Correlation of Serological and Biochemical Groupings of *Yersinia enterocolitica* with the β -Lactamases of the Strains

By MARGARET MATTHEW

Microbiology Division, Glaxo Research Limited, Greenford UB6 0HE, Middlesex

G. CORNELIS AND G. WAUTERS

Department of Microbiology, University of Louvain, B1200 Brussels, Belgium

(Received 16 February 1977; revised 22 April 1977)

The β -lactamase isoelectric focusing patterns of 37 strains of *Yersinia enterocolitica* from various serological and biochemical groups were examined. Strains of different serological groups generally had different patterns, but those of serological groups 1, 2, 3 and 9 were identical.

INTRODUCTION

The species *Yersinia enterocolitica* includes strains that are heterogeneous (Wauters, 1970, 1973); five biochemical types have been defined (Niléhn, 1969; Wauters, 1970, 1973), and up to 34 O-antigenic factors have been recognized (Wauters, Le Minor & Chalon, 1971; Wauters *et al.*, 1972; Winblad, 1967, 1968). All *Y. enterocolitica* strains examined by Cornelis, Wauters & Vanderhaeghe (1973) produced β -lactamase (EC 3.5.2.6). Purification of β -lactamases from strains of serological groups 3 and 9 showed that each strain produced two different enzymes, designated A and B (Cornelis & Abraham, 1975). The two enzymes hydrolysed β -lactam substrates at different rates, were differently inhibited by β -lactam inhibitors (Cornelis, 1975; Cornelis & Abraham, 1975), and were presumed to play different roles in resistance of the organisms to β -lactam antibiotics. A visual comparison of β -lactamases, by examining the patterns of enzymically-active bands revealed after analytical isoelectric focusing (Matthew *et al.*, 1975), has shown that chromosomally-mediated β -lactamases are specific for genus, species and sub-species (Matthew & Harris, 1976). The purpose of the present work was to assess the correlation between the β -lactamase isoelectric focusing patterns of various strains of *Y. enterocolitica* and the biochemical and serological groupings of the organisms.

METHODS

Strains. The strains of *Y. enterocolitica* (Table 1) were classified into serotypes as described by Winblad (1967, 1968) and Wauters *et al.* (1971, 1972), and into biotypes according to the criteria proposed by Niléhn (1969), slightly modified by Wauters (1970). Two strains of *Escherichia coli* K12 were also included: 84Nx(RY3) and NCIB9482(R-TEM).

Preparation and analytical isoelectric focusing of intracellular β -lactamases. The methods used were those described by Matthew *et al.* (1975).

RESULTS

The β -lactamase isoelectric focusing patterns of all the strains examined consisted of several bands (Fig. 1). Some of these focused close together and were regarded as a group of bands representing a single β -lactamase. In isoelectric focusing an apparently single

Table 1. *Strains of Yersinia enterocolitica from which intracellular β -lactamases were prepared*

Series no.	Source no. *	Sero-logical group†	Bio-chemical group‡	Species of isolation	Origin	
					Worker	Country
1	IP92 [NCTC10462]	1	3	Chinchilla	Frederiksen	Denmark
2	IP110	1	3	Chinchilla	Akkermans	Netherlands
3	IP8	2	5	Hare	Lucas	France
4	IP178	2	5	Hare	McDiarmid	U.K.
5	F96-1	2	5	Hare	Fievez	Belgium
6	A14700	2	5	Hare	Aldova	Czechoslovakia
7	W222	3	4	Human	Wauters	Belgium
8	W223	3	4	Human	Wauters	Belgium
9	W224	3	4	Pig	Wauters	Belgium
10	W230	3	4	Pig	Wauters	Belgium
11	W237	3	4	Pig	Wauters	Belgium
12	W239	3	4	Human	Wauters	Belgium
13	IP134 ['Winblad']	3	4	Human	Winblad	Sweden
14	IP97	5,27	2	Chinchilla	Frederiksen	Denmark
15	IP130	5,27	2	Monkey	Mair	U.K.
16	IP199	5,27	2	Chinchilla	County Vet.	California, U.S.A.
17	IP1607	5,27	2	Human	Zen-Yogi	Japan
18	M771	5,27	2	Human	Moinet	Belgium
19	IP636 [NCTC10598]	8	1	Human	Sonnenwirth	U.S.A.
20	IP1453	8	1	Human	Sonnenwirth	U.S.A.
21	IP1628	8	1	Human	Weaver	U.S.A.
22	IP2026	8	1	Human	Weaver	U.S.A.
23	IP410	9	2	Human	Ahvonon	Finland
24	W227	9	2	Human	Wauters	Belgium
25	W228	9	2	Human	Wauters	Belgium
26	W238	9	2	Human	Wauters	Belgium
27	W244	9	2	Human	Wauters	Belgium
28	IP123	5	1	Cow	Vallee	France
29	IP102	6	1	Human	Frederiksen	Denmark
30	A Z1523	16§	1	Mouse	Aldova	Czechoslovakia
31	IP867	16§	2	Human	Graux	Belgium
32	IP955	17	1	Water	Lassen	Norway
33	IP841	11,24		Human	Weaver	U.S.A.
34	IP103	12,26		Sheep	Frederiksen	Denmark
35	v. 2.73(RY3)	9	2	Human	Vandepitte	Belgium
36	v. 2.73(R-)	9	2	Human	Derived from v. 2.73(RY3)	
37	H66	3	4	Human	Greven-Brauns	Belgium

* Strains designated IP were received from the collection of Prof. H. H. Mollaret, Institut Pasteur, Paris.

