The plasmid profile of *Yersinia ruckeri*, the causative agent of redmouth disease in salmonid fish (4, 15), has been described previously (3, 19; T. M. Cook and P. Gemski, Abstr. XIIIth Int. Congr. Microbiol., 1982, p. 97). These investigators found a high-molecular-mass (40- to 50-megadalton [MDa]) plasmid in some serovar I strains and only small (1- to 5.5-MDa) plasmids among the serovar II strains.

Lesel et al. (8) have established the existence of redmouth disease in fish farms in France. The presence of a plasmid has never been shown in French *Y. ruckeri* strains. In this study, we proved the presence of a plasmid and compared the plasmid profile and *BamHI* restriction pattern of 18 French *Y. ruckeri* strains with those of the American type strain. Southern hybridization of a *Y. ruckeri* plasmid with the other isolated *Y. ruckeri* plasmids was carried out to determine the level of DNA homology among these plasmids.

The 18 French *Y. ruckeri* strains (Table 1) that were used in this study were isolated from various outbreaks of redmouth disease in rainbow trout (8). Biochemical and morphological testing of all isolates was carried out as described previously (1, 10, 18). From the information presented by De Grandis et al. (2), the sorbitol-positive strains belong to serovars II and V. Although the serovars of our strains were not determined, the ability of strain LMSP 160036 to ferment sorbitol led to the conclusion that this strain belongs to a serovar different from those of the other 17 sorbitol-negative strains. The American strain (ATCC 29473; American Type Culture Collection, Rockville, Md.) is a virulent, sorbitol-negative Hagerman serovar I strain.

First, we looked at the plasmid profiles of the 18 French strains and the American strain. Bacteria were grown in brain heart infusion broth at 25°C for 24 to 48 h. Plasmids were extracted by the method of Portnoy et al. (12) and subjected to agarose gel electrophoresis as described by Maniatis et al. (9). The American strain and the 18 French strains harbored a plasmid with a similar molecular mass (62 MDa) (Fig. 1). Furthermore, the sorbitol-positive strain had three additional plasmid bands of 70, 32, and 25 MDa (Fig. 1, lane 1). Some of these bands may represent open circular forms of plasmid DNA (20). Molecular masses were calculated from the relative positions of the 47- and 60-MDa plasmids of *Y. pestis* and confirmed with another 60-MDa plasmid from *Y. pseudotuberculosis* (16) (data not shown).

Second, we wanted to compare these plasmids according to their *BamHI* restriction patterns. The plasmids were obtained as described above and restricted with the *BamHI* endonuclease under the conditions recommended by the supplier (Amersham Laboratories, Buckinghamshire, England). The plasmids of all the sorbitol-negative French and American strains exhibited the same restriction profile (Fig. 2A). The *BamHI*-cleaved plasmids of strain LMSP 160036...
TABLE 1. Yersinia strains used in this study

<table>
<thead>
<tr>
<th>Strain (serotype)*</th>
<th>Source</th>
<th>Origin</th>
<th>Sorbitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. ruckeri</td>
<td>LMSP 160005. . . .</td>
<td>Salmo gairdneri</td>
<td>France</td>
</tr>
<tr>
<td></td>
<td>LMSP 160036</td>
<td>Salmo gairdneri</td>
<td>France</td>
</tr>
<tr>
<td></td>
<td>ATCC 29473</td>
<td>Salmo gairdneri</td>
<td>Idaho</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>IP383 (O:9)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>IP383b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WA (O:8)</td>
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<tr>
<td></td>
<td>IP864 (O:3)</td>
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<tr>
<td></td>
<td>8081 (O:8)</td>
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</tr>
<tr>
<td></td>
<td>9576 (O:Tacoma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>IP2637 (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IP2637c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y. pestis</td>
<td>6/69M</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/69Mc</td>
<td></td>
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</tr>
</tbody>
</table>

* Abbreviations: LMSP, Laboratoire des Microorganismes Collection, Saint-Pée, France; ATCC, American Type Culture Collection, Rockville, Md.; CNY, Centre National des Yersinia (Institut Pasteur) Collection, Paris, France.

** This strain was cured of the virulence plasmid by selection on magnesium oxalate agar at 37°C.

had a different pattern; none of its restriction fragments comigrated with a fragment of the restricted 62-MDa plasmid from the sorbitol-negative strains (Fig. 3A, lane 5). These results indicate that the 62-MDa plasmid from the American type strain and the 17 French sorbitol-negative Y. ruckeri strains share the same restriction pattern. This pattern was different from the BamHI plasmid profile of the sorbitol-positive Y. ruckeri strain.

