The Phylogeny of the Genera Chryseomonas, Flavimonas, and Pseudomonas. Supports Synonymy of These Three Genera

YOJIRO ANZAI,* YUKO KUDO, and HIROSHI OYAIZU

Nippon Roche Research Center, Kamakura, Kanagawa 247, and Department of Applied Biological Chemistry, Graduate School of Agriculture and Agricultural Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

The 16S rRNA sequences of Chryseomonas luteola, the type species of the genus Chryseomonas, and Flavimonas oryzihabitans, the type species of the genus Flavimonas, were determined. These sequences were compared with the sequences of 27 representative strains of the genus Pseudomonas. C. luteola and F. oryzihabitans were located in the cluster that contains Pseudomonas aeruginosa, the type species of genus Pseudomonas Migula 1894, and the levels of 16S rRNA sequence homology between P. aeruginosa and the other two species were more than 93.9%. All of the strains of the genus Pseudomonas sensu stricto whose sequences have been determined were included in the P. aeruginosa cluster. These results suggested that Chryseomonas, Flavimonas, and Pseudomonas are synonymous, and we concluded that Chryseomonas and Flavimonas are junior subjective synonyms of Pseudomonas.

Chryseomonas polytricha was established by Holmes et al. in 1986 for members of group Ve-2 (3). However, C. polytricha appeared to be a junior subjective synonym of Pseudomonas luteola Kodama et al. 1985 (6), and in 1987 Holmes et al. established a new combination, Chryseomonas luteola, because of this synonymous taxonomic status (4). Pseudomonas oryzihabitans was established by Kodama et al. in 1985 (6). Holmes et al. proposed that P. oryzihabitans should be transferred to a new genus, the genus Flavimonas, in 1987 and established a new combination, Flavimonas oryzihabitans (4). Holmes et al. recognized that the genera Chryseomonas and Flavimonas are close relatives of the genus Pseudomonas. The establishment of the genera Chryseomonas and Flavimonas by Holmes et al. in 1987 was grounded on low levels of DNA-DNA homology between Chryseomonas and Flavimonas strains and Pseudomonas strains. However, DNA-DNA homology has been used to distinguish bacterial species and has never been powerful enough to reveal phylogenetic relationships. In recent years, it has been recognized that phylogenetic analysis based on 16S rRNA sequences is necessary to draw boundaries between bacterial taxa.

In the present study the phylogenetic relationships among the genera Pseudomonas, Chryseomonas, and Flavimonas were revealed based on the almost complete 16S rRNA sequences of 24 selected Pseudomonas strains and the type strains of C. luteola (the type species of the genus Chryseomonas) and P. oryzihabitans (the type species of the genus Flavimonas). Based on the phylogenetic analysis, we concluded that Chryseomonas and Flavimonas are junior subjective synonyms of Pseudomonas.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study are shown in Table 1. For the sequencing study, these strains were cultured in nutrient broth (Difco) for 18 to 24 h at 30°C.

Sequencing of 16S rRNA. Total DNA was extracted by the phenol method from cells cultured in liquid medium by using sodium dodecyl sulfate followed by RNase treatment. The 16S rRNA-encoding region of the DNA was amplified from the total DNA by using Taq DNA polymerase (Boehringer, Mannheim, Germany) and two primers that attached to positions 10 to 25 (5'-TGAATTCGTATCTGCTGTC-3') and 1020 to 1036 (5'-CAGGACTACCAGGGTATCTAATGCGC-3'); complementary to positions 1031 to 1057, 1100 to 1126, and 1217 to 1243 of the nucleotide sequence (GenBank accession number X00684), Pseudomonas fluorescens NCIB 10630 (U01916), and Pseudomonas mendocina ATCC 25411 (M95134) were obtained from the EMBL database for comparison. The genetic distances between sequences were estimated by using K$_{2+1}$ values (5). Then a phylogenetic tree was constructed by the neighbor-joining method (12), an evaluation of the tree was carried out by using the bootstrap method and the Clustal V program, and a total of 1,000 bootstrapped trees were generated (1, 2). Deleted and unknown positions were eliminated for the construction of the tree. Positions (E. coli numbering) 70 to 100, 181 to 219, 447 to 487, 1004 to 1036, 1133 to 1141, and 1446 to 1456 were eliminated from the comparison because the secondary structures of these regions differed between strains.

