Genotyping of Human and Porcine *Yersinia enterocolitica*, *Yersinia intermedia*, and *Yersinia bercovieri* Strains from Switzerland by Amplified Fragment Length Polymorphism Analysis

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Received 25 August 2005/Accepted 16 March 2006

In this study, 231 strains of *Yersinia enterocolitica*, 25 strains of *Y. intermedia*, and 10 strains of *Y. bercovieri* from human and porcine sources (including reference strains) were analyzed using amplified fragment length polymorphism (AFLP), a whole-genome fingerprinting method for subtyping bacterial isolates. AFLP typing distinguished the different *Yersinia* species examined. Representatives of *Y. enterocolitica* biotypes 1A, 1B, 2, 3, and 4 belonged to biotype-related AFLP clusters and were clearly distinguished from each other. *Y. enterocolitica* biotypes 2, 3, and 4 appeared to be more closely related to each other (83% similarity) than to biotypes 1A (11%) and 1B (47%). Biotype 1A strains exhibited the greatest genetic heterogeneity of the biotypes studied. The biotype 1A genotypes were distributed among four major clusters, each containing strains from both human and porcine sources, confirming the zoonotic potential of this organism. The AFLP technique is a valuable genotypic method for identification and typing of *Y. enterocolitica* and other *Yersinia* spp.

*Yersinia enterocolitica*, a member of the family Enterobacteriaceae, is a well-known food-borne pathogen. *Y. enterocolitica* is isolated more often from children with diarrhea than from adults (11, 25). In children, infection with *Y. enterocolitica* can cause gastroenteritis with mild diarrhea, abdominal pain, low fever, and pseudoappendicitis. In adults, *Y. enterocolitica* can cause pharyngitis and various systemic infections and is frequently associated with postinfection reactive arthritis than an infection with *Y. enterocolitica*. Biotype 1A strains carry chromosomally encoded virulence factors and are able to invade cultured epithelial cells by a mechanism different from the mechanism used by pYV-bearing strains (31). Furthermore, a recent study showed that with regard to virulence genes, biotype 1A strains isolated from clinical samples did not differ significantly from strains isolated from other sources (32). Moreover, since *Y. enterocolitica* is difficult to differentiate from other *Yersinia* species by routine phenotypic tests (3, 4), strains identified as *Y. enterocolitica* biotype 1A and biotype 3 may belong to related species, such as *Y. intermedia* or *Y. bercovieri* (this study). As these *Yersinia* species lack classical virulence factors, they are considered nonpathogenic. These isolates originated from either clinical samples or environmental and food samples (2, 12, 29). In *Y. bercovieri* an enterotoxin different from that in *Y. enterocolitica* has subsequently been found (30).

Pork meat is one of the potential sources of infection of humans by *Y. enterocolitica*. In Switzerland the prevalence of *Y. enterocolitica* in pork meat at the retail level was reported to be 15.4% (20). However, only 0.7% of the strains isolated belonged to the potentially pathogenic biotypes 2, 3, and 4, while the majority of the strains belonged to apparently apathogenic biotype 1A (20). At the farm level, the prevalence of *Y. enterocolitica* was 63%, and 36.8% of the strains were biotype 1A strains in 2001 (20). Thus, the importance of pork meat as a source of *Y. enterocolitica* infection in Switzerland is unclear, given the assumptions concerning the virulence spectra of the different biotypes.

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The aim of this study was to elucidate the genetic relatedness among Y. enterocolitica strains belonging to various bio-
types originating from human stool samples, swine feces, and pork meat with the amplified fragment length polymorphism
(AFLP) technique (33).

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains were collected from
swine feces (n = 41), pork meat (n = 99), and human stool samples (n = 113).
All strains from humans were isolated from individuals suffering from diarrhea
and were kindly provided by the Swiss Federal Office of Public Health. The pork
meat and swine feces isolates were obtained during an investigation of the
prevalence of latent zoonoses in pigs and pork from animal-friendly farms per-
formed by the Federal Veterinary Office (20). All isolates originated from
healthy pigs or from pork meat derived from healthy animals. They were col-
lected in various geographical areas of Switzerland during 2000 to 2002. Refer-
ence strains and collection strains are listed in Tables 1 and 2. Four Y. enter-
ocolitica biotype 1B strains were obtained from abroad as this biotype was not
represented in our sample collection.

