The correlation between morphological and phylogenetic classification of myxobacteria

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In order to determine whether morphological criteria are suitable to affiliate myxobacterial strains to species, a phylogenetic analysis of 16S rDNAs was performed on 54 myxobacterial strains that represented morphologically 21 species of the genera *Angiococcus*, *Archangium*, *Chondromyces*, *Cystobacter*, *Melittangium*, *Myxococcus*, *Polyangium* and *Stigmatella*, five invalid species and three unclassified isolates. The analysis included 12 previously published sequences. The branching pattern confirmed the deep trifurcation of the order *Myxococcales*. One lineage is defined by the genera *Cystobacter*, *Angiococcus*, *Archangium*, *Melittangium*, *Myxococcus* and *Stigmatella*. The study confirms the genus status of '*Corallococcus*', previously '*Chondrococcus*', within the family *Myxococcaceae*. The second lineage contains the genus *Chondromyces* and the species *Polyangium* ('*Sorangium*') *cellulosum*, while the third lineage is comprised of *Nannocystis* and a strain identified as *Polyangium vitellinum*. With the exception of a small number of strains that did not cluster phylogenetically with members of the genus to which they were assigned by morphological criteria ('*Polyangium thaxteri*' Pl t3, *Polyangium cellulosum* ATCC 25531^T, *Melittangium lichenicola* ATCC 25947^T and *Angiococcus disciformis* An d1), the phenotypic classification should provide a sound basis for the description of neotype species in those cases where original strain material is not available or is listed as reference material.

Keywords: *Myxococcales*, 16S rDNA analysis, phylogeny

INTRODUCTION

The myxobacteria are among the few higher prokaryotic taxa in which morphology and the presence of a complex life cycle of its members can be considered a reflection of their common ancestry. These organisms were first shown to form a phylogenetically coherent cluster by partial 16S rRNA analysis (Ludwig *et al.*, 1983), as a result of which myxobacterial species from five genera formed three major sublines of descent, represented by *Nannocystis exedens*, '*Sorangium cellulosum*' and *Myxococcus fulvus* and its relatives of the genera *Stigmatella* and *Cystobacter*. Later, when a number of 16S rDNA oligonucleotide catalogues of hitherto less well-represented genera became available, the myxobacteria, together with the bdellovibrios (Hespell *et al.*, 1984) and the Gram-negative sulfate- and sulfur-reducing organisms (Fowler *et al.*, 1986),

were described as one of the major eubacterial divisions (Woese *et al.*, 1985). This phylum was subsequently described as the δ -subdivision of the division of purple bacteria (Oyaizu & Woese, 1985) when its members were found to form an individual branch within the radiation of the division of purple and non-purple bacteria and their relatives, equivalent in phylogenetic depth (i.e. in the range of 16S rRNA identity values separating the most divergent species of this taxon) to the α -, β - and γ -subdivisions. This division was later described as the class *Proteobacteria* (Stackebrandt *et al.*, 1988), with the subdivisions reclassified as subclasses. The most comprehensive phylogenetic analysis of myxobacteria was published by Shinkets & Woese (1992), who determined the almost complete 16S rRNA sequences of 12 strains belonging to 11 species of nine genera. The results of that study confirmed the partition of species as found in the original study by Ludwig *et al.* (1983) but broadened the '*Sorangium*' (this name has not been validated and '*Sorangium cellulosum*' has been reclassified as *Polyangium cellulo-*

The EMBL accession numbers of the 16S rDNA sequences reported in this paper are AJ233897–AJ233950.

sum) subline by the inclusion of *Chondromyces* and *Polyangium* and the *Myxococcus* subline by the addition of *Angiococcus*, *Archangium*, *Melittangium* and '*Coralloccoccus*'.

