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# Population Genetics of Human, Animal, and Environmental *Yersinia* Strains

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**Multilocus enzyme electrophoresis was used to analyze 244 strains of nine *Yersinia* species isolated from the environment, animals, and humans at 18 genes encoding metabolic enzymes. All 18 enzymes were polymorphic. Among the 137 electrophoretic types (ETs) distinguished, the mean allelic diversity per locus was 0.531. *Yersinia frederiksenii* ETs were divided into three major clusters that were separated by a large genetic distance, and one ET was more closely related to *Yersinia enterocolitica*. Thus, strains classically identified as *Y. frederiksenii* may represent more than one species. Furthermore, two strains identified as *Yersinia kristensenii* proved to be more closely related to *Yersinia mollaretii*. Environmental strains formed independent groups. A very interesting ET consisting of as many as 61 isolates of *Yersinia enterocolitica* was detected, and the epidemiologic relevance of this ET is discussed. Human strains of *Y. enterocolitica* biotype 4 and *Yersinia pseudotuberculosis* were recognized as being closely related to animal strains of the same species. Therefore, animal strains of these two species may be considered potential human pathogens.**

Bacteria belonging to the genus *Yersinia* are widely distributed in the environment. Some species are foodborne pathogens that cause gastroenteritis, mesenteric lymphadenitis, and terminal ileitis. These organisms may also cause extraintestinal diseases, such as septicemia associated with respiratory problems, liver abscesses, arthritis, cholangitis, pneumonia, and pharyngitis (6).

Multilocus enzyme electrophoresis (MEE) was first adapted for use with prokaryotes by Selander et al. (16), and this technique makes it possible to differentiate strains on the basis of allelic variation at individual gene loci for taxonomic and epidemiologic purposes. With MEE, strains are characterized by the electrophoretic motility of a large number of metabolic enzymes, which are detected by chromogenic reactions. The electromorphs of each enzyme represent different alleles at the corresponding structural gene locus. If a sufficient number of different enzymes (genes) are included in the study, each isolate is characterized by a pattern of electromorphs called an electrophoretic type (ET), which may be equated with the bacterial genotype (17). Therefore, MEE may be used for both epidemiologic studies and population genetics.

Caugant et al. (5) and Schill et al. (15) have used MEE to measure genetic distances between strains of *Yersinia enterocolitica* and between strains of *Yersinia ruckeri*, respectively.

The aims of this study were to measure levels of genetic relatedness among a large number of strains belonging to different species of the genus *Yersinia* and to analyze the genetic distances among strains belonging to the same species but having different origins. Furthermore, this investigation was undertaken to ascertain whether an epidemiological correlation among human, animal, and environmental isolates could be demonstrated.

## MATERIALS AND METHODS

**Bacterial isolates.** We analyzed 238 strains of *Yersinia* spp. that originated from the environment (79 strains), animals (26 strains), and humans (133 strains). Six reference strains were also included in the study.

All of the environmental strains, as well as 11 animal strains and 108 human strains, were isolated in Switzerland. The other 23 human isolates were obtained from Belgium, Denmark, France, Greece, Norway, and Spain. A group of 15 animal strains were isolated in Belgium (Table 1). Among the human isolates, 120 were obtained from feces, 5 were obtained from blood, 2 were obtained from sputum, 1 was obtained from a lymph node, 1 was obtained from a peritoneal swab, and 2 were of unknown human origin.

After biochemical identification, the 244 *Yersinia* strains were classified as belonging to the following species: *Yersinia enterocolitica* (168 strains), *Yersinia intermedia* (38 strains), *Yersinia frederiksenii* (13 strains), *Yersinia pseudotuberculosis* (9 strains), *Yersinia kristensenii* (7 strains), *Yersinia bercovieri* (6 strains), *Yersinia aldovae* (1 strain), *Yersinia mollaretii* (1 strain), and *Yersinia ruckeri* (1 strain) (Table 1).

**Phenotypic characterization.** Biochemical tests were performed by using the trial procedure of Wauters (21) and Wauters et al. (23). The lecithinase test (21) was performed instead of the lipase test. Pyrazinamidase activity was tested in order to distinguish potentially pathogenic strains from nonpathogenic strains (10). Strains were sent to the World Health Organization Center for *Yersinia* at the Institut Pasteur, Paris, France, for serotyping and phage typing. Serotyping was performed by using 60 O antisera against *Y. enterocolitica* and related species (22); phage types were determined as described by Nicolle et al. (14).

**Genetic analysis.** For the most part, MEE was performed as described by Selander et al. (16). The loci of the following 18 enzymes were considered: adenylate kinase, catalase, fumarase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, glyceraldehyde phosphate dehydrogenase, glutamic-oxalacetic transaminase, indophenol oxidase, isocitrate dehydrogenase, leucine aminopeptidase, malate dehydrogenase, mannitol-1-phosphate dehydrogenase, mannose phosphate isomerase, nucleoside phosphorylase, peptidase of L-phenylalanyl-L-leucine, phosphoglucomutase, 6-phosphogluconate dehydrogenase, and phosphoglucose isomerase.