Strains were stored by using a bacterial bead storage system (Technical Ser-
vices Consultants Limited, Lancashire, United Kingdom) at −70°C and were
cultured on Columbia agar (Biomerieux, Marcy l’Etoile, France) containing 5% sheeps
blood at 38°C for 24 h. All strains were identified with the API 20E system
(Biomerieux) and were biotyped and serotyped by using a previously published
protocol (32). Further biochemical identification was performed by the Swiss
National Center of Enteropathogens, using established procedures (7).

Isolation of chromosomal DNA. For each strain analyzed a loopful from a
fresh culture on solid medium was suspended in 200 µl of phosphate-buffered
saline (0.14 M NaCl, 0.027 M KCl, 0.10 M phosphate) (pH 7.4). DNA was
extracted with a High Pure PCR template preparation kit (Roche Diagnostics,
Mannheim, Germany) used according to the manufacturer’s protocol. The DNA
concentration was determined by agarose gel electrophoresis using a XIV 100-bp
standard. The gels were scanned and the signals were analyzed with the GelDoc
software BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium). Only DNA frag-
ments that were 80 to 450 bp long were considered in the comparisons. Briefly,
after normalization, interprofile similarities were calculated with the Pearson
product-moment correlation algorithm, and relationships were displayed in a
dendrogram based on the unweighted pair group with mathematical average
method. A threshold of 92%, as determined from the reproducibility analysis,
was used to perform the cluster analysis.

RESULTS

AFLP-based discrimination of species and Y. enterocolitica
biotypes and serotypes. A total of 266 strains of Yersinia species were
analyzed by the AFLP technique, including 231 Y. enterocolitica strains, 25 Y. intermedia strains, and 10 Y. bercovi-
eri strains. The AFLP profiles of these species comprised
between 50 and 120 bands, and the number and distribution of
these bands varied and formed the basis of the cluster analysis
(Fig. 1). At the 24% similarity level, four distinct clusters were
formed, three of which represented the species Y. intermedia, Y.
bercovieri, and Y. enterocolitica; the remaining cluster contained
three strains from human and porcine sources that were identified
initially as Y. enterocolitica biotype 1A but proved to be not sero-
typeable and yielded AFLP profiles clearly different from those of
all other Y. enterocolitica strains examined (Fig. 1).

At higher S levels, Y. enterocolitica strains formed smaller
groups that were largely concordant with their bio- or serotype
designations (Fig. 1). Strains assigned to biotype 1A were
clearly distinguished from the other biotypes examined at an S

### TABLE 1. Reference Y. enterocolitica strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Biotype</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP Ye 106</td>
<td>1A</td>
<td>O:7, O:8</td>
</tr>
<tr>
<td>IP Ye 21991</td>
<td>1A</td>
<td>O:5</td>
</tr>
<tr>
<td>IP Ye 102</td>
<td>1A</td>
<td>O:6, O:30</td>
</tr>
<tr>
<td>IP Ye 1501</td>
<td>1A</td>
<td>O:34</td>
</tr>
<tr>
<td>ATCC 23715</td>
<td>1B</td>
<td>O:8</td>
</tr>
<tr>
<td>ATCC 9262</td>
<td>1B</td>
<td>O:8</td>
</tr>
<tr>
<td>CIP 9027</td>
<td>1B</td>
<td>O:8</td>
</tr>
<tr>
<td>ATCC 55075</td>
<td>2</td>
<td>O:9</td>
</tr>
<tr>
<td>CIP 8142</td>
<td>2</td>
<td>O:9</td>
</tr>
<tr>
<td>CCTM 3247</td>
<td>3</td>
<td>O:5, O:27</td>
</tr>
<tr>
<td>IP Ye 134</td>
<td>4</td>
<td>O:3</td>
</tr>
<tr>
<td>IP Ye 1105</td>
<td>4</td>
<td>O:8</td>
</tr>
<tr>
<td>CCGU 8233</td>
<td>4</td>
<td>O:3</td>
</tr>
</tbody>
</table>

* ATCC, American Type Culture Collection; CIP, Collection de l’Institut Pasteur; IP, Institut Pasteur; CCGU, Culture Collection of the University of Göteborg, Sweden; CCTM, Centre de Collection de Types Micro-
biens, Université de Lausanne, Lausanne, Switzerland.