The availability of type strains for species of the order *Myxococcales* is a point of contention. The Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and subsequent Notification and Validation Lists contain 33 species and nine genera. However, only 12 species of seven genera are available from culture collections. The majority of species (15) are deposited as herbarium material, many of which have lost the ability to grow and to form fruiting bodies, which is the primary basis of species classification. For some species, no type material has been designated at all (e.g. *Polyangium aureum*, *Polyangium luteum*, *Polyangium parasiticum*, *Polyangium sorediatum*, *Polyangium spumosum* and *Melittangium boletus*). The situation is also confused by the fact that several species are listed with a reference strain in the chapter 'Fruiting Gliding Bacteria: The Myxobacteria' in *Bergey's Manual of Systematic Bacteriology* (McCurdy, 1989), a taxon that has no standing in nomenclature according to Rule 19 of the Bacteriological Code (Lapage *et al.*, 1975). Many type strains are deposited as non-culturable, dried herbarium material (accepted by Rule 18a of the Bacteriological Code), which excludes the possibility of using these strains in comparative phylogenetic and biochemical studies. Morphology and habitats are then the only characteristics that can be used to verify the affiliation of new isolates to known species.

In this study, most of the 54 myxobacterial strains investigated by comparative 16S rDNA analysis were tentatively assigned to described species and non-validated species on the basis of morphological characteristics. Unfortunately, reference material of many type strains was not available, which limited the present investigation to some extent. In the light of the future effort to replace non-living herbarium material with culturable neotype strains, it was the goal of this study to broaden the phylogenetic basis of species definitions within the order *Myxococcales* and to test the importance of morphology in the classification of these organisms.

METHODS

Bacterial strains. Most isolates analysed in this study were new isolates (collection Hans Reichenbach, GBF). All strains were cultivated aerobically on yeast agar (VY/2 agar) plates at 30 °C (Reichenbach & Dworkin, 1992). The strains were classified by morphological criteria: shape, size and structure of vegetative cells, myxospores, fruiting bodies and swarm colonies. *Myxococcus stipitatus* strains were tentatively identified by the bright yellow fluorescence of their swarm colonies under UV irradiation at 366 nm (Lampky & Brockman, 1977). *Myxococcus xanthus* and *Myxococcus virescens* were distinguished from each other by the greenish-yellow pigmentation of the latter which, however, is easily lost during cultivation. Fruiting-body mor-

phology and structure, important characteristics in myxobacterial taxonomy, are often substantially modified or disappear completely after several transfers, which may lead to misidentification.

16S rDNA sequence determination and analysis. Genomic DNA was extracted from the strains and used for the PCR-mediated amplification of the 16S rDNA (Rainey *et al.*, 1996). The purified PCR products were sequenced as described previously (Rainey *et al.*, 1996). The sequences were determined by electrophoresis by using a model 373A automatic DNA sequencer (Applied Biosystems).

The phylogenetic position of the myxobacterial strains was determined by using the ae2 editor (Maidak *et al.*, 1996). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were reconstructed by using the neighbour-joining treeing algorithms contained in the PHYLIP package (Felsenstein, 1993) as well as the algorithm of DeSoete (1983). Bootstrap values were determined by using the PHYLIP package (Felsenstein, 1993). The accession numbers of the 16S rDNAs of reference strains were: *Myxococcus xanthus*, M34114; *Myxococcus coralloides*, M94278; *Melittangium lichenicola*, M94277; *Angiococcus disciformis*, M94280; *Archangium gephyra*, M94273; *Cystobacter fuscus*, M94276; *Stigmatella aurantiaca*, M94281; *Polyangium* ('*Sorangium*') *cellulosum*, M94280; *Chondromyces crocatus*, M94275; *Chondromyces apiculatus*, M94274; *Polyangium* sp. Pl 4943, M94280; and *Nannocystis exedens*, M94279.

RESULTS AND DISCUSSION

The almost complete primary structure of the 16S rDNA, consisting of a region between positions 44 and 1465 (*Escherichia coli* nomenclature; Brosius *et al.*, 1978) was determined for 54 myxobacterial strains isolated at various times from different locations. The 16S rDNA sequences have been deposited in the EMBL database under the accession numbers indicated in Table 1. These sequences, representing 21 species of nine genera and some invalid taxa and non-classified isolates, were aligned against the 16S rDNA database of 12 myxobacterial strains, including eight type species of seven genera (Oyaizu & Woese, 1985; Shimkets & Woese, 1992), and phylogenetic trees were generated. The 16S rDNA of *Nannocystis exedens* Na e1, sequenced in this study, was 99.8% identical to the 16S rRNA sequence of the same strain published by Shimkets & Woese (1992). The branching patterns of the trees generated were highly similar with the two algorithms used and small differences occurred only in the order of branches among highly related strains. High bootstrap values (>80%) supported the trifurcation of the suborders and many other branching points (Fig. 1). The three major lineages were separated from each other by 16S rDNA identity values ranging from 73 to 85%.