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TABLE 1. Characteristics of 244 isolates of *Yersinia* spp.

| Species | ET | Isolate | Source | Country | Biotype | Serotype | Phage type |
|---|---|---|---|---|---|---|---|
| *Y. frederiksenii* | 1 | 7 | Water | Switzerland | | 16 | Xz |
| | 2 | 56 | Water | Switzerland | | 16,29 | Xo |
| | 3 | 59 | Water | Switzerland | | 7,13,19 | Xo |
| | 4 | 17 | Water | Switzerland | | 16 | Xo |
| | 5 | 1 | Water | Switzerland | | 16,29 | Xz |
| | 6 | 177 | Human | Switzerland | | AA[a] | Xo |
| | 7 | 55 | Water | Switzerland | | AA | Xo |
| | 83 | 29 | Water | Switzerland | | 35 | Xz |
| | 84 | 12 | Human | Switzerland | | 4,14,16,29 | Xz |
| | 85 | 308 | Human | Switzerland | | 41,43 | ND[b] |
| | 86 | 79 | Water | Switzerland | | 25,35 | Xz |
| | 87 | 85 | Water | Switzerland | | 25,35 | Xz |
| | 88 | 25 | Water | Switzerland | | 16 | Xz |
| *Y. bercovieri* | 8 | H 16 | Human | Switzerland | | AA | Xo |
| | 9 | 860 | Human | Switzerland | | 25,35 | Xo |
| | 10 | 307 | Human | Switzerland | | 5 | ND |
| | 11 | F 10 | Human | Switzerland | | ND | ND |
| | 12 | J 41 | Human | Switzerland | | ND | ND |
| | 13 | IP 7506 | Reference | | | 16 | Xo |
| *Y. aldovae* | 14 | IP 6005 | Reference | | | NT[c] | Xo |
| *Y. enterocolitica* | 15 | 2 | Human | Switzerland | 4 | 3 | VIII |
| | | 4 | Human | Switzerland | 4 | 3 | VIII |
| | | 5 | Human | Switzerland | 4 | 3 | VIII |
| | 16 | 28 | Water | Switzerland | 4 | 3 | VIII |
| | | 30 | Human | Switzerland | 4 | 3 | VIII |
| | 17 | 50 | Human | Switzerland | 4 | 3 | VIII |
| | 18 | 62 | Human | Switzerland | 4 | 3 | VIII |
| | 19 | 60 | Human | Switzerland | 4 | 3 | VIII |
| | | 219 | Human | Switzerland | 4 | 3 | VIII |
| | | 295 | Human | Switzerland | 4 | ND | ND |
| | | F4 | Human | Switzerland | 4 | ND | ND |
| | | F47 | Human | Switzerland | 4 | ND | ND |
| | | V3421 | Human | Switzerland | 4 | ND | ND |
| | | 222401 | Human | Switzerland | 4 | 3 | VIII |
| | | 224120 | Human | Switzerland | 4 | 3 | VIII |
| | | 227189 | Human | Switzerland | 4 | 3 | VIII |
| | | 235471 | Human | Switzerland | 4 | 3 | VIII |
| | | 244127 | Human | Switzerland | 4 | 3 | VIII |
| | | 291158 | Human | Switzerland | 4 | 3 | VIII |
| | | 783 | Human | Switzerland | 4 | 3 | VIII |
| | 20 | 99 | Human | Switzerland | 4 | 3 | VIII |
| | | 261 | Human | Switzerland | 4 | 3 | VIII |
| | | F 16 | Human | Switzerland | 4 | ND | ND |
| | 21 | Co881968 | Human | Switzerland | 2 | 9 | X3 |
| | | 293621 | Human | Switzerland | 2 | 9 | X3 |
| | | 491 | Human | Switzerland | 4 | ND | ND |
| | 22 | 86 | Human | Switzerland | 4 | 3 | VIII |
| | | 148 | Human | Switzerland | 4 | 3 | VIII |
| | | 220 | Human | Switzerland | 4 | 3 | VIII |
| | | 229 | Human | Switzerland | 4 | 3 | VIII |
| | | He 885899 | Human | Switzerland | 2 | 9 | X3 |
| | | 881881 | Human | Switzerland | 2 | 9 | X3 |
| | | 881894 | Human | Switzerland | 2 | 9 | X3 |
| | | 882022 | Human | Switzerland | 2 | 9 | X3 |
| | | 882139 | Human | Switzerland | 2 | 9 | X3 |
| | | 882378 | Human | Switzerland | 2 | 9 | X3 |
| | | 882389 | Human | Switzerland | 2 | 9 | X3 |
| | | 881968 | Human | Switzerland | 2 | 9 | X3 |
| | | 882150 | Human | Switzerland | 2 | 9 | X3 |
| | 23 | 134 | Human | Switzerland | 4 | 3 | VIII |
| | | 176 | Human | Switzerland | 4 | 3 | VIII |
| | | 182 | Human | Switzerland | 4 | 3 | VIII |
| | | 184 | Human | Switzerland | 4 | 3 | VIII |
| | | 222 | Human | Switzerland | 4 | 3 | VIII |
| | | 226 | Human | Switzerland | 4 | 3 | VIII |