### TABLE 2. Sample strains used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains</th>
<th>Biotype</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. enterocolitica</td>
<td>46</td>
<td>1A</td>
<td>NT or diverse</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>4</td>
<td>1B</td>
<td>O:8</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>26</td>
<td>2</td>
<td>O:9</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>2</td>
<td>3</td>
<td>O:5</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>2</td>
<td>3</td>
<td>O:3</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>29</td>
<td>7</td>
<td>O:3</td>
</tr>
<tr>
<td>Y. intermedia</td>
<td>14</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Y. bercovieri</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* NT, not typeable.
* All biotype 1B strains were obtained from countries other than Switzerland.
level of 37%, and the biotype 1A strains were very heterogeneous and were distributed in four clusters. The biotype 1B strains were clearly distinguished at an S level of 47% from the other virulent biotypes, biotypes 2, 3, and 4. At S levels ranging from 83 to 87% strains representing bioserotypes 2/O:9, 3/O:3, and 4/O:3 clustered together; bioserotype 2/O:5 isolates were distributed in two clusters. Most of the 13 reference strains or collection strains were in the corresponding biotype clusters; the only exception was the bioserotype 3/O:5,O:27 strain, which clustered according to its serotype. A correlation with the source of the isolate and AFLP clustering was less apparent. Cluster D of biotype 2 contained only strains from porcine feces, but cluster C, also comprising biotype 2 isolates, contained strains from both porcine and human sources.

The 92% cutoff value was derived from the reproducibility analysis, and the data clearly indicated that matching biotypes and serotypes could be further differentiated by the AFLP results. This is shown in Fig. 2, which shows the AFLP profiles.
of all biotype 1A strains and the types formed at the 92% S level. All biotype 1A serotype O:5 strains were from human sources and represented the same AFLP type. Notably, four other AFLP types contained human isolates that were genetically indistinguishable from pork meat isolates.

**DISCUSSION**

Our study showed that AFLP is a valuable method for species identification and epidemiological typing of *Yersinia* species at the molecular-genetic level. This was shown previously for other bacteria (1, 13, 18, 23), including *Yersinia* spp., as recently shown by Fearnley et al. (14). In our study we found that phenotypic identification of *Yersinia* spp. is problematic; all strains and isolates studied here were initially identified as *Y. enterocolitica* by an API 20E commercial identification kit. However, AFLP analysis clearly placed the species *Y. enterocolitica, Y. bercovieri,* and *Y. intermedia* in separate clusters. A subsequent thorough phenotypic analysis using specialized reference methods at the Swiss National Center for Enteropathogens confirmed the three species shown in Fig. 1. The taxonomic position of another group of *Yersinia* spp. that resembled *Y. enterocolitica* but had an AFLP type that was clearly distinct from that of *Y. enterocolitica* and the other known species examined requires further study. The *Y. intermedia* isolates used in this study were recovered only from pork meat, whereas the *Y. bercovieri* isolates were obtained only from human diarrhea samples. The method used for isolation of *Yersinia* spp. from pork meat involved the use of cefsulodin-irgasan-novobiocin agar, which has been reported to inhibit the growth of *Y. bercovieri,* and this may account for the absence of this species in porcine material. Alternatively, pigs may not be a host for *Y. bercovieri* (12).

As recently shown by Fearnley et al., the notable correlations between biotype, serotype, and AFLP type of *Y. enterocolitica* strains are consistent with the clonal nature of this species (14). In particular, the close relationship of *Y. enterocolitica* bioserotype 3/O:3 and bioserotype 2/O:9 demonstrated in this study has also been shown by using ribotype analysis (17). However, in the AFLP study of Fearnley et al. (14), a distinction was found only at the serotype level. This could have been due to the difficulty of identifying biotype 2, which differs from biotype 3 only in indole production. Moreover, the *Y. enterocolitica* bioserotype 2/O:5 strains form two distinct AFLP clusters. Both of these clusters are more closely related to *Y. enterocolitica* biotype 4 than to biotype 2, serotype O:9 strains. This is in agreement with the results of other genetic studies (17). For other enteric bacteria, AFLP results have been found to correlate with the results of multilocus sequence typing, an established tool for investigation of genetic population structures (21, 27). Our data suggest that AFLP analysis may be useful for inferring clonal relationships of *Y. enterocolitica*.