All strains identified as members of the order *Myxococcales* on the basis of morphological criteria were also found to be phylogenetically related to members of this order by analysis of their 16S rDNA. The inclusion of the isolates did not change the topology of

Table 1. Strains investigated in this study, their morphology-based classification and 16S rDNA accession numbers

Isolate	Morphology-based classification	16S rDNA accession no.
An d1	<i>Angiococcus disciformis</i>	AJ233909
An d4	<i>Angiococcus disciformis</i>	AJ233910
An d6	<i>Angiococcus disciformis</i>	AJ233911
Ar g1	<i>Archangium gephyra</i>	AJ233912
Ar g2	<i>Archangium gephyra</i>	AJ233913
Cm a14	<i>Chondromyces apiculatus</i>	AJ233938
Sy t2	<i>Chondromyces lanuginosus</i>	AJ233939
Cm p17	<i>Chondromyces pediculatus</i>	AJ233940
Cm a13	' <i>Chondromyces robustus</i> '	AJ233942
Cm a22	' <i>Chondromyces robustus</i> '	AJ233941
Cc e12b	' <i>Corallococcus exiguus</i> '	AJ233931
Cc e100	' <i>Corallococcus exiguus</i> '	AJ233932
Cb f6	<i>Cystobacter fuscus</i>	AJ233897
Cb f10	<i>Cystobacter fuscus</i>	AJ233898
Cb f39	<i>Cystobacter fuscus</i>	AJ233899
Cb fe13	<i>Cystobacter ferrugineus</i>	AJ233900
Cb fe18	<i>Cystobacter ferrugineus</i>	AJ233901
Cb fe27	<i>Cystobacter ferrugineus</i>	AJ233902
Cb m2	<i>Cystobacter minor</i>	AJ233903
Cb m6	<i>Cystobacter minor</i>	AJ233904
Cb vi29	' <i>Cystobacter violaceus</i> '	AJ233905
Cb vi34	' <i>Cystobacter violaceus</i> '	AJ233906
Me b7	<i>Melittangium alboraceum</i>	AJ233907
Me b8	<i>Melittangium boletus</i>	AJ233908
Cc c494	<i>Myxococcus</i> (' <i>Corallococcus</i> ') <i>coralloides</i>	AJ233914
Cc c806	<i>Myxococcus</i> (' <i>Corallococcus</i> ') <i>coralloides</i>	AJ233915
Mx f1	<i>Myxococcus flavescens</i>	AJ233916
Mx f2	<i>Myxococcus fulvus</i>	AJ233917
Mx f421	<i>Myxococcus fulvus</i>	AJ233918
Mx f428	<i>Myxococcus fulvus</i>	AJ233919
Cc m7	<i>Myxococcus macrosporus</i>	AJ233920
Cc m8	<i>Myxococcus macrosporus</i>	AJ233921
Mx s33	<i>Myxococcus stipitatus</i>	AJ233922
Mx s42	<i>Myxococcus stipitatus</i>	AJ233923
Mx s52	<i>Myxococcus stipitatus</i>	AJ233924
Mx v2	<i>Myxococcus virescens</i>	AJ233925
Mx v76	<i>Myxococcus virescens</i>	AJ233926
Mx v1	<i>Myxococcus virescens</i>	AJ233927
Mx v144	<i>Myxococcus virescens</i>	AJ233928
DSM 435; Mx x1	<i>Myxococcus xanthus</i>	AJ233929
Mx x132	<i>Myxococcus xanthus</i>	AJ233930
Na a1	' <i>Nannocystis aggregans</i> '	AJ233945
Na e1	<i>Nannocystis exedens</i>	AJ233946
Na e571	<i>Nannocystis exedens</i>	AJ233947
Pl t3	' <i>Polyangium thaxteri</i> '	AJ233943
Pl vt1	<i>Polyangium vitellinum</i>	AJ233944
Pd e3	<i>Stigmatella erecta</i>	AJ233933
Pd e19	<i>Stigmatella erecta</i>	AJ233934
Sg a1	<i>Stigmatella aurantiaca</i>	AJ233935
Sg a15	<i>Stigmatella aurantiaca</i>	AJ233936
Sg a28	<i>Stigmatella aurantiaca</i>	AJ233937
NOSO-1	Unidentified	AJ233948
NOCB-2	Unidentified	AJ233949
NOCB-4	Unidentified	AJ233950

