Taxonomic studies of the genera Acidomonas, Acetobacter and Gluconobacter by 5S ribosomal RNA sequencing

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Ribosomal 5S rRNA (rRNA) was isolated from 12 strains belonging to the genera Acidomonas, Acetobacter and Gluconobacter and sequenced. A dendrogram constructed from the data indicated that methylotrophic and non-methylotrophic strains of the genus Acetobacter formed two separate clusters. The non-methylotrophic members of the genus Acetobacter were phylogenetically closer to Gluconobacter than to the methylotrophic strains of Acetobacter. The methylotrophic strains of Acetobacter were recovered as a clade with the type strain of Acidomonas methanolica. These data support an earlier proposal which reclassified methylotrophic strains of Acetobacter into the genus Acidomonas.

Introduction

The genus Acetobacter is composed of aerobic, Gram-negative, non-sporeforming, acidophilic rod-shaped bacteria, which utilize a wide variety of organic compounds (De Ley et al., 1984). Some strains of this genus are able to use one-carbon compounds. The validity of placing methylotrophic and non-methylotrophic species in a single genus has been questioned for several genera (Green & Bousfield, 1982; Doronina & Govorukhina, 1987). For example, pink-pigmented facultative methylotrophic bacteria, previously assigned to the genus Pseudomonas have been given a separate generic status mainly as a result of their ability to grow on methanol and methylamines (Patt et al., 1976; Hood et al., 1987). The same feature was used for distinguishing the genera Hyphomicrobium and Hyphomonas (Gebers et al., 1986).

Recently, Urakami et al. (1989) proposed that the methylotrophic species of Acetobacter should be transferred to a new genus Acidomonas on the basis of chemotaxonomic data. To address this question, we have performed a phylogenetic study of the genus Acidomonas and related genera by comparing 5S rRNA sequences.

Methods

Strains and growth conditions. The strains used in this study and their sources are listed in Table 1. Methylotrophic strains of Acetobacter and Acidomonas methanolica were grown at 30°C, with shaking, in a mineral medium (pH 4.0; Loginova et al., 1981) containing 0.5% methanol. Non-methylotrophic strains of Acetobacter and Gluconobacter were grown at 30°C in a medium containing 0.3% peptone, 0.5% yeast extract and 1.5% (w/v) glucose; this medium was adjusted to pH 4.5 with HCl.

Isolation and sequencing of 5S rRNA. 5S rRNAs were isolated and their nucleotide sequences were determined as described previously (Bulygina et al., 1990). [5'-32P] cytidine 3',5'-diphosphate (pCp, Isotope, USSR) and T, phage RNA-ligase (Ferment, USSR) were used for 3'-end-labelling.

Phylogenetic analysis. The 5S rRNA nucleotide sequences were aligned as previously described (Wolters & Erdmann, 1988) and used to calculate the mutation distance matrix (Md). The distance between two sequences was expressed as a proportion of differing positions. A dendrogram was constructed using a UPGMA method (Sneath & Sokal, 1973). A tentative unrooted phylogenetic tree was constructed using the 'maximum topological similarity' (MTS) method (Chumakov

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The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers M76569 to M76580.
assumptions on the relative rate of evolutionary change in different MTS methods is that, unlike cluster methods, it does not require any assumptions on the relative rate of evolutionary change in different lineages, and provides straightforward computationally efficient algorithms to construct a phylogenetic tree. This method determines the topology of all subtrees from the four species, and joins these trees together. The first step is fulfilled by the use of a four-node rule, which allows one to find two pairs of neighbours in any subset of four species based on the distance between them. The second step, linking subtrees in a single tree, is achieved using different heuristic algorithms.