The data obtained in this study also show the high discriminatory potential of the AFLP technique for genotyping *Yersinia* spp., and 149 genotypes were defined by use of the cutoff for reproducibility, a common way by which strains are distinguished using this approach. In agreement with the results of Fearnley et al. (14), *Y. enterocolitica* biotypes 2, 3, and 4 are closely related; nonetheless, we found 10 AFLP types among the 83 strains examined. This compares favorably with the results of multilocus enzyme electrophoresis (12) and pulsed-field gel electrophoretic (PFGE) studies (9).

Strains of *Y. enterocolitica* bioserotype 4/O:3 are typically considered the most common enteropathogenic type in this species (6, 19, 28). The closely related strains of *Y. enterocolitica* bioserotype 4/O:3 from human and porcine sources confirmed the importance of swine as a reservoir for pathogenic *Y. enterocolitica,* as also shown by PFGE (15). However, bioserotype 4/O:3 was not isolated from pork meat obtained at the retail level in Switzerland. Therefore, pork meat was not considered an important source of infection (20), a conclusion which contradicts the findings of a recent study in Finland (15). In our study, we found porcine strains of *Y. enterocolitica* biotype 1A that were genetically indistinguishable from isolates from human sources. This observation supports the hypothesis that pigs and pork products are likely sources of human infection in Switzerland and that *Y. enterocolitica* bioserotypes other than the classical pathogenic type 4/O:3 are potential causes of food-borne yersiniosis.

*Y. enterocolitica* biotype 1A strains appeared to be particularly diverse as determined by AFLP analysis, an observation in agreement with ribotyping, multilocus enzyme electrophoresis, and PFGE typing data (10, 12, 17, 22). The high percentage of biotype 1A strains recovered from porcine samples could have been due to the cold enrichment procedure used for isolation of the porcine samples, which increased the likelihood of detecting *Y. enterocolitica* biotype 1A strains (31). Furthermore, the low storage temperatures used for meat could facilitate the growth of biotype 1A strains. One AFLP-based cluster comprised only *Y. enterocolitica* bioserotype 2/O:5 strains from porcine samples. We hypothesized that these strains might be less virulent. However, the remaining cluster of this bioserotype comprised both human and porcine isolates, indicating that bioserotype alone is not reliable as a predictive marker of pathogenic potential.

In conclusion, AFLP was found to be a reliable method for identification and epidemiological subtyping of *Yersinia* spp. A correlation with phenotypic markers was observed, indicating a clonal relationship. The results demonstrated that several indistinguishable genotypes were obtained from human diarrhea and porcine material and suggested that pigs and their products are a source of human infections in Switzerland, as demonstrated previously for pigs and sheep in England and Wales.

**FIG. 2.** Dendrogram and AFLP banding patterns of 131 pork and human samples of *Y. enterocolitica* biotype 1A. The arrows indicate isolates from human sources. The stars indicate reference strains. The black lines indicate a similarity level of ≥92%. There are four major clusters, clusters G, H, I, and K. Six clusters of *Y. enterocolitica* biotype 1A strains from human and porcine sources exhibiting levels of similarity of ≥90% are highlighted by gray boxes in the banding pattern. The cluster containing biotype 1A, serotype O:5 strains is highlighted with a rectangle. The other serotypes are not indicated since the serotypes of several biotype 1A isolates could not be determined.
(14). The indistinguishable genotypes are distributed among several bioserotypes of *Y. enterocolitica*.

**ACKNOWLEDGMENTS**

This study was supported by the Swiss Federal Veterinary Office. We thank Denise Howald, Elisabeth Luethi, and Christian Kohler of the Federal Veterinary Office and Herbert Haechler and Grethe Saergesser of the Swiss National Centre of Enteropathogenic Bacteria (NENT) for excellent technical support. We also thank Marianne Kueffer, Swiss Federal Office of Public Health, for collecting human isolates of *Yersinia*. In the context of a case-control study, these strains were kindly made available by D. Buhl of the Institute für klinische Mikrobiologie und Immunologie, St. Gallen, Switzerland, O. Dubuis, P. Friedrich, and F. Mueller of Viölliger AG, Basel, Switzerland, B. Lowsky of Enzymelabor Dr. H. Weber AG, St. Gallen, Switzerland, and R. Zbinden of the Medical Microbiology, University of Zurich, Switzerland.

**REFERENCES**