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Fruiting and non-fruiting myxobacteria: A phylogenetic perspective of cultured and uncultured members of this group

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Abstract

The diversity of myxobacteria present in campus garden soil was surveyed by both cultivation-based and cultivation-independent methods. Detailed phylogenetic analysis of cultured and uncultured myxobacteria 16S rRNA gene sequences revealed that many undescribed relatives of the myxobacteria exist in nature. Molecular systematic analyses also revealed that myxobacterial genera described to date on the basis of the morphology of multi-cellular fruiting bodies were mostly monophyletic. However, these known taxa comprised only in a small part of the sequences recovered directly from soil in a cultivation-independent approach, indicating that the group is much more diverse than previously thought. We propose that the myxobacteria exist in two forms: the fruiting and the non-fruiting types. Most of the uncultured myxobacteria may represent taxa which rarely form fruiting bodies, or may lack some or all of the developmental genes needed for fruiting body formation. In order to identify non-fruiting myxobacteria, new morphology-independent cultivation and isolation techniques need to be developed.
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1. Introduction

Myxobacteria are Gram-negative gliding bacteria (McCurdy, 1989) whose cells grow cooperatively on a food source, and can then form multi-cellular, myxospore-containing fruiting bodies after exhaustion of the food supply (Dworkin, 1996; Shimkets, 1990). As a result of their specialist lifestyle, myxobacteria are able to live in many places. They prefer to grow in the soil, the dung of herbivores, in bark and in rotting wood (Dawid, 2000; Reichenbach, 1999). Cultivation strategies for myxobacteria usually involve incubation of natural samples on a selective, agar-containing medium, followed by cell swarming and fruiting body formation; occasionally, fruiting bodies themselves can be collected from nature (Dawid, 2000; Reichenbach and Dworkin, 1992). However, fruiting body formation is but one stage of their life cycle, and these structures often quickly collapse after their formation (Reichenbach and Dworkin, 1992). As a result, the distribution and diversity of myxobacteria in nature is still substantially unclear more than a century after they were first described by Thaxter in 1892.

The present taxonomy of myxobacteria is based solely on cultured, fruiting myxobacteria. After several updates, the recognized myxobacteria are now classified into three suborders, six families, 17 genera and approximately 50 species (Reichenbach, 2004; Shimkets et al., 2005). Compared to previous classifications (McCurdy, 1989; Reichenbach, 1993), new myxobacterial taxa have recently been described either from new isolates, reclassification of misplaced taxa after phylogenetic analysis of their 16S rRNA gene sequences, or identification of novel and distinguishing morphological characteristics. For instance, the present suborder Sorangineae contains six genera, *Haploangium* (now identified as *Polyangium* by Reichenbach in 2004), *Chondromyces*, *Polyangium*, *Sorangium* and two new genera *Byssophaga* (a cellulose-degrading myxobacterium

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initially identified by Thaxter in 1897 and rediscovered by Reichenbach in 2004) and *Jahnia* (*Jahnia thaxteri*, previously termed *Polyangium thaxteri*). The genus *Nannocystis*, which had been placed in the suborder Sorangineae has now been elevated to form a new suborder Nannocystineae, together with the halophilic or slightly halophilic myxobacterial strains that were recently isolated from Japanese coastal areas (Iizuka et al., 1998, 2003a,b; Fudou et al., 2002), as well as a newly renamed species *Kofleria flava* (previously *Polyangium vitellinum* Pl vt1) (Reichenbach, 2004). The more recently described myxobacteria usually have special properties reflecting unique habitats. For instance, Sanford et al. (2002) isolated anaerobic myxobacteria strains from soil which were classified as members of the suborder Cystobacterineae, based on their ability to grow using acetate as an electron donor and 2-chlorophenol as an electron acceptor. These and other unusual myxobacteria have broadened our understanding of the taxonomy of the group, which has yet to be explored in detail. For example, even when using conventional methods, Watve et al. (1999) found that the number of myxobacteria in soil samples from India was probably several-fold higher than the number of samples typically recovered elsewhere.

