDC Atlanta	S
59	<i>E. gergoviae</i>	6.77			H. sputum, CDC Atlanta	S
60	<i>E. gergoviae</i>	7.77			H. urine, Clermont-Ferrand	S

* The strains were provided by Dr C. Richard from the Collection du Service des Entérobactéries de l'Institut Pasteur de Paris (Professeur L. Le Minor) and were grown in L broth (Goullet, 1973).

† According to Richard (1973, 1977). NC, No capsule.

‡ H, Human origin. ATCC, American Type Culture Collection; CDC, Center for Disease Control; CIP, Collection de l'Institut Pasteur, Paris.

§ E, Bacteria harvested during the exponential phase of growth; S, bacteria harvested during the stationary phase.

Fig. 1. *Klebsiella pneumoniae*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 1). Horizontal slab polyacrylamide-agarose gel electrophoresis was performed using 7% (w/v) acrylamide and discontinuous Tris/glycine buffer, pH 8.7. Substrates used were: 1, α -naphthyl acetate; 2, α -naphthyl butyrate; 3, indoxyl acetate; 4, β -naphthyl acetate; 5, β -naphthyl butyrate. Relative intensity of staining: $\blacksquare > \square \text{ with horizontal lines} > \square \text{ with vertical lines} > \square \text{ with diagonal lines} > \square$. (b) Esterase patterns of 14 strains (numbered as in Table 1) arranged in order of increasing mobility of E_1 esterase. $\blacksquare$, E_1 band; $\square \text{ with horizontal lines}$, m_1 band; $\square \text{ with vertical lines}$, m_2 band; $\square \text{ with diagonal lines}$, m_3 band; $\square$, m_4 band; —, F band; ---, S band. The M_F values were used only comparatively.

0 °C. Cell debris was removed by centrifugation at 20000 *g* for 20 min at 4 °C. The crude extracts, containing 40 to 60 mg protein ml⁻¹, were stored at -25 °C until use.

Protein estimation. Protein was determined by the biuret method (Gornall *et al.*, 1949) with bovine serum albumin as standard.

Inhibition by di-isofluoropropyl phosphate (DFP). DFP (Sigma) at concentrations between 10⁻⁵ and 10⁻³ M was added to the crude extracts and the mixtures were allowed to stand for 30 min at room temperature immediately before electrophoresis.

Heat inactivation. Crude extracts mixed with an equal volume of buffer (0.06 M-Tris/glycine pH 8.7) were heated at 60 °C for 10 min.

Polyacrylamide-agarose gel electrophoresis, estimation of electrophoretic mobility (M_F value) and esterase staining. These have all been described previously (Goulet, 1975, 1977).

Polyacrylamide gradient gel electrophoresis. Molecular sizes of esterases were investigated by 4 to 30% (w/v) polyacrylamide gradient gel electrophoresis (Andersson *et al.*, 1972; Margolis & Kenrick, 1968) using Pharmacia gradient gel PAA 4/30 and Tris (0.09 M)/borate (0.08 M)/Na₂EDTA (0.003 M) buffer (pH 8.35) without denaturing agents. For molecular weight estimations, bovine serum albumin (Miles, Kankakee, Ill., U.S.A.) and *E. coli* alkaline phosphatase (Worthington, Freehold, N.J., U.S.A.) were used as reference proteins. Electrophoresis was performed at 5 °C in the Pharmacia electrophoresis apparatus GE-4. After electrophoresis, gels were stained for esterase activity and bands were marked with a copper wire. Standard proteins were subsequently stained by Coomassie blue. Esterase and protein positions were determined relative to the cathode end of the gel by direct measurement.

Fig. 2. *Klebsiella oxytoca*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 15). (b) Esterase patterns of 14 strains arranged in order of increasing mobility of E₁ band. Experimental conditions, relative intensity of staining and key as in legend to Fig. 1. ■, E₂ band; ■, E₃ band.

RESULTS

Polyacrylamide-agarose gel electrophoresis

Esterase zymograms gave reproducible patterns characteristic of the four species tested. Electrophoretic relationships between the strains were established by multiple runs comparing esterase bands in adjacent positions on the same gel.