† Classified as described by Winblad (1967, 1968) and Wauters *et al.* (1971, 1972).

‡ Classified according to the criteria proposed by Niléhn (1969), slightly modified by Wauters (1970).

§ As suggested by the different biotypes, these two strains of serological group 16 exhibit different biochemical behaviour.

|| These strains do not fit exactly in any of the described biotypes.

β -lactamase commonly focuses as a group of bands, often a main band accompanied by satellites: the reason for this appearance is unknown (Matthew *et al.*, 1975). When β -lactamases from more than one strain of a certain serological group were focused, the β -lactamase patterns for each of these strains were identical (Fig. 1, serological groups 1; 2; 3; 5,27; 8; 9).

Serological groups 1, 2, 3 and 9. All the strains belonging to these groups had the same β -lactamase isoelectric focusing pattern, with the exception of strain H66 (see below). The pI 8.7 band corresponded to the broad-spectrum β -lactamase designated by Cornelis & Abraham (1975) as type A; these authors' type B enzyme was represented by bands with pI 5.3 and 5.7. Cornelis & Abraham (1975) assumed that both enzymes were chromosomally-

Fig. 1. Analytical isoelectric focusing patterns of intracellular β -lactamases produced by *Yersinia enterocolitica*. Particulars of the strains are given in Table 1. Plasmid RY3 was transferred from *Y. enterocolitica* v. 2.73(RY3) into *E. coli* κ 12. *Escherichia coli* κ 12(R-TEM) is a reference strain that produces the TEM-1 plasmid-mediated β -lactamase (Matthew & Hedges, 1976).

mediated and we found that none of the bands matches that of any β -lactamase known to be plasmid-mediated (Matthew & Hedges, 1976; Matthew, unpublished observations). The TEM-1 plasmid-mediated β -lactamase had a pI similar to that of Cornelis & Abraham's B enzyme, but the β -lactamase pattern of the naturally-occurring serological group 9 strain v. 2.73(RY3) (Cornelis *et al.*, 1973; Fig. 1, strain 35) indicated that the two enzymes are not identical. Strain H66 is atypical of group 3, since it produced no type A β -lactamase (Fig. 1, strain 37). In common with other strains of groups 1, 2, 3 and 9, strain H66 gave a weak β -lactamase band at pI 6.7.

Serological group 5,27. This corresponds to serological group 5b of Cornelis (1975). Our results confirm his finding that strains of this group [except those that were indole(-)] produced only the type B β -lactamase.

Other serological groups. None of the β -lactamases produced by strains of the other serological groups studied were identical with the type A and type B enzymes, although strains from serological groups 8 and 17 gave a doublet of bands with pI values close to those of the B β -lactamase. However with the exception of strain IP841 (serological group

11,24) and strain IP103 (serological group 12,26), all strains apparently produced more than one β -lactamase. Strains of different serotype produced different β -lactamases.

DISCUSSION

Matthew & Harris (1976) suggested that the isoelectric focusing patterns of chromosomally-mediated β -lactamases were specific for sub-species. The patterns of the A and B β -lactamases from *Y. enterocolitica* strains of serological groups 1, 2, 3, 9 (and enzyme B for group 5,27) suggest that these strains may have a close taxonomic relationship, in spite of the biochemical differences between them. Knapp & Thal (1972) questioned whether strains of serological group 2, usually isolated from hares, should be included in the species *Y. enterocolitica*, but our results suggest that they should.

The strains belonging to serological groups 1, 2, 3, 9 and 5,27 are all regularly encountered in specific hosts and all have an established pathogenicity. Apart from the β -lactamase patterns, strains of serological groups 1, 2 and 3 have other features in common. Most of the strains are lysogenic (Nicolle *et al.*, 1967): many phages liberated by group 1 and 3 strains plaque on group 2 strains and many phages liberated by group 2 strains plaque on strains of groups 1 and 3 but not on representatives of other serological groups (Niléhn & Ericson, 1969). In addition, all these strains possess the O:3 antigen (Wauters *et al.*, 1971; Winblad, 1967, 1968). None of the other strains, except those belonging to group 8, have been shown to be pathogenic. Isoelectric focusing of β -lactamases from *Y. enterocolitica* thus tends to group together the pathogenic strains, despite their wide differences in geographical origin.