*Continued on following page*

TABLE 1—*Continued*

| Species | ET | Isolate | Source | Country | Biotype | Serotype | Phage type |
|---|---|---|---|---|---|---|---|
| | | 235 | Human | Switzerland | 4 | 3 | VIII |
| | | 244 | Human | Switzerland | 4 | 3 | VIII |
| | | 302 | Human | Switzerland | 4 | ND | ND |
| | | 304 | Human | Switzerland | 4 | ND | ND |
| | | 772367 | Human | Switzerland | 4 | ND | ND |
| | | 784681 | Human | Switzerland | 4 | ND | ND |
| | | 793225 | Human | Switzerland | 4 | ND | ND |
| | | 793510 | Human | Switzerland | 4 | ND | ND |
| | | 802979 | Human | Switzerland | 4 | ND | ND |
| | | 810128 | Human | Switzerland | 4 | ND | ND |
| | | S 55 | Human | Switzerland | 4 | ND | ND |
| | | 830196 | Human | Switzerland | 4 | ND | ND |
| | | S 148 | Human | Switzerland | 4 | ND | ND |
| | | MM 231 | Human | Switzerland | 4 | ND | ND |
| | | ZH 2 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 3 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 4 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 7 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 8 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 17 | Pig | Switzerland | 4 | 3 | VIII |
| | | ZH 19 | Pig | Switzerland | 4 | 3 | VIII |
| | | W 495 | Pig | Belgium | 4 | 3 | VIII |
| | | W 502 | Pig | Belgium | 4 | 3 | VIII |
| | | W 511 | Pig | Belgium | 4 | 3 | VIII |
| | | W 512 | Pig | Belgium | 4 | 3 | VIII |
| | | S 54/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 55/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 57/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 58/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 59/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 60/89 | Pork | Belgium | 4 | 3 | VIII |
| | | S 63/89 | Pork | Belgium | 4 | 3 | VIII |
| | | E 181/89 | Human | Belgium | 4 | 3 | VIII |
| | | E 192/89 | Human | Belgium | 4 | 3 | VIII |
| | | E 210/89 | Human | Belgium | 4 | 3 | VIII |
| | | E 211/89 | Human | Belgium | 4 | 3 | VIII |
| | | E 220/89 | Human | Belgium | 4 | 3 | VIII |
| | | IP 20203 | Human | Norway | 4 | 3 | VIII |
| | | IP 20221 | Human | Norway | 4 | 3 | VIII |
| | | IP 20231 | Human | Denmark | 4 | 3 | VIII |
| | | IP 20418 | Human | Greece | 4 | 3 | VIII |
| | | IP 20422 | Human | Greece | 4 | 3 | VIII |
| | | IP 20423 | Human | Greece | 4 | 3 | VIII |
| | | IP 20443 | Human | France | 4 | 3 | VIII |
| | | IP 20459 | Human | France | 4 | 3 | VIII |
| | | IP 20460 | Human | France | 4 | 3 | VIII |
| | | IP 20462 | Human | France | 4 | 3 | VIII |
| | | IP 20464 | Human | France | 4 | 3 | VIII |
| | | IP 20527 | Human | France | 4 | 3 | VIII |
| | | IP 20531 | Human | France | 4 | 3 | VIII |
| | | IP 20533 | Human | France | 4 | 3 | VIII |
| | | IP 20534 | Human | France | 4 | 3 | VIII |
| | | IP 20540 | Human | Spain | 4 | 3 | VIII |
| | | IP 20542 | Human | Spain | 4 | 3 | VIII |
| | | IP 20547 | Human | France | 4 | 3 | VIII |
| | 24 | I 47 | Human | Switzerland | 3 | ND | ND |
| | | G 42 | Human | Switzerland | 2 | 9 | X3 |
| | | 310 | Human | Switzerland | 2 | 9 | X3 |
| | 25 | S 64/89 | Pork | Belgium | 4 | 3 | VIII |
| | 26 | 305 | Human | Switzerland | 4 | ND | ND |
| | 27 | H 6665 | Human | Switzerland | 4 | ND | ND |
| | 28 | 800221 | Human | Switzerland | 4 | ND | ND |
| | 29 | S 56/89 | Pork | Belgium | 4 | 3 | VIII |
| | 30 | S 62/89 | Pork | Belgium | 4 | 3 | VIII |
| | 31 | 224 | Human | Switzerland | 3 | 5,27 | Xz |
| | 32 | He 857023 | Human | Switzerland | 3 | 5 | Xz |
| | 33 | 296 | Human | Switzerland | 4 | 3 | VIII |
| | 34 | 175 | Human | Switzerland | 4 | 3 | VIII |