Klebsiella pneumoniae. One major band, designated E₁, hydrolysed α -naphthyl acetate and, to a lesser degree, indoxyl acetate, α -naphthyl butyrate and β -naphthyl acetate (Fig. 1). This band was inactivated by heat (10 min at 60 °C) and inhibited by 10⁻⁴ M-DFP. Four minor bands resistant to 10⁻³ M-DFP were also observed: band m₁ migrated faster than band E₁ and was detected by indoxyl and α -naphthyl acetates, band m₂ was detected by β -naphthyl acetate, band m₃ was stained by α - and β -naphthyl acetates and band m₄ by β -naphthyl acetate only. Bands m₁ and m₂ were heat-resistant. Bands m₂ and m₄ overlapped in strains 6 and 7. The electrophoretic mobility of bands m₂ and m₃ showed no 'between-strain' variation (with the exception of band m₂ from strain 6). In addition, two minor bands, F (fast) and S (slow), were detected by β -naphthyl acetate.

Klebsiella oxytoca. Three major bands, designated E₁, E₂ and E₃ in order of decreasing electrophoretic mobility, hydrolysed indoxyl acetate (Figs 2 and 3a). Band E₁ showed the same esterolytic activity and sensitivity to heat and to DFP as the corresponding band of *K. pneumoniae*. Bands E₂ and E₃ reacted slightly with α -naphthyl acetate. Band E₂ resisted 10⁻³ M-DFP and band E₃ was heat-resistant (Fig. 3b). Two minor bands, m₂ and m₃, were similar to those of *K. pneumoniae*. Band F was not detected.

Enterobacter aerogenes. The major band E₁ and minor bands m₂ and m₃ (Fig. 4) were practically identical to the corresponding bands of *K. pneumoniae* and *K. oxytoca*. A faint

Fig. 3. Effects of heat and DFP on the activity of esterases E₁, E₂, E₃ of *Klebsiella oxytoca* (strain 23). Esterases were stained with indoxyl acetate and scanned at 660 nm. (a) Untreated preparation. (b) Treated preparation: ---, treated with 10⁻³ M-DFP; —, heated at 60 °C for 10 min.

Fig. 4. *Enterobacter aerogenes*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 33). (b) Esterase patterns of 16 strains arranged in order of increasing mobility of E₁ band. Experimental conditions, relative intensity of staining and key as in legend to Fig. 1. ■■■, band similar to m₁, but more coloured, for strains 29, 33 and 34; ▤, m₅ band.

esterase band, m₁, hydrolysing indoxyl and α -naphthyl acetates and resistant to heat and DFP was similar to that observed in *K. pneumoniae*. An additional band with characteristics similar to m₁ was observed in strains 29, 33 and 34. Differences in mobility between some minor bands were small and bands m₂ and m₃ overlapped in most strains. Separation and characterization of bands were then achieved by varying the concentration of acrylamide and by differential heat inactivation. Band m₅ reacted weakly with α - and β -naphthyl butyrates. Band F was very faint. Each of the 16 strains showed a distinct esterase electrophoretic pattern.

Fig. 5. *Enterobacter gergoviae*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 54). (b) Esterase patterns of 16 strains arranged in order of increasing mobility of E₄ band. Experimental conditions, relative intensity of staining and key as in legend to Fig. 1. ■, E₄ band; —, E₁ band; //, m₇ band.

Enterobacter gergoviae. Typically, strains of *E. gergoviae* showed two major esterase bands (Fig. 5): one, designated E₁, possessed hydrolysing activity similar to that observed in the other three species tested; the second, designated E₄, showing very strong esterase activity, hydrolysed all five substrates tested and was partially inhibited by 10⁻³ M-DFP. Bands E₁ and E₄ from strains 45, 53, 55, 56, 57, 58 and 59 overlapped when 7% acrylamide gels were used for electrophoresis but were clearly separated by polyacrylamide gradient gel electrophoresis (see below). Band E₄ was not resolved in strains 50, 51, 52 and 60. Two minor bands were resistant to 10⁻³ M-DFP – band m₆ hydrolysing β -naphthyl acetate and band m₇ hydrolysing α -naphthyl and indoxyl acetates. The electrophoretic mobility of bands E₁ ($M_r \approx 60$) and m₇ ($M_r \approx 57$) showed no 'between-strain' variation. In some strains a diffuse band ($M_r \approx 28$) reacted with all five substrates (not shown).

Polyacrylamide gradient gel electrophoresis

The major esterases E₁, E₂, E₃ and E₄ were easily detectable in gradient gels by indoxyl acetate. The E₁ and E₄ bands of *E. gergoviae*, which for some strains overlapped in polyacrylamide-agarose gels, were well separated in polyacrylamide gradient gels. Comparison of electrophoresis for 400, 900, 1500, 2000 and 2500 V h showed that asymptotic migration, necessary to determine molecular weight (Rodbard *et al.*, 1971), was obtained for esterases E₂ and E₃ of *K. oxytoca* (Fig. 6). Using bovine serum albumin and *E. coli* alkaline phosphatase as standard proteins, the molecular weights of esterases E₂ and E₃ were estimated to be 58000 (± 1000) and 72000 (± 1800), respectively.