In a numerical taxonomic study, Stevens & Mair (1973) divided *Y. enterocolitica* into two major groups, A and B. Subgroup A₁ contained strains isolated from humans, monkeys and pigs and corresponded to biotype 4 (Niléhn, 1969; Wauters, 1970); subgroup A₂ contained strains from various sources, including all those isolated from chinchilla; subgroup A₃ contained mainly strains isolated from hares. Group B was composed solely of hare strains of biotype 5, markedly different from group A₃ strains. Our hare strains all belonged to biotype 5, serological group 2, and had identical β -lactamase patterns, but our sample was too small to indicate whether this pattern predominates in hare isolates. Our results cannot be compared directly with those of Stevens & Mair (1973) because we examined different strains. But it seems that focusing of β -lactamases is a tool, additional to serological and differential biochemical tests, that should prove useful in the taxonomic classification of *Y. enterocolitica*.

We thank Janet E. Horne and Jill Sparks for technical assistance and S. R. Jones for taking the photographs.

REFERENCES

- CORNELIS, G. (1975). Distribution of β -lactamases A and B in some groups of *Yersinia enterocolitica* and their role in resistance. *Journal of General Microbiology* **91**, 391-402.
- CORNELIS, G. & ABRAHAM, E. P. (1975). β -Lactamases from *Yersinia enterocolitica*. *Journal of General Microbiology* **87**, 273-284.
- CORNELIS, G., WAUTERS, G. & VANDERHAEGHE, H. (1973). Présence de β -lactamase chez *Yersinia enterocolitica*. *Annales de Microbiologie (Institut Pasteur)* **124B**, 139-152.
- KNAPP, W. & THAL, E. (1972). Die biochemische Charakterisierung von *Yersinia enterocolitica* (syn. 'Pasteurella X') als Grundlage eines vereinfachten O-Antigenschemas. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I, Originale A)* **223**, 88-105.
- MATTHEW, M. & HARRIS, A. M. (1976). Identification of β -lactamases by isoelectric focusing: correlation with bacterial taxonomy. *Journal of General Microbiology* **94**, 55-67.
- MATTHEW, M., HARRIS, A. M., MARSHALL, M. J. & ROSS, G. W. (1975). The use of analytical isoelectric focusing for detection and identification of β -lactamases. *Journal of General Microbiology* **88**, 169-178.
- MATTHEW, M. & HEDGES, R. W. (1976). Analytical isoelectric focusing of R factor-determined β -lactamases: correlation with plasmid compatibility. *Journal of Bacteriology* **125**, 713-718.

- NICOLLE, P., MOLLARET, H., HAMON, Y. & VIEU, J. F. (1967). Étude lysogénique, bacteriocinogénique et lysotypique de l'espèce *Yersinia enterocolitica*. *Annales de l'Institut Pasteur* **112**, 86-92.
- NILÉHN, B. (1969). Studies on *Yersinia enterocolitica*, with special reference to bacterial diagnosis and occurrence in human acute enteric disease. *Acta pathologica et microbiologica scandinavica*, Suppl. **206**.
- NILÉHN, B. & ERICSON, C. (1969). Studies on *Yersinia enterocolitica*. Bacteriophages liberated from chloroform-treated cultures. *Acta pathologica et microbiologica scandinavica* **75**, 177-187.
- STEVENS, M. & MAIR, N. S. (1973). A numerical taxonomic study of *Yersinia enterocolitica* strains. In *Contributions to Microbiology and Immunology*, vol. 2, *Yersinia, Pasteurella and Francisella*, pp. 17-22. Edited by S. Winblad. Basel: Karger.
- WAUTERS, G. (1970). *Contribution à l'étude de Yersinia enterocolitica*. Thesis, University of Louvain, Belgium.
- WAUTERS, G. (1973). Correlation between ecology, biochemical behaviour and antigenic properties of *Yersinia enterocolitica*. In *Contributions to Microbiology and Immunology*, vol. 2, *Yersinia, Pasteurella and Francisella*, pp. 38-41. Edited by S. Winblad. Basel: Karger.
- WAUTERS, G., LE MINOR, L. & CHALON, A. M. (1971). Antigènes somatiques et flagellaires des *Yersinia enterocolitica*. *Annales de l'Institut Pasteur* **120**, 631-642.
- WAUTERS, G., LE MINOR, L., CHALON, A. M. & LASSEN, J. (1972). Supplément au schéma antigénique de *Yersinia enterocolitica*. *Annales de l'Institut Pasteur* **122**, 951-956.
- WINBLAD, S. (1967). Studies on serological typing of *Yersinia enterocolitica*. *Acta pathologica et microbiologica scandinavica*, Suppl. **187**, 115.
- WINBLAD, S. (1968). Studies on O-antigen factors of *Yersinia enterocolitica*. In *International Symposium on Pseudotuberculosis, Symposia Series in Immunobiological Standardisation*, vol. 9, pp. 337-342. Basel: Karger.