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TABLE 1—*Continued*

| Species | ET | Isolate | Source | Country | Biotype | Serotype | Phage type |
|---|---|---|---|---|---|---|---|
| | 35 | 311 | Human | Switzerland | 4 | ND | ND |
| | 36 | 239015 | Human | Switzerland | 4 | 3 | VIII |
| | 37 | 49 | Human | Switzerland | 4 | 3 | VIII |
| | 38 | 51 | Human | Switzerland | 4 | 3 | VIII |
| | 39 | 6 | Water | Switzerland | 1 | 44,45 | Xo |
| | 40 | 10 | Water | Switzerland | 1 | AA | Xz |
| | 41 | 41 | Water | Switzerland | 1 | 7,8,13 | Xz |
| | | 54 | Water | Switzerland | 1 | 5 | Xz |
| | 42 | 64 | Water | Switzerland | 1 | 6,47 | Xz |
| | 43 | 66 | Water | Switzerland | 1 | 46 | Xz |
| | 44 | 52 | Water | Switzerland | 1 | 7,13 | Xo |
| | 45 | 19 | Water | Switzerland | 1 | 25,35 | Xo |
| | | 20 | Water | Switzerland | 1 | 25,35 | Xz |
| | | 26 | Water | Switzerland | 1 | 14 | Xo |
| | 46 | 32 | Human | Switzerland | 1 | 39,41,42,43 | Xz |
| | 47 | 35 | Water | Switzerland | 1 | 7,13 | Xo |
| | 48 | 39 | Water | Switzerland | 1 | 6,47 | Xz |
| | 49 | 31 | Water | Switzerland | 1 | 7,13 | Xo |
| | 50 | 36 | Water | Switzerland | 1 | 7,13 | Xo |
| | 51 | 76 | Water | Switzerland | 1 | 5,8,50,51 | Xo |
| | 52 | 110 | Water | Switzerland | 1 | 48 | Xo |
| | 53 | 300 | Human | Switzerland | 1 | NA[d] | Xo |
| | 54 | 21 | Water | Switzerland | 1 | AA | Xz |
| | 55 | 58 | Water | Switzerland | 1 | 16 | Xo |
| | 56 | 214 | Human | Switzerland | 1 | 6,47 | Xz |
| | | 306 | Human | Switzerland | 1 | 5 | ND |
| | 57 | 309 | Human | Switzerland | 1 | 7,8,19 | ND |
| | 58 | J 45 | Human | Switzerland | 1 | ND | ND |
| | 59 | 247 | Human | Switzerland | 1 | 5 | Xz |
| | 60 | 303 | Human | Switzerland | 1 | 7,8,19 | Xz |
| | 61 | 70 | Human | Switzerland | 1 | 29,39,41,42,43 | Xz |
| | 62 | 72 | Human | Switzerland | 1 | AA | Xz |
| | 63 | 77 | Water | Switzerland | 1 | AA | Xo |
| | 64 | 1621 | Human | Switzerland | 1 | 6 | Xz |
| | 65 | E 700/88 | Human | Switzerland | 1 | 7,8,19 | Xz |
| | 66 | 242 | Human | Switzerland | 1 | 7,8,19 | Xo |
| | 67 | 274 | Human | Switzerland | 1 | 5 | Xz |
| | 68 | 109 | Water | Switzerland | 1 | 7,8,13,19 | Xo |
| | 69 | 301 | Human | Switzerland | 1 | 5,5,27 | Xz |
| | 70 | 298 | Human | Switzerland | 1 | 6 | Xz |
| | | F 12 | Human | Switzerland | 1 | ND | ND |
| | | H 2 | Human | Switzerland | 1 | 5 | Xz |
| | 71 | F13 | Human | Switzerland | 1 | PA[e] | Xz |
| | 72 | S 297556 | Human | Switzerland | 1 | 5 | Xo |
| | 73 | 113 | Human | Switzerland | 1 | 52,52,53 | Xz |
| | 74 | S 61/89 | Pork | Belgium | 1 | ND | ND |
| | 75 | 218 | Human | Switzerland | 1 | 7,13,7 | Xo |
| | 76 | ATCC 23715 | Reference | | 1 | 8 | ND |
| | 77 | 40 | Water | Switzerland | 1 | 10,34 | Xo |
| | 78 | 42 | Water | Switzerland | 1 | 10,34 | Xo |
| | | 47 | Water | Switzerland | 1 | 10,34,46 | Xo |
| | 79 | 48 | Water | Switzerland | 1 | 16 | Xz |
| | 80 | 65 | Water | Switzerland | 1 | 46 | Xz |
| | 81 | 69 | Water | Switzerland | 1 | 7,13,19 | Xo |
| | 82 | 67 | Water | Switzerland | 1 | 46 | Xz |
| *Y. kristensenii* | 89 | 16 | Water | Switzerland | | 11 | Xo |
| | 90 | 101 | Water | Switzerland | | 12,25 | Xz |
| | 91 | 1628 | Human | Switzerland | | ND | ND |
| | 92 | 3193 | Human | Switzerland | | ND | ND |
| | 93 | 3379 | Human | Switzerland | | ND | ND |
| | 94 | 96 | Water | Switzerland | | 38,52 | Xo |
| | 95 | 98 | Water | Switzerland | | 17 | Xo |
| *Y. mollaretii* | 96 | IP 7263 | Reference | | | 7,8 | Xz |
| *Y. intermedia* | 97 | 11 | Water | Switzerland | 4 | 8,19 | Xo |
| | 98 | 14 | Water | Switzerland | 1 | 46 | Xo |