Third, to study the homology between the 62-MDa plasmids of the Y. ruckeri strains, Southern hybridization was carried out. Electrophoresed BamHI-digested plasmid DNA of all the Y. ruckeri strains was partially depurinated by washing the gel with 0.25 M HCl for 15 min at room temperature (7) and was transferred to a nitrocellulose filter, as described previously (17). BamHI-restricted bacteriophage λ DNA was included as a negative control. The plasmid DNA from Y. ruckeri LMSP 160005, which was purified by ultracentrifugation in a CsCl-ethidium bromide density gradient (7), was labeled in vivo with 35S-labeled deoxynucleotides (Amersham Laboratories, Buckinghamshire, England) by nick translation (to a specific activity of 1.107 cpm/μg of DNA). Hybridization of the nitrocellulose filter with the radiolabeled probe was performed as described by Quilici et al. (14). The LMSP 160005 probe recognized all the BamHI fragments of the 62-MDa plasmids from all the French and American sorbitol-negative strains, but only a weak recognition occurred with the DNA from the restricted LMSP 160036 plasmids (Fig. 2B). These results

![Image of Southern hybridization results](http://aem.asm.org/content/54/10/2595.full.html)

FIG. 2. (A) BamHI restriction patterns obtained after electrophoresis of Y. ruckeri strains in 0.7% agarose. Lanes: 1, LMSP 160005; 2, LMSP 160006; 3, ATCC 29473; 4, LMSP 160043; 5, LMSP 160046; 6, LMSP 160050; 7, LMSP 160054; 8, LMSP 160055; 9, LMSP 160057; 10, LMSP 160060; 11, LMSP 160065; 12, LMSP 160066; 13, LMSP 160069; 14, LMSP 160070; 15, LMSP 160071; 16, LMSP 160072; 17, LMSP 160073; 18, LMSP 160074; 19, LMSP 160036; 20, λ phage DNA. (B) Autoradiograph of Southern hybridization of the filter with 35S-labeled plasmid DNA from Y. ruckeri LMSP 160005. Lanes are as described above for panel A.
confirmed the DNA homology between the 62-MDa plasmid of the American strain and the 62-MDa plasmid of the French sorbitol-negative strains.

In the genus Yersinia, the virulence of Y. pestis, Y. pseudotuberculosis, and Y. enterocolitica is associated with the presence of a 42- to 47-MDa plasmid (5, 6, 11, 21). Portnoy et al. (13) have suggested that this plasmid is derived from a common ancestor. In this study, we tried to establish the relatedness between the 42- to 47-MDa plasmid and the Y. ruckeri plasmids by comparing their BamHI restriction patterns and by hybridizing some Y. ruckeri plasmids with the 47-MDa plasmid of Y. enterocolitica serotype O:9, which was used as a probe. The plasmid profile of our Y. ruckeri strains was compared with the patterns of Y. pestis, Y. pseudotuberculosis, and Y. enterocolitica (Table 1). After electrophoresis of the plasmids, none of the Y. ruckeri plasmids comigrated with the 42- to 47-MDa plasmid (Fig. 1). To study them further, the Y. ruckeri and other yersinia plasmids were cleaved with the BamHI endonuclease, as described above. The pattern of the 42- to 47-MDa plasmid of the pathogenic Yersinia strains shared only one restriction band with the profile of the 62-MDa plasmid (data not shown). The plasmid restriction patterns of Y. enterocolitica IP383, serotype O:9, three sorbitol-negative Y. ruckeri strains, and strain LMSP 160036 are shown in Fig. 3A. To establish the DNA homology between the plasmids of Y. ruckeri and those of other members of the tribe Yersiniaeae, Southern hybridization was done. The hybridization of restricted Y. ruckeri plasmids with the plasmid probe IP383 showed a complete absence of recognition of the sequences by this probe (Fig. 3B). These results confirm that the plasmid of Y. ruckeri is completely different from the plasmid of the virulent members of the genus Yersinia.

The data reported here demonstrate the presence of a common 62-MDa plasmid in all the French sorbitol-negative Y. ruckeri strains and the American Hagerman type strain studied. The only sorbitol-positive strain, which harbored a different plasmid profile and restriction pattern, shared little sequence homology with this common plasmid. The absence of DNA homology between the 62-MDa plasmid and the common yersinia 42- to 47-MDa plasmid suggests a different plasmid origin. De Grandis et al. (2) have described the difference in chromosomal DNA relatedness and biochemical reactions between Y. ruckeri and the other Yersinia species. These results and our observations seem to confirm the conclusion of these investigators that the taxonomic classification of these bacteria needs to be modified.

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LITERATURE CITED