Interestingly, recently isolated strains do not usually have typical myxobacterial morphological characteristics, but instead have atypical or inconspicuous fruiting body structures. We surmise that there may be many other bacteria within the myxobacterial clade that have yet to be discovered because they form fruiting bodies rarely, or not at all. In our previous work, we explored the diversity of the myxobacterial suborder, Cystobacterineae in soil by using both conventional isolation as well as cultivation-independent, molecular phylogenetic methods (Wu et al., 2005). On the one hand, from the soil sample we were able to isolate only five Cystobacterineae strains (two *Myxococcus* spp., two *Corallococcus* spp. and a *Cystobacter* sp.) using classical isolation techniques (in addition a *Nannocystis* sp. and a *Sorangium* sp. of the suborder Sorangineae were also identified). By contrast, cloning and sequencing 16S rRNA gene sequences from the same soil sample using myxobacteria-specific primers revealed that far greater diversity was present. Fifty-three Cystobacterineae-related clones were sequenced and could be divided into at least 12 subgroups (with 95% or less sequence similarity), of which four spanned the diversity of all previously described members of this suborder. This result indicated that there are many undiscovered, greatly diversified myxobacteria in nature. In the present study, after a further molecular survey of the suborder Sorangineae in soil, we carried out a detailed phylogenetic analysis of both cultured and uncultured myxobacteria. Our data suggest that the myxobacteria exist in nature in two forms: fruiting and non-fruiting types, which probably correspond to cultured and uncultured myxobacteria. The phylogenetic diversity, molecular taxonomy and classification of this interesting bacterial group is also discussed.

2. Materials and methods

2.1. Construction of a Sorangineae-enriched 16S rRNA gene library from a soil sample

The total DNA surveyed for the construction of a Sorangineae-enriched 16S rRNA gene library was the same as that used in the surveying of uncultured Cystobacterineae sequences reported previously (Wu et al., 2005). Briefly, the soil sample was ground with a mortar and pestle under liquid nitrogen. The ground soil was mixed with an extraction buffer (100 mM Tris–HCl; 100 mM EDTA; 100 mM sodium phosphate; 1.5 M NaCl; 1% [w/v] CTAB; pH 8.0) containing 1 mg/ml protease K, and was then subjected to three freeze-thaw cycles followed by incubation at 65 °C for 2 h. Following extraction with chloroform–isopentanol, total DNA was precipitated with 0.6 vol of cold isopentanol, washed with 70% ice-cold ethanol, and purified on a Sephedex G-200 column.

We designed specific primers (Wu et al., 2005) for 16S rRNA genes of the suborders Cystobacterineae and Sorangineae to determine the diversity of myxobacteria in the environment. Identification of group-specific primers was possible because cultivated members of these suborders do not display that much sequence divergence in this gene (Spröer et al., 1999; Yan et al., 2003). By contrast, it was not possible to design a primer set specific for 16S rRNA genes of members of the new suborder Nannocystineae (only 19 entries in GenBank) due to our inability to identify conserved sequence that was diagnostic for the group. Members of the Nannocystineae are more diverse in both 16S rDNA sequence content and fruiting body morphology (Reichenbach, 2004). The primer set of Sorangineae specific W4 (GTA AGA CAG AGG GTG CAA ACG T; corresponding to positions 492–510 of the 16S rRNA in *Escherichia coli*, Brosius et al., 1978) and bacterial universal Pr (GGC TGG ATC ACC TCC TTT CT; corresponding to the position of 1524–1541 bp) was evaluated initially by using DNA from pure bacterial cultures as references. At an annealing temperature of 69 °C, all the Sorangineae cultures were amplified, whereas the Cystobacterineae strains and the negative controls *E. coli* and *Methylobacterium* sp. 0093-1 were not amplified. *E. coli* produced positive signals with the primer set at an annealing temperature lower than 64 °C, and *Methylobacterium* sp. 0093-1 at a temperature lower than 67 °C. Accordingly, the primer set W4/Pr was used to amplify Sorangineae-enriched 16S rRNA gene sequences from the soil community DNA using a touchdown PCR protocol (Wu et al., 2005) to increase the diversity of Sorangineae products. The products were about 1000 bp in length. The amplified products containing 16S rRNA gene sequences were purified using a QIAquick Gel Extraction Kit (Qiagen, USA) and were ligated with pGEM-T easy Vector (Promega, USA) following the protocols recommended by the manufacturer. They were electroporated into *E. coli* JM109 using the published procedure (Sambrook and Russell, 2001).

About 3000 white transformants were collected and stored as a Sorangineae-enriched 16S rRNA gene library.

