Environmental Occurrence of the Whipple’s Disease Bacterium
(Tropheryma whippelii)
MATTHIAS MAIWALD,1* FRANK SCHUHMACHER,1 HANS-JÜRGEN DITTON,1 AND AXEL VON HERBAY2

Hygiene-Institut der Universität, Abteilung Hygiene und Medizinische Mikrobiologie,1 and Pathologisches Institut der Universität,2 69120 Heidelberg, Germany

Received 8 September 1997/Accepted 5 November 1997

Whipple’s disease is a systemic disorder in which a gram-positive rod-shaped bacterium is constantly present in infected tissues. After numerous unsuccessful attempts to culture this bacterium, it was eventually characterized by 16S rRNA gene analysis to be a member of the actinomycetes. The name *Tropheryma whippelii* was proposed. Until now, the bacterium has only been found in infected human tissues, but there is no evidence for human-to-human transmission. Here we report the detection of DNA specific for the Whipple's disease bacterium in 25 of 38 wastewater samples from five different sewage treatment plants in the area of Heidelberg, Germany. These findings provide the first evidence that *T. whippelii* occurs in the environment, within a polymicrobial community. This is in accordance with the phylogenetic relationship of this bacterium as well as with known epidemiological aspects of Whipple’s disease. Our data argue for an environmental source for infection with the Whipple's disease bacterium.

Whipple’s disease, which was first described in 1907 as intestinal lipodystrophy (18), is a multisystem disorder characterized by the presence of gram-positive, rod-shaped bacteria in infected human tissues (2). Numerous attempts to culture the bacterium associated with Whipple’s disease have failed (2), and eventually its phylogenetic position within the actinomycetes was established by 16S rRNA gene analysis (11, 19). The name *Tropheryma whippelii* was proposed (11).

The clinical presentation of Whipple’s disease is heterogeneous. Frequently, patients suffer for years from arthralgias, chronic diarrhea, and weight loss, and less often from dementia or cardiac insufficiency. If untreated, the disease is usually fatal, but with appropriate antibiotic therapy the prognosis is favorable (2). However, the pathogenesis of Whipple’s disease appears to involve more than just an infection with *T. whippelii*. Immunological abnormalities are presumed to play a necessary role as predisposing factors (2, 7, 8), a view which is strengthened by the detection of *T. whippelii* in association with AIDS (5).

Two outstanding questions in the epidemiology of Whipple’s disease are the bacterium’s natural habitat and the route of infection. Until now, *T. whippelii* has never been found outside of infected human hosts, and although an oral route of infection has often been suspected (10), it has not been proven. There is no evidence for human-to-human transmission.

A reassessment of its phylogeny revealed a close relationship of the Whipple’s disease bacterium to typical environmental bacteria, such as the cellulomonads and the rare group B peptidoglycan organisms (6). This would support the hypothesis that *T. whippelii* may be a soil or water inhabitant. This may also explain the difficulties involved with its culture, as an estimated 80 to 99% of bacteria occurring in such natural environments are not culturable on artificial media (14, 17) and uncultured members of the actinomycete line of descent have been found in different environments and geographical locations (12). Soil and water bacteria tend to concentrate in sewage to form communities in which a large variety of different species are found. Therefore, the search for the Whipple’s disease bacterium was concentrated at sewage treatment plants.

From September 1995 to July 1996, a total of 38 effluent samples from the sedimentation ponds of five different sewage treatment plants were examined by PCR for the presence of the Whipple’s disease bacterium. The sewage treatment plants are located within a distance of 5 to 25 km from Heidelberg, Germany (Fig. 1). Effluent was sampled in 1,000-ml single-use plastic flasks and filtered as described previously (4) in portions of 25 ml through cellulose acetate prefilters (25-mm diameter, 5-μm pore size; Sartorius, Göttingen, Germany). It was then passed through polyvinylidened fluoride filters (25-mm diameter, 0.45-μm pore size; Millipore, Bedford, Mass.). The DNA from the 0.45-μm-pore-size filters was extracted with the EnvironAmp Legionella sample preparation kit (Perkin-Elmer, Norwalk, Conn.), with the addition of 0.2% bovine serum albumin to the water in the final extraction step. Distilled water for negative controls was sampled in the same flasks and treated in the same way as the sewage.