Characteristics of the major bands are summarized in Table 2.

Fig. 6. Plots of mobilities of esterases E_1 , E_2 and E_3 from *K. oxytoca* (strain 23) and esterase E_4 from *E. gergoviae* (strain 57) against time of electrophoresis in a polyacrylamide gradient gel: ○, esterase E_1 ; ●, esterase E_2 ; □, esterase E_3 ; ■, esterase E_4 .

Table 2. *Characteristics of principal esterase bands of K. pneumoniae, K. oxytoca, E. aerogenes and E. gergoviae*

Band	Substrates* hydrolysed†					Inactivation† after 10 min at 60 °C	Inhibitory concn of DFP (M)	Apparent molecular weight§
	α NA	α NB	IA	β NA	β NB			
E_1	+++	+	++	+	—	—	10^{-4}	
E_2	+	—	++	—	—	—	$R\ 10^{-3}\ddagger$	58000 ± 1000
E_3	+	—	++	—	—	++	10^{-3}	72000 ± 1800
E_4	++++	++	+++	+++	++	—	10^{-3}	

* α NA, α -naphthyl acetate; α NB, α -naphthyl butyrate; IA, indoxyl acetate; β NA, β -naphthyl acetate; β NB, β -naphthyl butyrate.

† +++++, +++++, +++, +, decreasing intensities of esterase band; —, no activity.

‡ Resistant to 10^{-3} M-DFP.

§ Mean $\pm$ s.d.; 6 runs.

Table 3. M_F values of esterase bands of *K. pneumoniae*, *K. oxytoca*, *E. aerogenes* and *E. gergoviae*

Mean M_F values are given together with the standard deviation. Sample sizes are given in parentheses.

Band	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>E. aerogenes</i>	<i>E. gergoviae</i>
E_1	76.79 ± 5.80 (14)	72.50 ± 2.35 (14)	73.13 ± 3.89 (16)	60.50 ± 0 (16)
E_2		61.86 ± 6.94 (14)		
E_3		31.93 ± 4.84 (14)		
E_4				64.33 ± 1.97 (12)
m_1	78.88 ± 0.35 (8)		64.53 ± 6.55 (16)	
m_2	64.43 ± 1.60 (14)	67.93 ± 0.28 (14)	62.81 ± 0.75 (16)	
m_3	56.00 ± 0 (14)	50.14 ± 5.02 (14)	62.06 ± 1.44 (16)	
m_4	65.86 ± 5.17 (14)			
m_5			54.25 ± 7.19 (16)	
m_6				89.31 ± 1.54 (16)
m_7				57.00 ± 0 (16)
F	95.14 ± 0.53 (14)		95.00 ± 0 (16)	95.00 ± 0 (16)
S	27.86 ± 4.69 (14)	22.64 ± 5.20 (11)	13.73 ± 4.57 (15)	

DISCUSSION

Four major esterase bands and nine minor bands were defined from extracts of strains of *K. pneumoniae*, *K. oxytoca*, *E. aerogenes* and *E. gergoviae*. The distribution of major and minor bands among the strains (Table 3) showed that the four species were characterized by distinct esterase patterns. *Enterobacter gergoviae* exhibited substantial differences from *E. aerogenes* (Table 3). *Klebsiella oxytoca* differed from *K. pneumoniae* in the presence of major bands E₂ and E₃ and the absence of bands m₁, m₄ and F, whereas *K. pneumoniae* and *E. aerogenes* differed from each other only in two minor bands – band m₄ detected in *K. pneumoniae* strains and band m₅ detected in *E. aerogenes* strains.

The present study strongly supports the phenotypic individuality of *E. gergoviae* (Richard, 1979; Richard *et al.*, 1976) and provides new evidence for the differentiation of *K. oxytoca* from *K. pneumoniae* (Brenner *et al.*, 1977; Gavini *et al.*, 1977; Hugh, 1959; Jain *et al.*, 1974; Lautrop, 1956; Von Riesen, 1976). The similarity of the esterase patterns of *K. pneumoniae* and *E. aerogenes* correlates well with the biochemical and serological similarities of these two species (Bascomb *et al.*, 1971; Johnson *et al.*, 1975; Richard, 1977; Steffen *et al.*, 1972).