*Continued on following page*

TABLE 1—*Continued*

| Species | ET | Isolate | Source | Country | Biotype | Serotype | Phage type |
|---|---|---|---|---|---|---|---|
| | 99 | 3 | Water | Switzerland | 4 | 8,19 | Xz |
| | 100 | 53 | Water | Switzerland | 1 | 10,K1 | Xo |
| | 101 | 15 | Water | Switzerland | 1 | 47 | Xo |
| | 102 | 43 | Water | Switzerland | 1 | 36 | Xo |
| | 103 | 46 | Water | Switzerland | 1 | NA | Xo |
| | 104 | 74 | Water | Switzerland | 4 | 50,51 | Xo |
| | 105 | 24 | Water | Switzerland | 1 | NA | Xo |
| | 106 | 34 | Water | Switzerland | 1 | 38 | Xo |
| | 107 | 37 | Water | Switzerland | 1 | 48 | Xo |
| | 108 | 38 | Water | Switzerland | 1 | 4,14,16 | Xz |
| | 109 | 18 | Water | Switzerland | 1 | 38,52 | Xo |
| | 110 | 75 | Water | Switzerland | 4 | 5,8 | Xo |
| | 111 | 97 | Water | Switzerland | 4 | 8,19 | Xo |
| | 112 | 111 | Water | Switzerland | 1 | 17 | Xo |
| | 113 | 61 | Water | Switzerland | 4 | AA | Xo |
| | | 63 | Water | Switzerland | 4 | 8 | Xo |
| | 114 | 102 | Water | Switzerland | 1 | 10,K1 | Xo |
| | 115 | 105 | Water | Switzerland | 1 | 4 | Xz |
| | 116 | 93 | Water | Switzerland | 1 | 14,37 | Xo |
| | 117 | 95 | Water | Switzerland | 1 | 16,29 | Xz |
| | 118 | 73 | Water | Switzerland | 1 | 52,53,54 | Xz |
| | | 78 | Water | Switzerland | 1 | 14 | Xz |
| | | 88 | Water | Switzerland | 1 | 14,37 | Xo |
| | 119 | 103 | Water | Switzerland | 2 | 14,37 | Xo |
| | | 104 | Water | Switzerland | 1 | 14,37 | Xo |
| | 120 | 112 | Water | Switzerland | 1 | 52,53,54 | Xz |
| | 121 | 117 | Water | Switzerland | 1 | 25,35 | Xo |
| | 122 | 115 | Water | Switzerland | 3 | 50,51 | Xo |
| | 123 | 116 | Water | Switzerland | 1 | 50,51 | Xo |
| | 124 | 8 | Water | Switzerland | 1 | 34,36,46,47 | Xo |
| | 125 | 44 | Water | Switzerland | 1 | 21,PSTII | Xo |
| | 126 | 22 | Water | Switzerland | 1 | 10,K1 | Xo |
| | 127 | 33 | Water | Switzerland | 1 | 38 | Xo |
| | 128 | 23 | Water | Switzerland | 1 | 17 | Xo |
| | 129 | 9 | Water | Switzerland | 8 | 52,53,54 | Xo |
| | 130 | 246 | Human | Switzerland | 2 | 4,33,13a,13b,11,24 | Xo |
| *Y. pseudotuberculosis* | 131 | 834318 | Human | Switzerland | | ND | |
| | 132 | M 21310 | Cattle | Switzerland | | ND | |
| | | M 21118 | Cattle | Switzerland | | ND | |
| | | D 7635 | Cattle | Switzerland | | ND | |
| | | D 7645 | Cattle | Switzerland | | ND | |
| | 133 | ZH 28 | Human | Switzerland | | ND | |
| | 134 | M 14 | Human | Switzerland | | ND | |
| | 135 | ZH Y.pst | Human | Switzerland | | ND | |
| | 136 | La 2931 | Reference | | | 1 | |
| *Y. ruckeri* | 137 | ATCC 29833 | Reference | | | 1 | |

[a] AA, autoagglutinable.
[b] ND, not determined.
[c] NT, nontypeable.
[d] NA, nonagglutinable.
[e] PA, polyagglutinable.

The following modifications were made: electrophoresis for glucose-6-phosphate dehydrogenase was carried out in buffer D, and electrophoresis for fumerase was carried out in buffer F.

Specific staining for catalase and specific staining for glutamic-oxalacetic transaminase were performed by using the method of Harris and Hopkinson (9).

**Statistical analysis.** Statistical analysis of the data was performed with a computer program designed by T. S. Whittam and R. K. Selander. The genetic diversity ($h$) for each enzyme locus was calculated as follows: $h = (1 - \Sigma x_i^2)[n/(n-1)]$, where $x_i$ is the frequency of the $i$th allele and $n$ is the number of ETs. The mean genetic diversity ($H$) was the arithmetic average of all of the $h$ values for the 18 enzyme loci. The genetic distance between a pair of ETs was expressed as the proportion of loci at which dissimilar alleles occurred (mismatches) (12). Clustering of ETs was performed by the average-linkage method from a matrix of coefficients of pairwise genetic distances (18).

## RESULTS

**Enzyme loci and alleles.** All 18 enzyme loci were polymorphic. The number of alleles per locus ranged from four

(nucleoside phosphorylase) to nine (glutamic-oxalacetic transaminase and phosphoglucose isomerase) and the average number of alleles per locus was 6.72.

**ETs.** Among the 244 isolates studied we identified 137 ETs, with a mean genetic diversity of 0.531. For the 168 strains of *Y. enterocolitica* 68 ETs were identified. For the 73 strains of *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, *Y. bercovieri*, and *Y. pseudotuberculosis*, we identified 34, 13, 7, 6, and 6 ETs, respectively. One ET was identified for each of the three reference strains which were placed in *Y. aldovae*, *Y. mollaretii*, and *Y. ruckeri*.

**Cluster analysis and dendrogram.** The genetic relationships among the 244 isolates and the allocation of these isolates to the species mentioned above as determined by classical methods are shown in Fig. 1.

The mean genetic diversity among the 168 isolates of *Y. enterocolitica* was 0.372. The dendrogram in Fig. 1 shows that there were two major clusters among the 68 *Y. enterocolitica* ETs; these clusters were separated by a genetic distance of 0.57. Cluster A consisted of ETs 15 through 38, and cluster B consisted of ETs 39 through 82. Cluster A was composed of 117 isolates belonging to biotypes 2, 3, and 4, which were genetically very closely related. Clusters A and B were well separated. Cluster B consisted of 51 isolates, all of which belonged to biotype 1. These results are similar to those of Caugant et al. (5), who identified two separate clusters among *Y. enterocolitica* strains.

