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

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Ribosomal 5SRNA (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).

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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.

Recently, Urakami *et al.* (1989) proposed that the methylotrophic species of *Acetobacter* should be transferred to a new genus *Acidomonas* on the basis of chemo- and genotaxonomic 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′-^{32}P] cytidine 3′,5′-diphosphate (pCp, Isotope, USSR) and T_4 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

Table 1. *Strains used*

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

* 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.

& Yushmanov, 1988; Yushmanov & Chumakov, 1988). A computer program based on this method has been described previously (Chumakov, 1988) and was used in this study. The advantage of the MTS method 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 methanolicus*) 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 dfferences 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 $\Delta T_{m(e)}$ for *Acetobacter* and *Gluconobacter* (Table 2).

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

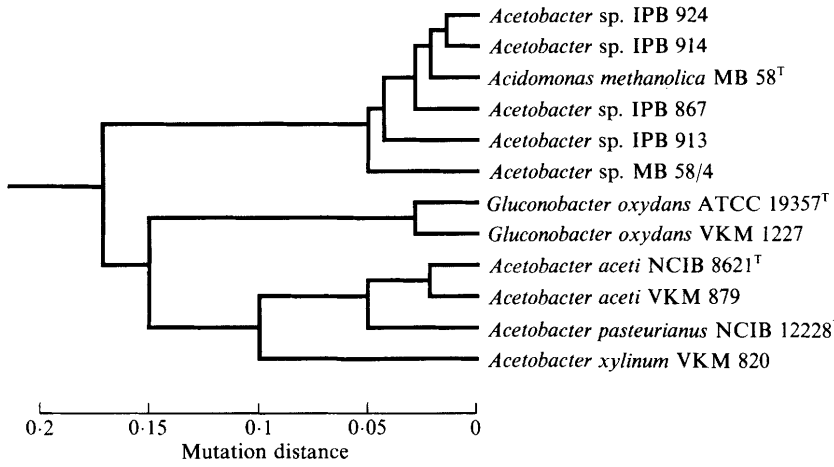

Fig. 1. UPGMA dendrogram derived from a mutation distance matrix and showing the relationships between different species of *Acidomonas*, *Acetobacter* and *Gluconobacter*.

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

Genus	G + C* content (%)	% DNA–DNA pairing* with genus:			$\Delta T_{m(e)}$† (°C)		Mutation distance between genera		
		Ab	*Gb*	*Am*	*Ab*	*Gb*	*Ab*	*Gb*	*Am*
Acetobacter	53–64	11–18			6		0·106		
Gluconobacter	55–60	8–24	10–19		5	2	0·145	0·032	
Acidomonas	63–66	7–19	8–15	–	–	–	0·175	0·175	0·063

* 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

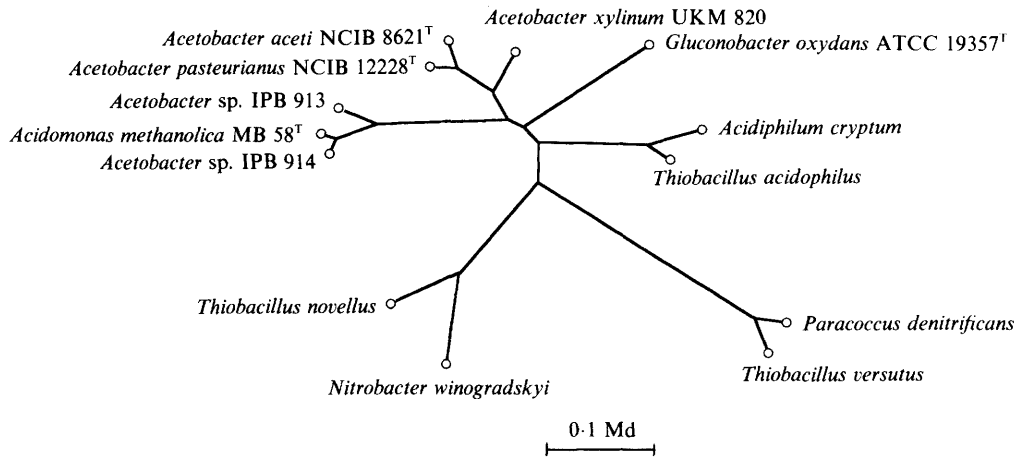

Fig. 2. Unrooted phylogenetic tree based on 5S rRNA sequences and showing the relationships of the family Acetobacteriaceae to representatives of the alpha subdivision of Proteobacteria. The sequences, which are not reported in this paper, are from Walters & Erdman (1988).

between members of the genera *Acetobacter* and *Acidomonas*.

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