RESULTS AND DISCUSSION

The genus Pseudomonas Migula 1894 was described so that it included polarly flagellated strictly aerobic rods with a respi-
TABLE 1. Bacterial strains examined and their 16S rRNA accession numbers and the sequencing methods used

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain no.</th>
<th>Accession no.</th>
<th>Method used for sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chryseomonas luteola</td>
<td>IAM 13000T</td>
<td>D84002</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas agarici</td>
<td>ATCC 25941</td>
<td>D84005</td>
<td>Direct</td>
</tr>
<tr>
<td>Pseudomonas chlororaphis</td>
<td>IAM 12353T</td>
<td>D84008</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas comigata</td>
<td>ATCC 29756</td>
<td>D84012</td>
<td>Direct</td>
</tr>
<tr>
<td>Pseudomonas fulva</td>
<td>IAM 12022T</td>
<td>D84013</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas glycinea</td>
<td>IAM 12402T</td>
<td>D84014</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas iraminosa</td>
<td>IAM 1529T</td>
<td>D84015</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas olendorovae</td>
<td>IAM 1058T</td>
<td>D84018</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas pavonaceae</td>
<td>IAM 1155</td>
<td>D84019</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>IAM 1236T</td>
<td>D84020</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas stramines</td>
<td>IAM 1589T</td>
<td>D84023</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas stutzeri</td>
<td>IAM 12668T</td>
<td>D84024</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas syruga</td>
<td>IAM 12356T</td>
<td>D84025</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas taenorensis</td>
<td>IAM 1653T</td>
<td>D84027</td>
<td>Cloning</td>
</tr>
<tr>
<td>Pseudomonas tolasii</td>
<td>ATCC 36187T</td>
<td>D84028</td>
<td>Cloning</td>
</tr>
</tbody>
</table>

* IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; ATCC, American Type Culture Collection, Rockville, Md.; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom.

Anaty type of metabolism in which oxygen is used. Defined in this way, the genus was very heterogeneous, and several subgroups of species have been recognized, including five subgroups based on rRNA homology data (rRNA groups I to V) (9) and groups of species based on cellular fatty acid compositions and quinone systems (groups 1 to 9) (8). There was a good correlation between rRNA groups I to V and groups 1 to 9 based on cellular fatty acids and quinone systems. In the past two decades Pseudomonas species belonging to rRNA groups II to V or fatty acid and quinone groups 2 to 9 have been transferred to other genera (10, 13, 14, 16, 17, 19, 20). At this time, the genus Pseudomonas is restricted to Palleroni rRNA group I, and only the group I strains belong to the genus Pseudomonas sensu stricto. Therefore, in the present study only the strains belonging to Palleroni rRNA group I were used for phylogenetic analyses.

Several subgroups could be differentiated in Palleroni rRNA group I. P. aeruginosa and Pseudomonas fluorescens were placed in different clusters as a result of oligonucleotide cataloging of 16S rRNA (18). Based on the results of rRNA-DNA hybridization, rRNA group I was divided into three groups, whose representative species were P. aeruginosa, P. fluorescens, and Pseudomonas syringae (5). In this study, the almost complete 16S rRNA sequences of 27 strains of the genus Pseudomonas were determined, and a phylogenetic analysis was carried out. A phylogenetic tree was drawn on the basis of these new sequences and four sequences obtained from the database (Fig. 1). The total number of nucleotides compared was 1,073. This tree had two main clusters, although the bootstrap values for two branches were not high enough (66 and 67%) to give a high level of confidence. The first cluster contained 16 strains and included P. aeruginosa DSM 50071T, Pseudomonas alcaligenes IAM 12411T, P. mendocina ATCC 25411T, and NCIB 10541, Pseudomonas stutzeri IAM 12668T, and Pseudomonas putida IAM 1236T; all of these strains except P. putida IAM 1236T belonged to the P. aeruginosa subgroup of Palleroni rRNA group I. The second cluster contained 14 strains and included Pseudomonas agarti ATCC 25941T, Pseudomonas chlororaphis IAM 12354T, Pseudomonas aureofaciens IAM 12353T, P. syringae ATCC 19310T, and P. fluorescens IAM 12022T, which belonged to the P. fluorescens or P. syringae subgroup of Palleroni rRNA group I. The results of this phylogenetic analysis based on 16S rRNA sequences were compared with the results obtained by oligonucleotide cataloging of 16S rRNA and the rRNA-DNA hybridization studies.