Fig. 1. Neighbour-joining tree of 16S rDNAs showing the phylogenetic position of the type strains of different genera of the order *Myxococcales* and isolates that were assigned to myxobacterial species on the basis of morphological characteristics (e.g. fruiting bodies, myxospores, colour). The sequences of Gram-negative, sulfate-reducing bacteria were used to root the dendrogram. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 100 bootstrapped trees. The bar represents 10 nucleotide substitutions per 100 nucleotides.

the phylogenetic tree significantly, with three major branches as originally defined on the basis of the 16S rDNA cataloguing approach (Ludwig *et al.*, 1983). Some of the families have been described, i.e. *Myxococcaceae* Jahn 1924, *Polyangiaceae* Jahn 1924 emend. Brockman 1989, *Archangiaceae* Jahn 1924 and *Cystobacteraceae* McCurdy 1970, while 'Sorangiaceae' has been proposed by Reichenbach & Dworkin (1992), although the genus name 'Sorangium' has not yet been validated.

The morphology-based affiliation of isolates to genera agreed for most strains, while a higher degree of discrepancies could be observed at the species level. However, for most genera, the intragenetic differences between the 16S rDNA primary structures of members were very small, in some cases below 1%. In these cases, the branching pattern was based on sequence differences within highly variable regions of the molecule, which do not necessarily reflect phylogenetic relationships (Stackebrandt & Goebel, 1994) and

should not be taken as evidence that species affiliation is erroneous.

Genus *Cystobacter*

This genus contains three species, *Cystobacter fuscus* (the type species), *Cystobacter ferrugineus* and *Cystobacter minor*. The epithet *minus* is incorrect and should be corrected to *minor*, L. neut. Only reference but no type material is available for these species. While strains of *Cystobacter minor* were phylogenetically well separated from the other two species and must probably be considered members of a new genus (see below), strains of *Cystobacter fuscus* and *Cystobacter ferrugineus* were phylogenetically intermixed at a 16S rDNA identity level above 99.4%.

Genus *Melittangium*

The genus *Melittangium* is a closely related sister taxon of the genus *Cystobacter sensu stricto*, from which it was separated by 98.2% 16S rDNA identity. The branching point was supported by a bootstrap value of 90%. *Melittangium* contains three species but type material is not available for the type species of the genus, *Melittangium boletus*. The *Melittangium* cluster contained two strains, which on the basis of morphological characteristics most closely matched *Melittangium boletus* (strain Me b8) and *Melittangium alboraceum* (Me b7). The type strain of *Melittangium lichenicola* clustered with strains of '*Corallococcus*' and was probably not correctly identified.

Genera *Archangium* and *Angiococcus*

This cluster embraced the type species of *Archangium* as well as strains assigned to the genera *Archangium*, *Cystobacter* and *Angiococcus*. Closely related to this cluster was a neighbouring taxon, the strains of which are related the neotype strain of *Angiococcus disciformis*, ATCC 33172^T. *Angiococcus disciformis* strain An d1 clearly does not belong to the same species as the neotype strain of the species, strain ATCC 33172^T. The swarm and cell morphology of the latter correspond to those of the genus *Cystobacter*, to which the species has been transferred in the latest edition of *Bergey's Manual of Systematic Bacteriology*. Since in our hands the neotype strain no longer produced fruiting bodies and myxospores, comparison of fruiting bodies was not possible. The description of the neotype strain (Hook *et al.*, 1980) suggests that this strain indeed represents the species originally described by Thaxter (1904). The strain was a phylogenetic neighbour of *Cystobacter ferrugineus* Cb fe13 and two moderately related strains of a tentative morpho-species '*Cystobacter violaceus*'. Strains of the invalid species '*Cystobacter violaceus*' grouped with strains of *Cystobacter minor*. The *Cystobacter minor* strains are morphologically similar to *Angiococcus disciformis* ATCC 33172^T, supporting the phylogenetic clustering

of members of the taxa. The most unrelated strain was '*Cystobacter violaceus*' Cb vi29, showing as little as 97.4% identity to other members of the cluster.