In our collection of 168 *Y. enterocolitica* isolates, the loci of two enzymes, nucleoside phosphorylase and indophenol oxidase, were monomorphic. Among the 21 biotype 4 strains isolated from pigs, all tongue isolates and most of the meat isolates belonged to ET 23 and were identical to 43 human isolates.

ET 16 consisted of one human strain and one environmental strain. The latter was the only environmental isolate belonging to biotype 4 and serotype O:3.

The 13 *Y. enterocolitica* biotype 2 isolates belonged to ETs 21, 22, and 24, which could be distinguished only at the isocitrate dehydrogenase gene loci.

ETs 21, 22, and 24 included isolates belonging to different biotypes. In ETs 21 and 22, strains belonging to biotype 4 were clustered together with strains belonging to biotype 2 (one and four strains belonging to biotype 4 were clustered together with two and nine strains belonging to biotype 2, respectively). Among the ET 24 isolates two strains belonged to biotype 2, and one strain belonged to biotype 3. ET 22 included nine *Y. enterocolitica* biotype 2 strains isolated from patients in the region of Lausanne, Switzerland, in July and August 1988. This genetic identity led the laboratory in Lausanne to confirm the presence of a minor epidemic (9a).

Cluster B, represented by *Y. enterocolitica* biotype 1, did not overlap with cluster A. Only 5 of 44 ETs included more than one isolate. Cluster B was divided into groups b1, b2, b3, and b4. Groups b1 and b4 (ETs 39 through 54 and 77 through 82, respectively) were mainly composed of environmental strains, whereas group b2 (ETs 55 through 75) was mainly composed of human isolates. ET 74 represented the only porcine strain of *Y. enterocolitica* biotype 1. Group b3 (ET 76) represented reference strain ATCC 23715; the great genetic distance between this strain and the other *Y. enterocolitica* strains did not make it a very representative strain in our collection. Strains belonging to *Y. enterocolitica* biotype 1 could be identified by using glutamic-oxalacetic transaminase, which had just one allele in *Y. enterocolitica* biotype 1 strains.

The 13 *Y. frederiksenii* isolates formed three major clusters (Fig. 1, clusters I, II, and III). Clusters I and II were separated by a very great genetic distance (0.7). The genetic distance between these two groups and group III was even greater (more than 0.8). In fact, *Y. frederiksenii* group III was more closely related to *Y. kristensenii* than to *Y. frederiksenii* groups I and II. Interestingly, isolate 29 was classified by using classical biochemical reactions as *Y. frederiksenii*, but according to MEE data this isolate, which formed ET 83, was more closely related to *Y. enterocolitica*. The isolates of *Y. pseudotuberculosis*, as well as those of *Y. bercovieri*, were closely related to one another. The human strains of *Y. kristensenii* were genetically more closely related to one another than the environmental strains of the same species. Furthermore, ETs 94 and 95 were also more closely related to *Y. mollaretii*. ETs 14, 96, and 137 each represented the genotype of a single isolate of *Y. aldovae*, *Y. mollaretii*, and *Y. ruckeri*, respectively. Among the 39 *Y. intermedia* strains, 34 distinct ETs were identified. All but three ETs among the 39 isolates represented only one genotype. ET 130 represented the only human isolate of *Y. intermedia* and differed from the other ETs belonging to the same species at as many as nine loci.

**Genetic structure in relation to serotype and phage type.** The 244 isolates of *Yersinia* species belonged to many different serotypes. Serotype O:3 was represented only among *Y. enterocolitica* biotype 4 strains. This finding was at variance with the results of Caugant et al. (5), who found strains belonging to serotype O:3 that were members of other *Yersinia* species.

A few serotypes were found among strains belonging to different *Yersinia* species, and these strains were genetically very distantly related to one another. For example, serotype 25,35 was present in *Y. bercovieri* (ET 9), *Y. enterocolitica* (ET 45), *Y. frederiksenii* (ETs 86 and 87), and *Y. intermedia* (ET 121).

In our collection of 244 strains the following four phage types were found: VIII, X3, Xo, and Xz. In cluster A, *Y. enterocolitica* biotype 4 strains were lysed by phage type VIII, *Y. enterocolitica* biotype 2 strains were lysed by phage type X3, and *Y. enterocolitica* biotype 3 strains were lysed by a variable phage image (Xz). Cluster B was composed of 20 phage type Xo isolates and 25 phage type Xz isolates; for six cluster B isolates phage typing was not performed.

## DISCUSSION

The dendrogram revealed variable genetic distances among the different *Yersinia* species, and this result is consistent with the results of DNA hybridization studies which showed different levels of DNA homology among the species belonging to the genus *Yersinia* (1–4, 20).

Furthermore, the dendrogram revealed some noteworthy findings which are not revealed by classical identification. Wauters et al. (23) established previously that biotypes 3A and 3B actually represent two species, *Y. mollaretii* and *Y. bercovieri*, which are now differentiated on the basis of the results of an extended biochemical test procedure. Our dendrogram (Fig. 1) confirms this finding. *Y. mollaretii* (ET 96) and *Y. bercovieri* (ETs 8 through 13) are well differentiated from *Y. enterocolitica* biotype 3 (ETs 24, 31, and 32).