The author thanks Dr C. Richard for *Klebsiella* and *Enterobacter* strains, and Madame Colette Gaillard and Mademoiselle Catherine Lamesch for technical assistance. This work was supported by grants from the Université de Paris VII and from the Institut National de la Santé et de la Recherche Médicale (no. 78.1.136.1).

REFERENCES

- ANDERSSON, L. O., BORG, H. & MIKAELSSON, M. (1972). Molecular weight estimations of proteins by electrophoresis in polyacrylamide gels of graded porosity. *FEBS Letters* **20**, 199–202.
- BASCOMB, S., LAPAGE, S. P., WILLCOX, W. R. & CURTIS, M. A. (1971). Numerical classification of the tribe Klebsiellae. *Journal of General Microbiology* **66**, 279–295.
- BRENNER, D. J., FARMER, J. J., HICKMAN, F. W., ASBURY, M. A. & STEIGERWALT, A. G. (1977). Taxonomic and nomenclature changes in *Enterobacteriaceae*. Center for Disease Control, Department of Health, Education and Welfare, Publication No. 78-8356. Atlanta, Georgia, U.S.A.: C.D.C.
- BUCHANAN, R. E. & GIBBONS, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- GAVINI, F., LECLERC, H., LEFEBVRE, B., FERRAGUT, C. & IZARD, D. (1977). Etude taxonomique d'Enterobactéries appartenant ou apparentées au genre *Klebsiella*. *Annales de Microbiologie* **128B**, 45–49.
- GORNALL, A. G., BARDAWILL, D. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry* **177**, 751–766.
- GOULLET, PH. (1975). Esterase zymograms of *Proteus* and *Providencia*. *Journal of General Microbiology* **87**, 97–106.
- GOULLET, PH. (1977). Relationships between electrophoretic patterns of esterases from *Salmonella*. *Journal of General Microbiology* **98**, 535–542.
- HUGH, R. (1959). Oxytoca group organisms isolated from the oropharyngeal region. *Canadian Journal of Microbiology* **5**, 251–254.
- JAIN, K., RADSACK, K. & MANNHEIM, W. (1974). Differentiation of the *Oxytoca* group from *Klebsiella* by deoxyribonucleic acid–deoxyribonucleic acid hybridization. *International Journal of Systematic Bacteriology* **24**, 402–407.
- JOHNSON, R., COLWELL, R. R., SAKAZAKI, R. & TAMURA, K. (1975). Numerical taxonomy study of the *Enterobacteriaceae*. *International Journal of Systematic Bacteriology* **25**, 12–37.
- LAUTROP, H. (1956). Gelatin-liquefying *Klebsiella* strains (*Bacterium oxytoca* (Flüggel)). *Acta pathologica et microbiologica scandinavica* **39**, 375–384.
- MARGOLIS, J. & KENRICK, K. G. (1968). Polyacrylamide gel electrophoresis in a continuous molecular sieve gradient. *Analytical Biochemistry* **25**, 347–362.
- ORSKOV, I. (1974). Genus VI *Klebsiella* Trevisan 1885. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 321–324. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- RICHARD, C. (1973). Etude antigénique et biochimique de 500 souches de *Klebsiella*. *Annales de biologie clinique* **31**, 295–303.
- RICHARD, C. (1977). Présence chez *Enterobacter aerogenes* d'antigènes capsulaires apparentés à ceux de *Klebsiella*: intérêt de l'utilisation du méthydroxybenzoate dans le diagnostic différentiel *E. aerogenes* – *K. pneumoniae*. *Annales de Microbiologie* **128A**, 289–295.
- RICHARD, C. (1979). Entérobactéries inhabituelles. *Bulletin de l'Institut Pasteur* **77**, 83–98.
- RICHARD, C., JOLY, B., SIROT, J., STOLERU, G. H. & POPOFF, M. (1976). Etude de souches d'*Entero-*

- bacter* appartenant à un groupe particulier proche de *E. aerogenes*. *Annales de Microbiologie* **127A**, 545–548.
- RODBARD, D., KAPADIA, G. & CHRAMBACH, A. (1971). Pore gradient electrophoresis. *Analytical Biochemistry* **40**, 135–157.
- STEFFEN, D. L., COCKS, G. T. & WILSON, A. C. (1972). Micro-complement fixation in *Klebsiella* classification. *Journal of Bacteriology* **110**, 803–808.
- VON RIESEN, V. L. (1976). Pectinolytic, indole-positive strains of *Klebsiella pneumoniae*. *International Journal of Systematic Bacteriology* **26**, 143–145.