The phylogenetic separateness of *Cystobacter minor* and '*Cystobacter violaceus*' from the type species, together with unique morphological properties of these strains, supports the proposal to elevate this cluster to genus status.

Genus *Myxococcus*

All of the 13 strains identified as members of *Myxococcus* clustered with the type strain of *Myxococcus xanthus*, ATCC 19368^T (Oyaizu & Woese, 1985) [listed as ATCC 25232^T in the latest edition of the ATCC catalogue of strains (1994)]. The intragenic structure recognized five subclusters, which were separated by 16S rDNA identity values of about 98.2% and showed bootstrap values above 55%. The first subcluster contained two isolates identified as members of *Myxococcus xanthus*, strains Mx x132 and Mx x1 (=DSM 435). They shared more than 99% identity with the type strain, ATCC 25232^T. Other strains that were highly related to *Myxococcus xanthus* were identified as *Myxococcus virescens* (four strains), a strain of *Myxococcus flavescens* and a single strain of *Myxococcus* ('*Corallococcus*') *macrosporus* (strain Cc m8). The genus name '*Corallococcus*' has been proposed by Reichenbach & Dworkin (1992) for former '*Chondrococcus*' species, but neither genus name has been validated so far. The second subcluster consisted of a group of three strains, two of which were identified as *Myxococcus stipitatus* (Mx s42 and Mx s52) and a strain of *Myxococcus fulvus* (Mx f2). Two strains of *Myxococcus fulvus* (Mx f421 and Mx f428) and a strain of *Myxococcus stipitatus* (Mx s33) formed subcluster 3, while the other two subclusters were defined by single strains of *Myxococcus* ('*Corallococcus*') *macrosporus* (Cc m7) and *Angiococcus disciformis* strain An d1. The data may indicate that the subtle differences in 16S rDNA sequences do not allow a reliable species differentiation. They may also suggest that *Myxococcus xanthus* and *Myxococcus virescens* can not be distinguished reliably by pigmentation alone, viz. orange versus greenish-yellow, a colour difference that may disappear during cultivation anyway. DNA–DNA reassociation should establish whether the two species should be united. *Myxococcus flavescens* may belong to the same complex. The *Myxococcus fulvus* and *Myxococcus stipitatus* strains used in this study appeared to be typical representatives of each species, which are clearly distinguished by smaller, often oval myxospores and the yellow fluorescence mentioned above for *Myxococcus stipitatus*. Yet, strains of either species were placed at different locations in the dendrogram. The position of the two *Myxococcus macrosporus* strains is less of a surprise. Both produced only degenerated fruiting bodies and therefore could really represent *Myxococcus virescens* strains. As both strains have large myxospores, 2–2.5 µm in diameter,

they should cluster together and with *Myxococcus virescens*. Again, DNA–DNA reassociation studies should resolve the problem of species affiliation. *Angiococcus disciformis* strain An d1 has a typical myxobacterial swarm with soft, snaking veins and spherical myxospores but, in contrast to all other members of the family, produces fruiting bodies with sporangioles. Strain An d1 does not belong to the same species and genus represented by the neotype strain of *Angiococcus disciformis*, ATCC 33172^T.