However, according to our dendrogram (Fig. 1) the current phenotypic species differentiation procedure does not yet completely reflect the genetic relationships among strains of this genus. For instance, strains of *Y. frederiksenii* were clustered into three major groups that were separated by great genetic distances. This result provides evidence that

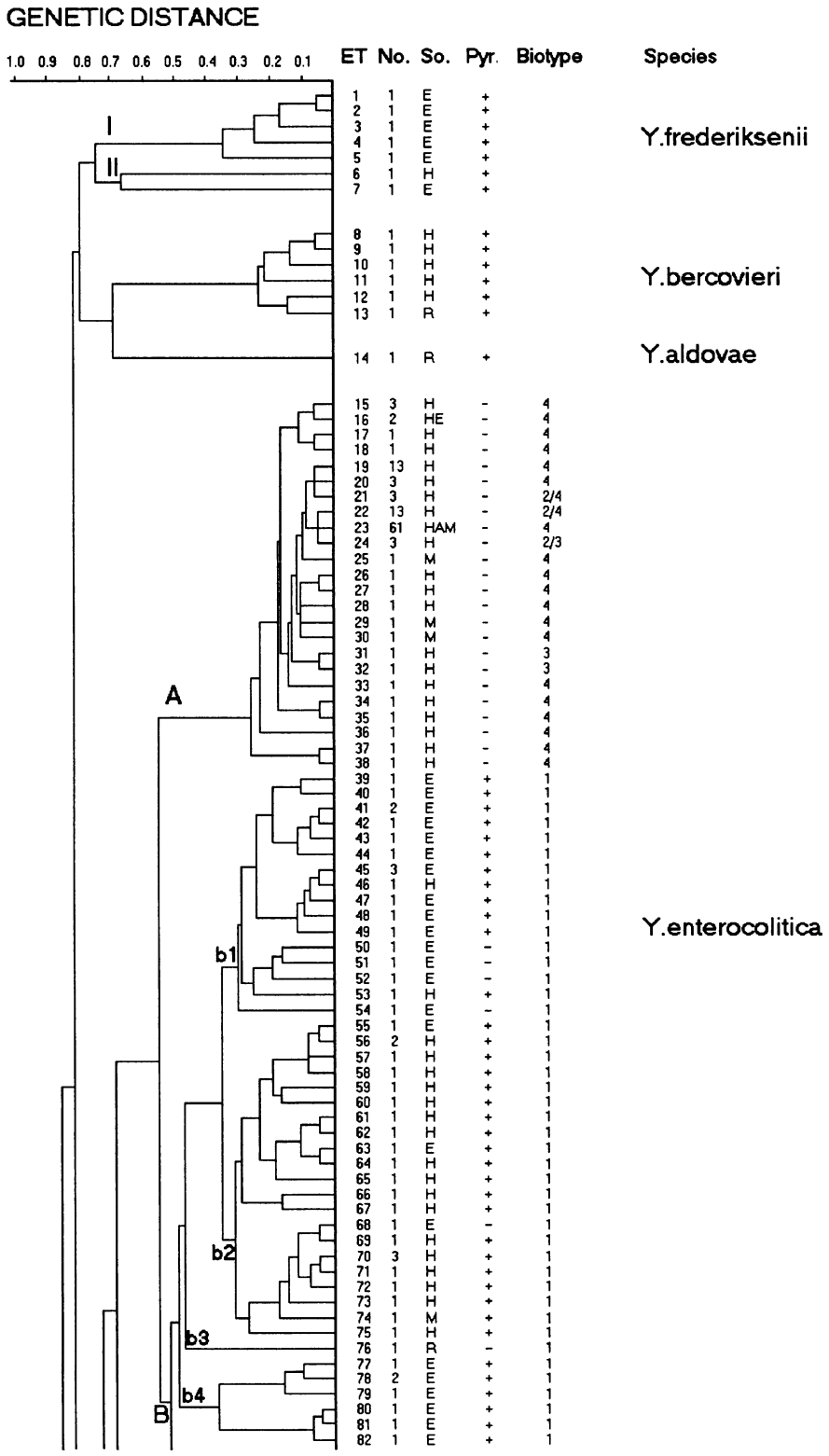


FIG. 1. Genetic relationships among 137 *Yersinia* species ETs. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distances based on 18 enzyme loci. Abbreviations: No., number of strains with the same ET; So., source of the strains (A, animal; E, environment; H, human; M, meat products; R, reference strain); Pyr., pyrazinamidase test (+, positive; −, negative).