### Results and Discussion

The 5S rRNA nucleotide sequences of bacteria determined in this study have been submitted to GenBank and have been assigned the accession numbers M76569 to M76580. The nucleotide sequences of *Acidomonas methanolica* (Acetobacter methanicus) MB 58 and Acetobacter sp. 914 were published previously (Bulygina et al., 1990). The mutation distance matrix from these sequences was used to construct a dendrogram, reflecting the relationships between the genera *Acidomonas*, Acetobacter and Gluconobacter (Fig. 1). This dendrogram suggests that the non-methylotrophic representatives of the genus Acetobacter are actually phylogenetically closer to Gluconobacter than to the group of methylotrophic Acetobacter strains. The bacteria therefore appear to fall into three distinct clusters: methylotrophic strains of the genus Acetobacter and *Acidomonas methanolica*, Gluconobacter, and non-methylotrophic species of Acetobacter.

Urakami *et al.* (1989) proposed a new genus *Acidomonas* for methylotrophic strains of Acetobacter based upon chemotaxonomic data, DNA base composition and DNA homology studies. However, in our opinion, these data could not be interpreted unambiguously. Chemotaxonomic features such as fatty acid and ubiquinone composition can be common to microorganisms belonging to different but closely related genera and thus the value of these features for estimating taxonomic rank is limited. Moreover, Urakami *et al.* (1989) used phenotypic and genotypic characteristics of only the type strains to distinguish the genus *Acidomonas* from related genera. According to their published data, the differences between the genera *Acidomonas*, Acetobacter and Gluconobacter are not clear cut when the intrageneric variation of phenotypic and genotypic features are taken into account. Furthermore, comparisons of the DNA homology values for various strains and species of these genera do not indicate any differences between them (Urakami *et al.*, 1989). Overlapping values are also observed between the genera *Acidomonas*, Acetobacter and Gluconobacter when the ubiquinone composition, fatty acid composition, flagella morphology and G+C content values are compared.

In our opinion, the main distinguishing characteristic that justifies establishing the genus *Acidomonas* is the ability to utilize one-carbon compounds (mainly methanol). This conclusion does not exclude the possibility of using chemo- and genotaxonomic methods for the differentiation of bacteria at the intergeneric level. In the case studied here, the overlap seems to be caused by the high level of divergence of the genus Acetobacter and the close relatedness of the genera *Acidomonas* and Gluconobacter. These results are also supported by the data of Gillis & De Ley (1980) who have shown overlaps in the intra- and intergeneric values of $\Delta T_{\text{m}}(e)$ for Acetobacter and Gluconobacter (Table 2).

In contrast, the 5S rRNA sequence data presented here clearly provide evidence that methylotrophic strains

### Table 1. Strains used

<table>
<thead>
<tr>
<th>Name</th>
<th>Source*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acidomonas methanolica</em> MB 58$^T$</td>
<td>IMET 10945</td>
<td>Uhlig <em>et al.</em>, 1986</td>
</tr>
<tr>
<td>Acetobacter sp. MB 58/4</td>
<td>IPB</td>
<td>Babel &amp; Muller, 1977</td>
</tr>
<tr>
<td>Acetobacter sp.</td>
<td>IPB 914</td>
<td>Dikanskaya, 1987</td>
</tr>
<tr>
<td>Acetobacter sp.</td>
<td>IPB 924</td>
<td>Dikanskaya, 1987</td>
</tr>
<tr>
<td>Acetobacter sp.</td>
<td>IPB 867</td>
<td>Dikanskaya, 1987</td>
</tr>
<tr>
<td>Acetobacter sp.</td>
<td>IPB 913</td>
<td>Dikanskaya, 1987</td>
</tr>
<tr>
<td><em>Acetobacter aceti</em> NCIB 8621$^T$</td>
<td>IMET 10732</td>
<td>De Ley <em>et al.</em>, 1984</td>
</tr>
<tr>
<td><em>Acetobacter aceti</em> VKM 879</td>
<td>CMD 178</td>
<td>Arkadjeva &amp; Pimenova, 1985</td>
</tr>
<tr>
<td><em>Acetobacter pasteurianus</em> NCIB 12228$^T$</td>
<td>IMET 10733</td>
<td>De Ley <em>et al.</em>, 1984</td>
</tr>
<tr>
<td><em>Acetobacter xylinum</em> VKM 820</td>
<td>CMD 180</td>
<td>IJSB, 1984; Yamada, 1983</td>
</tr>
<tr>
<td><em>Gluconobacter oxydans</em> ATCC 19357$^T$</td>
<td>CMD 185</td>
<td>De Ley <em>et al.</em>, 1984</td>
</tr>
<tr>
<td><em>Gluconobacter oxydans</em> VKM 1227</td>
<td>CMD 182</td>
<td>Arkadjeva &amp; Pimenova, 1985</td>
</tr>
</tbody>
</table>