The 'Corallococcus' cluster

This cluster is a sister taxon of the genus *Myxococcus*, containing the type strain of *Myxococcus* ('*Corallococcus*') *coralloides* ATCC 25202^T (previously '*Chondrococcus*') and, unexpectedly, the type strain of *Melittangium lichenicola*, ATCC 25946^T. Two strains that were identified as typical members of the emerging genus '*Corallococcus*', '*Corallococcus exiguus*' Cc e100 and Cc e216, were also found to be placed within this cluster, while two strains of *Myxococcus* ('*Corallococcus*') *coralloides*, Cc c494 and Cc c806, branched somewhat deeper. The '*Corallococcus*' strain cluster was separated from members of *Myxococcus* by 16S rDNA identity values between 97 and 98%. A bootstrap value of 100% strongly supported the bifurcation point of these two neighbouring taxa. The separate position of '*Corallococcus*' species, together with their distinct morphological characteristics and physiological differences, indicate that this taxon merits genus status.

Genus *Stigmatella*

This genus, containing two species for which type material is available, formed a phylogenetically well-defined taxon. Strains of the two species showed greater than 99.4% 16S rDNA sequence identity and the branching pattern of strains did not allow clear discrimination between the two species, *Stigmatella erecta* and *Stigmatella aurantiaca*. The two are very difficult to distinguish if fruiting body morphology has somewhat degenerated. A clear difference in genome size appears to support the species differentiation (Neumann *et al.*, 1992).

Suborder Sorangineae

Genus *Chondromyces*. The genus *Chondromyces* consists of five species, all of which have been deposited as herbarium material and hence are not available for phylogenetic analysis. However, *Chondromyces* strains are the most differentiated morphologically among the myxobacteria, meaning that strains can be allocated to described species with considerable confidence. Comparison of 16S rDNA sequences revealed that all isolates affiliated to the species *Chondromyces lanuginosus*, *Chondromyces apiculatus*, *Chondromyces crocatus* and *Chondromyces pediculatus* formed a phylo-

genetically coherent cluster. The phylogenetic distance among certain species, however, indicates that the genus is genomically more diverse than the other genera of the *Myxococcales*. While some species exhibited 16S rDNA identities above 98.5% ('*Chondromyces robustus*', *Chondromyces apiculatus* and *Chondromyces lanuginosus*), the species *Chondromyces crocatus* and *Chondromyces pediculatus* showed around 96.5% sequence identity to each other and to other species of the genus. A more detailed analysis of *Chondromyces crocatus* strains has recently demonstrated the phylogenetic identity of most of the analysed strains. Three strains were slightly less related, showing around 98.5% 16S rDNA identity to the other strains of the species (Jacobi *et al.*, 1996).

Also included in the *Chondromyces* cluster is a strain classified as '*Polyangium thaxteri*' on the basis of its unique morphology (see Reichenbach & Dworkin, 1992). This organism produces clusters or chains of large, spherical, orange-brown sporangioles, which may be borne on a short slime pedicle. Thus, the fruiting body has little resemblance to a *Chondromyces* fruiting body but is also clearly different from those described for *Polyangium* species.

Genus *Polyangium*. Ten species of this genus have been described, but with the exception of *Polyangium* ('*Sorangium*') *cellulosum*, culturable reference material is not available for any of them. For many species, not even a reference strain is available. While *Polyangium* ('*Sorangium*') *cellulosum* subsp. '*ferrugineum*' ATCC 25531 and *Polyangium* sp. PI 4943 (Shimkets & Woese, 1992) clustered distantly with members of *Chondromyces*, *Polyangium vitellinum* PI vt1 defined a separate line of descent. The morphology of strain PI vt1 most closely matches that of the type species, *Polyangium vitellinum*, in that the organism produces greenish-yellow swarms and large, spherical, yellow sporangioles within and on the substrate. Obviously, the phylogenetic position of *Polyangium vitellinum* is neither close to *Polyangium* ('*Sorangium*') *cellulosum* ATCC 25531 nor to any species of *Chondromyces*. Apparently, the genus-specific morphological characteristics of *Polyangium* do not bear phylogenetic significance, as strains identified as members of this genus were placed at three different positions within the suborder *Sorangineae* (see below). In fact, the fruiting bodies of the *Polyangium* species are simply clusters and piles of smaller and larger sporangioles, sometime closely packed and polyhedral, and they resemble the fruiting bodies of *Cystobacter* and *Angiococcus*. *Cystobacter* and *Polyangium* species have therefore previously been classified together, but can easily be separated by the shape of the vegetative cells. From this it follows that *Polyangium cellulosum* and possibly '*Polyangium thaxteri*' probably represent the nuclei of new genera. The genus name *Sorangium* should be revived for the cellulose degraders of the *Polyangium cellulosum* complex. A new genus will be defined for the '*Polyangium*' species of the PI 4943 type, which are very common myxobacteria in soil.