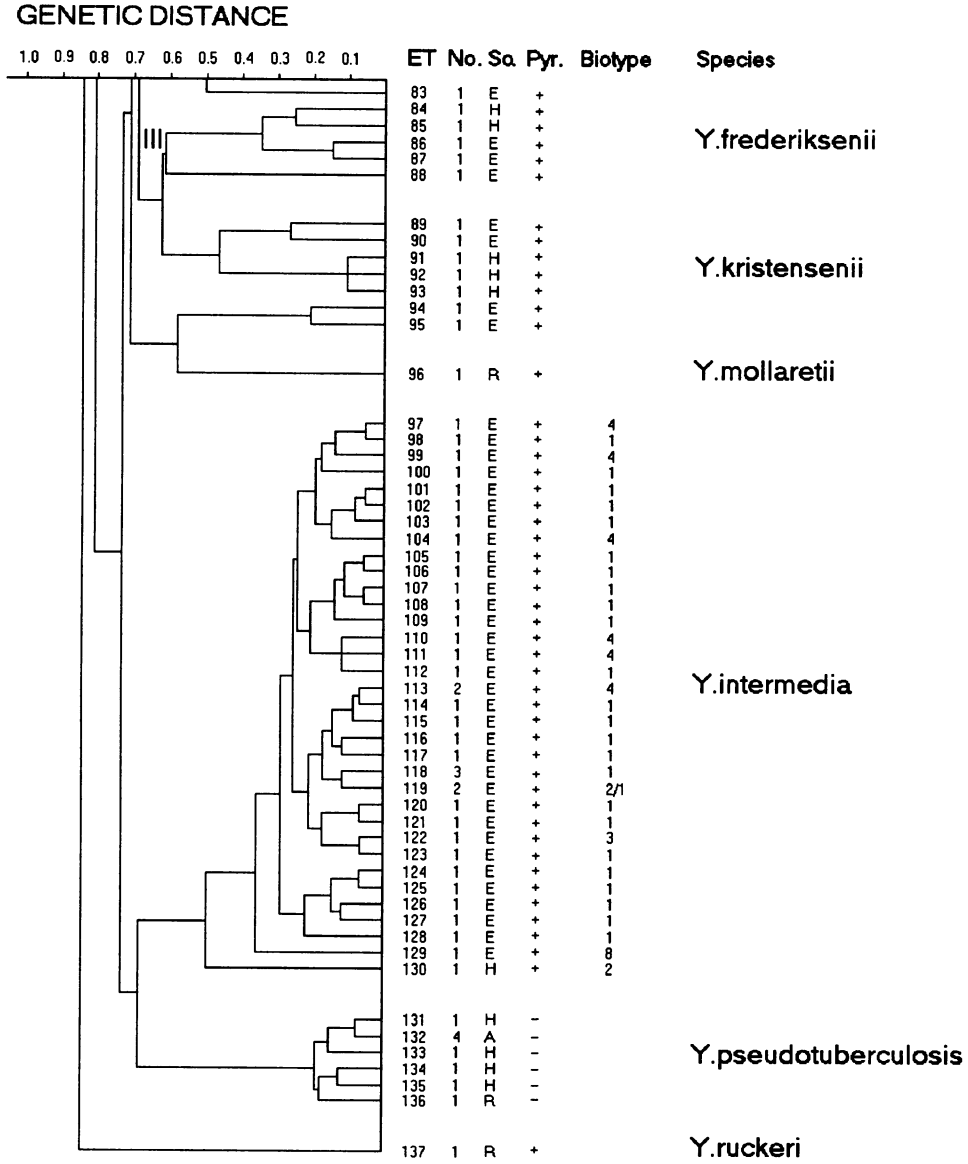


FIG. 1—*Continued.*

strains classically identified as *Y. frederiksenii* actually belong to more than one *Yersinia* species. DNA hybridization studies will be performed to confirm our assumption. The methods currently used to identify *Y. frederiksenii* should be reviewed. A similar problem of classification exists with strains of *Y. kristensenii*; two environmental isolates were genetically more closely related to *Y. mollaretii* than to other *Y. kristensenii* strains. As shown in the dendrogram (Fig. 1), strains of *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 4 obtained from animals (cattle and pigs, respectively) were genetically closely related to strains isolated from clinical material. This result shows that animal strains have to be considered potential human pathogens. This is consistent with the results of phenotype studies (7, 8, 11, 19), in which pigs were found to be healthy carriers of *Y. enterocolitica* biotype 4 and therefore possible sources of human infection. This finding is also consistent with restriction pattern studies of human and porcine isolates that showed the same plasmid digestion pattern (13). Moreover, we found that all *Y. enterocolitica* biotype 4 strains isolated from animals and humans were pyrazinamidase negative, which supports the hypothesis that pathogenic strains are transmitted from pigs to humans. All but six pyrazinamidase-negative *Y. enterocolitica* strains were grouped in cluster A, which contains mainly human and animal strains. Furthermore, there is another large group of *Y. enterocolitica* strains (group b2) which comprises mainly human strains that are not recognized by the pyrazinamidase test as potential pathogens. Environmental strains were clustered in separate groups; this fact was well documented for the *Y. enterocolitica* biotype 1 environmental strains that form groups b1 and b4 (Fig. 1). The few exceptions (i.e., ETs 46, 53, 63, and 68) may be explained by fortuitous transfer to human beings or the survival of human strains in the environment.

Among the strains isolated from the environment we found only one strain that was identical to a human strain,

(*Y. enterocolitica* biotype 4 ET 16). However, the time and the place of isolation of the two strains did not match, and therefore these organisms were not epidemiologically correlated.

ET 23 in cluster A represents as many as 61 strains of *Y. enterocolitica* that were isolated from humans, pigs, and pork and therefore had to be considered members of a very particular clone. All of these strains are biotype 4 strains, are pyrazinamidase negative, and therefore are recognized as potential pathogens. Particularly interesting is the fact that the strains belonging to this ET originated from different countries in Europe (Table 1). Therefore, ET 23 may represent a clone that is particularly adapted to humans and pigs. There are two more ETs that contain large numbers of isolates, ETs 19 and 22. The latter contains strains obtained from a small epidemic in Lausanne. Interestingly, these two ETs are closely related to ET 23. Therefore, this whole group of ETs might represent strains that exhibit high levels of pathogenicity. ET 22 contains strains belonging to *Y. enterocolitica* biotypes 4 and 2. This shows that even if these two biotypes are biochemically distinguishable genetically, they may be very closely related to one another.

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