In this study we determined the 16S rDNA sequence of "Pseudomonas pavonaceae" IAM 1155, and this strain was included on the branch that contained the Pseudomonas sensu stricto group. However, Van Landschoot et al. (15) reported that "P. pavonaceae" NCTC 10693 belongs on the Acinetobacter rRNA branch. The genus Acinetobacter is a genus of the family Moraxellaceae (11), and the Acinetobacter rRNA branch has been reported to be far from the P. fluorescens complex branch (11, 15). Strain IAM 1155 was received from H. Taka-hashi at ATU 224 (Laboratory of Fermentation and Microbiol-ogy, Faculty of Agriculture, University of Tokyo, Tokyo, Japan) in 1964. The history of the isolation of strain IAM 1155...
was not available, but this strain was apparently isolated in the Laboratory of Fermentation and Microbiology, Faculty of Agriculture, University of Tokyo. In contrast, NCTC 10693 is the strain which was originally characterized as "P. pavonaceae" by Levine and Soppeland (7). Therefore, the difference between our phylogenetic analysis results and the conclusions reported by Van Landschoot et al. (15) can be attributed to the difference in the strains used.

C. luteola (4) and F. ozychabditans (4) were originally described as P. luteola and P. ozychabditans, and the type strains were isolated from a human clinical specimen and a rice paddy, respectively (6). The transfers of P. luteola and P. ozychabditans from the genus Pseudomonas were grounded on low levels of DNA-DNA hybridization (1 to 5%) with the other Pseudomo-

nas species (4), although the physiological and chemotaxo-
nomic characteristics of these organisms are similar to those of the genus Pseudomonas. In our 16S rRNA sequence analysis, C. luteola IAM 13000 T and ATCC 43330 and F. ozychabditans IAM 1568 T were found in the P. aeruginosa cluster (Fig. 1). The levels of homology between C. luteola IAM 13000 T and P. aeruginosa DSM 50071 T and between F. ozychabditans IAM 1568 T and P. aeruginosa DSM 50071 T (P. aeruginosa is type species of the genus Pseudomonas) were 94.0 and 93.9%, respectively. In contrast, the levels of homology between P. fluo-

rescens IAM 12022 T and P. aeruginosa DSM 50071 T and be-

tween P. syringae ATCC 19310 T and P. aeruginosa DSM 50071 T were 92.8 and 93.1%, respectively. Thus, according to our phylogenetic analysis, C. luteola and F. ozychabditans are more closely related to P. aeruginosa, the type species of the genus Pseudomonas, than P. fluorescens and P. syringae are. Therefore, if P. fluorescens and P. syringae are included in the genus Pseudomonas, it is not reasonable to exclude P. luteola and P. ozychabditans from the genus Pseudomonas. Consequently, we conclude that Chryseomonas and Flavimonas are junior subjective synonyms of Pseudomonas and that the names P. luteola and P. ozychabditans should be used.

REFERENCES


monas acidovorans den Dooren de Jong 1926 and Pseudomonas testosteroni Marcus and Talsaday 1956 as Comamonas acidovorans comb. nov. and Co-

17. Willems, A., E. Felsen, B. Pot, E. Jantzen, B. Hoste, P. Vandamme, M. Gillis, K. Kersters, and J. De Ley. 1990. Acidovorax, a new genus for Pseudomonas facilis. Pseudomonas delafaii E. Felsen (EUF) group 13, EF group 16, and several clinical isolates, with the species Acidovorax facilis comb. nov., Ac-


crobiol. 1:179-195.
fer of seven species of the genus Pseudomonas homology group II to the new genus, with the type species Burkholderia cepacia (Palleroni and Holmes 1981) comb. nov. Microbiol. Immunol. 6:1251-1275.
20. Yabuuchi, E., I. Yano, H. Oyainz, Y. Hashimoto, T. Ezaki, and H. Ya-