Genus *Nannocystis*. The separate position of the genus *Nannocystis* has already been noted in the first phylogenetic study on myxobacteria (Ludwig *et al.*, 1983) and was later explained by rapidly evolving 16S rDNA (Shimkets & Woese, 1992). The presence of the sequence idiosyncrasies in the strains of *Nannocystis* investigated in this study was confirmed. The two strains of *Nannocystis exedens* (*Nannocystis exedens* Na el was sequenced twice, see above) shared more than 99.4% sequence identity, and this species is highly related to the new species '*Nannocystis aggregans*' (99% sequence identity).

Unassigned isolates. Three isolates could not be assigned to any of the acknowledged myxobacterial species on the basis of morphological criteria. The phylogenetic analysis confirmed their morphological uniqueness. While isolate NOSO-1, showing some morphological resemblance to species of *Polyangium*, branched deeply within the suborder *Sorangineae*, the isolates NOCB-2 and NOCB-4, resembling *Cystobacter* species, are distantly related to members of *Stigmatella*. These two new groups of isolates probably represent novel genera.

Conclusions

The present study confirms not only that the myxobacteria are a phylogenetically coherent group, but, to a large extent, the phylogenetic significance of morphological characteristics of myxobacteria. Strains classified on morphological grounds, such as *Archangium gephyra*, *Cystobacter fuscus*, *Nannocystis exedens* and *Stigmatella aurantiaca*, indeed clustered next to the respective neotype strains in the phylogenetic analysis. In two cases, no match was observed. The type strain of *Angiococcus disciformis*, ATCC 33127^T, clearly does not belong to the family *Myxococcaceae*. On the basis of cell and swarm morphology, it seems justified that the species has been transferred to the genus *Cystobacter*. This means that strain An d1, which belongs to the *Myxococcaceae*, represents a new genus. The second case refers to the neotype strain of *Melittangium lichenicola*, ATCC 25946^T, which clustered within the radiation of '*Corallococcus*' species and may have been misclassified. There are many fruiting-body-forming strains that conform to the description of the species and those strains certainly do not belong to the family *Myxococcaceae*.

The main problem identified in this study refers to the availability of type strains for myxobacterial species. The original descriptions of most species are based on fruiting bodies developing in crude cultures and many of these strains have never been cultivated in pure culture by the authors. Consequently, type strains are often only deposited as herbarium specimens that can no longer be cultured. In addition, our experience has shown that herbarium material will not be made available for isolation of DNA, on which 16S rDNA analysis depends. Obviously, the solution is not to rely on herbarium material but to define and deposit

neotype strains. As myxobacteria have a highly developed morphology, it should be possible to find strains that correspond to the original descriptions. There is, however, a basic difficulty. Fruiting-body morphology often degenerates or is lost completely during cultivation, with the consequence that the type or neotype strain no longer shows the characteristics of the species. In fact, this observation has made myxobacterial taxonomists hesitate to deposit neotypes. There are, however, two ways to circumvent the problem. Firstly, the use of preservation methods that allow the strains to produce fruiting bodies, e.g. drying fruiting bodies on filter paper at an early stage of cultivation. Secondly, the deposition of type strains together with careful and comprehensive photographic documentation of fruiting body and myxospore morphology, both being made available to interested investigators. Some difficulties will still remain: fruiting bodies often change their shape and structure during cultivation. Thus, members of several genera, e.g. *Stigmatella*, *Cystobacter* and *Melittangium*, may produce *Archangium*-type fruiting bodies. This may lead to erroneous classification, even by experienced specialists. The search for differentiating physiological properties has not been particularly successful so far. Eventually, systematic monitoring for differentiating characteristics at the chemotaxonomic level, e.g. protein patterns and lipids, and at the molecular level, e.g. species-specific oligonucleotide probes, may help to overcome the presently unsatisfactory taxonomic situation within the *Myxococcales*.

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