PCR amplification and detection were performed as described in detail elsewhere (15). Primers whip1 (5′-AGAGATACGCCCCCGCAA) and whip2 (5′-ATTCCGTCCACCCCTTGCCA), which amplify a fragment of 267 bp from the 16S rRNA gene of the bacterium, were used. In addition to hybridization with oligonucleotide whip3 (5′-TGGTACAGAGGGTGCAATA), a second hybridization in a separate reaction was performed with oligonucleotide whip4 (5′-GTAATGCGGGGCGACTCAG). The specificities of primers whip1 and whip2 and of probe whip3 have been tested previously (15), and testing of probe whip4 with the same set of 37 control bacteria gave the same results as those reported for whip3 (15). Of the 38 sewage samples tested, 25 were found to be positive by PCR and hybridization with both oligonucleotides (Table 1). Positive samples were found at each of the five sewage treatment plants. Most of the positive PCR products displayed relatively weak bands at the expected size of 267 bp. They also contained some extraneous bands, which were not of the ex-
expected size, probably due to the large amount of DNA from other organisms present in sewage. On hybridization, however, the PCR products displayed distinct bands, which were consistent between the two oligonucleotides (Fig. 2).

To further compare the sequences of the PCR products with the known sequence of the Whipple’s disease bacterium, nine PCR products selected from all five sewage treatment plants were cloned and sequenced. The PCR products were reamplified over 5 cycles by using specific primers with BamHI and EcoRI restriction endonuclease recognition sites (11), ligated into vector pDS1-NOC (3) and transformed by electroporation into Escherichia coli JM101. To screen for positive clones, a series of PCRs (primer whip2 and vector-specific primer 5\'-TGTCTTTGTAAGCGATAACAATTAT) in which pools of 10 E. coli colonies were amplified in each of the reactions was performed. The PCR products were tested by gel electrophoresis, Southern blotting, and hybridization with oligonucleotide whip4. Pools which produced hybridizing bands of the appropriate size were retested to find individual colonies. Plasmids from positive clones were extracted according to standard methods (13), and both strands of the inserts were sequenced manually with the AmpliCycle sequencing kit (Perkin-Elmer), with incorporation of [α-33P]dATP. For each of the nine sewage samples, the sequence of the PCR product was identical to the known sequence determined previously for the Whipple’s disease bacterium (5, 9).

This is the first documented encounter with the Whipple’s disease bacterium outside of the human body. The specific sequence was found in all of the sewage treatment plants from which samples were taken, indicating that the bacterium is a regular member of, and most likely is able to multiply in, such polymicrobial communities. This finding is in agreement with one previous report which found sequences highly similar to, but not identical with, those of the Whipple’s disease bacterium in the eutrophic water of a Mediterranean coastal lagoon (1).

The environmental occurrence of the Whipple’s disease bacterium at the sewage treatment plants is in agreement with the evolutionary relationships of the bacterium (6, 11). It is also consistent with the lack of geographical preference of reported cases of Whipple’s disease in Germany and with the relatively constant incidence of new cases per year (16). In addition, it is consistent with a predominance of outdoor professions among patients with the disease (2). These features and the absence of the Whipple’s disease bacterium in normal human tissues (11, 15) make it highly unlikely that this bacterium is a common

### TABLE 1. Results of PCR for the Whipple’s disease bacterium from sewage samples

<table>
<thead>
<tr>
<th>Sewage treatment plant</th>
<th>No. of samples Tested</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heidelberg</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Untere Hardt</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mannheim</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Kohlhof</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Grenzhof</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>25</td>
</tr>
</tbody>
</table>
commensal which is transferred between warm-blooded hosts in the same way as a variety of other pathogens. However, the complete range of occurrences in the environment and the exact localization of the habitats of the Whipple’s disease bacterium still remain to be determined.

Nucleotide sequence accession numbers. The GenBank/EMBL accession numbers of the published 16S rRNA gene sequences of the Whipple’s disease bacterium are M77832 (19), M87484 (11), and X99636 (6).

This work was supported by the Deutsche Forschungsgemeinschaft (grant Ma 1663/2-1).

We thank T. N. Petney (Heidelberg, Germany) for helpful comments on the manuscript and E. Fuchs (Heidelberg, Germany) for providing vector pDS1-NOC.

REFERENCES