*Strain source: IMET, Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Akademie der Wissenschaften, Jena, Germany; CMD, Collection of Microbiology Dept., Moscow State University, Russia; IPB, Collection of Institute for Protein Biosynthesis, Moscow, Russia.
Acidomonas, Acetobacter and Gluconobacter taxonomy

Fig. 1. UPGMA dendrogram derived from a mutation distance matrix and showing the relationships between different species of Acidomonas, Acetobacter and Gluconobacter.

Table 2. Chemotaxonomic characteristics and mutation distance values for the genera Acetobacter (Ab), Gluconobacter (Gb) and Acidomonas (Am)

<table>
<thead>
<tr>
<th>Genus</th>
<th>G + C content (%)</th>
<th>% DNA-DNA pairing* with genus:</th>
<th>ΔT_{min} † (°C)</th>
<th>Mutation distance between genera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ab</td>
<td>Gb</td>
<td>Am</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>53-64</td>
<td>11-18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluconobacter</td>
<td>55-60</td>
<td>8-24</td>
<td>10-19</td>
<td></td>
</tr>
<tr>
<td>Acidomonas</td>
<td>63-66</td>
<td>7-19</td>
<td>8-15</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Urakami et al. (1989)
† Calculated from data of Gillis & De Ley (1980)

of Acetobacter are correctly classified as Acidomonas. The degree of sequence similarity within Acetobacter, Gluconobacter and Acidomonas is much higher than between them (Table 2). This suggests that the rank of the group of methylotrophic strains may be equivalent to the rank of a genus.

Several conclusions about the genetic divergence within Acidomonas can be drawn from our data. The maximum mutation distances between representatives of Acidomonas are lower than the values between different species of Acetobacter, but are higher than the values between the different strains of the species of Acetobacter and Gluconobacter. This suggests that the most divergent strains may represent new species. These strains also differ from the type strain A. methanolica MB 58 by their motility, ability to reduce nitrate and growth requirements (Arkadjeva & Pimeniva, 1985).

It should be noted that dendrograms constructed by the use of cluster analysis methods cannot be regarded as phylogenetic trees. The methods used to construct phylogenetic trees invoke assumptions about the equivalence of divergence rates in different evolutionary lineages (Golding, 1983). This assumption seems to be realistic only when the range of difference is low, as is seen between the taxa in the present study (Chumakov, 1987). To determine the relationships of the new genus to other groups of the alpha subdivision of Proteobacteria (Stackebrandt et al., 1988) we constructed a tentative phylogenetic tree using a topological method which is insensitive to the differences in divergence rates (Chumakov & Yushmanov, 1988) (Fig. 2). In this tree the genera Acetobacter, Gluconobacter and Acidomonas are clearly different from each other but they form a single line of descent. All of these genera have a common ancestor and should probably be classified as the same family Acetobacteriaceae.

The results of our phylogenetic analysis firmly support the proposal of Urakami et al. (1989) to transfer methylotrophic strains from the genus Acetobacter into a new genus Acidomonas. At present, the only definitive data available to differentiate the bacteria of this group is that of the 5S rRNA sequence analysis. Among phenotypic features, only the ability to utilize single-carbon compounds (methylotrophy) distinguishes...
between members of the genera *Acetobacter* and *Acidomonas*.

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References