2.2. In situ colony hybridization and sequencing

The oligonucleotide probe W5 (GTA AGA CAG AGG GTG CAA ACG T; corresponding to positions 529-550 of 16S rRNA in *E. coli*) specific for the suborder Sorangineae was labeled at the 3′-end with fluorescein-dUTP by terminal transferase in accordance with the manufacturer's instructions for the Gene Image 3′-oligolabeling module (Amersham Biosciences, USA). To evaluate and optimize conditions for *in situ* colony hybridization, slot blot hybridization was performed with W5. The 16S rRNA gene PCR products from pure myxobacteria strains were blotted onto a Hybond™-N^+ membrane (Amersham Biosciences, USA). The membrane was soaked in the hybridization buffer [5× SSC; 0.1% (w/v) SDS; 20-fold dilution of liquid block supplied with the kit and 0.5% (w/v) dextran sulphate (MW 500,000)] for 1 h at a selected temperature. After the addition of 5 ng/ml of labeled oligonucleotide probe, the hybridization process was performed overnight. When the hybridization temperature was 61 °C, the 16S rRNA gene PCR products from Sorangineae strains produced obvious hybridization signals, while there were no signals for Cystobacterineae strains, *E. coli* or *Methylobacterium* sp. 0093-1. When the hybridization temperature was reduced to 58 °C, the Cystobacterineae strains produced weak signals, while there were still no signals for *E. coli* or *Methylobacterium* sp. 0093-1. To obtain maximum diversity, the lower stringency hybridization temperature of 58 °C was employed. After hybridization, the membrane was washed twice in 5× SSC, 0.1% (w/v) SDS at room temperature, for 5 min each time, and then washed twice in 1× SSC, 0.1% (w/v) SDS at 50 °C, for 15 min each time. The Gene Image CDP-Star detection module (Amersham Biosciences, USA) was used to detect the hybridization signal following the manufacture's instruction. KODAK X-OMAT™ AR (XAR5) films were exposed to the blots between intensifying screens for 1 h. Eighty-five positive clones were randomly selected for sequencing, which was carried out in the Shanghai Sangon Sequencing Center (Shanghai, China). After BLAST analysis against the GenBank database, the sequences that were related to the myxobacteria were sequenced from the other direction to obtain the sequence of the whole segment. The sequence data have been submitted to GenBank with Accession Nos. DQ646254 to DQ646330.

2.3. Phylogenetic analysis

For phylogenetic analyses, the 16S rRNA gene sequences of all myxobacteria available in GenBank were extracted. After a complete alignment using clustalX (version 1.83; Thompson et al., 1997), corresponding parts of 16S rRNA gene sequences from cultured and uncultured myxobacteria were used for inferring phylogenetic relationships. Phylogenetic trees were inferred using the interior branch test of the Neighbor-Joining method in MEGA (version 3.1; Kumar et al., 2004). Phylogenetic relationships were also confirmed by the likelihood and parsimony programs in the same software package. The bootstrapping supports for the trees were calculated from a sample of 1000 replicates. The 16S rRNA gene sequence of the δ-protoebacterium, *Desulfovibrio desulfuricans* (GenBank Accession No. M34113), was used as an outgroup to root the tree.

3. Results and discussion

3.1. Phylogenetic diversity of uncultured Sorangineae 16S rRNA gene sequences

In situ colony hybridization was performed on more than 1000 clones of the Sorangineae-enriched 16S rRNA gene library. Approximately 20% of the samples produced a signal, from which 85 clones were randomly chosen for sequencing. Among the sequenced clones, six fragments had no relationship to myxobacteria and were not studied further. The other 79 sequences (all were unique except for two, each of which occurred twice) showed 76.4–99.1% homology with known cultured myxobacteria. Seventy-four of the 77 unique myxobacteria-related clones belonged to the suborder Sorangineae (Fig. 1). The uncultured Sorangineae sequences in the campus garden soil exhibited a similar diversity to that of the uncultured Cystobacterineae, described previously (Wu et al., 2005). Interestingly, and despite some morphological diversity, sequences of all cultured Sorangineae were limited to a clade that included only 16 of the 74 Sorangineae-related sequences from soil. The 58 other soil-derived sequences were noticeably more diverse. Based on the phylogeny of the 74 unique Sorangineae sequences, we suggest that this particular soil habitat contained at least five major clades of the suborder Sorangineae, having 90% or less sequence similarity with one another (i.e., at the depth of separate families). Group I contained 33 uncultured sequences (accounting for 44.6% of the total 74 sequences) in two different branches, one of which included all of the reported cultured Sorangineae strains, i.e. *Chondromyces* spp., *Polyangium* spp., *Sorangium* spp. and *Byssophaga* spp. (Fig. 1). Among the 33 uncultured sequences, five clones (9-96, 13-17, 14-33, 20-8, 20-2) shared >96% similarity with the genus *Sorangium*, four clones (9-15, 13-73, 16-60, 21-42) were *Polyangium*-related, seven clones (9-66, 10-37, 13-5, 13-27, 14-7, 15-11, 21-33) were *Byssophage*-related, and the others were of unknown subgroups. There was no clone that was affiliated with the genus *Chondromyces*, suggesting that members of this genus were probably not present in the campus garden soil. This was consistent with the observation that *Chondromyces* strains are normally found on the bark of trees (Reichenbach and Dworkin, 1992; Dawid, 2000; Li et al., 2000). The other four groups in the suborder Sorangineae were comprised exclusively of uncultured

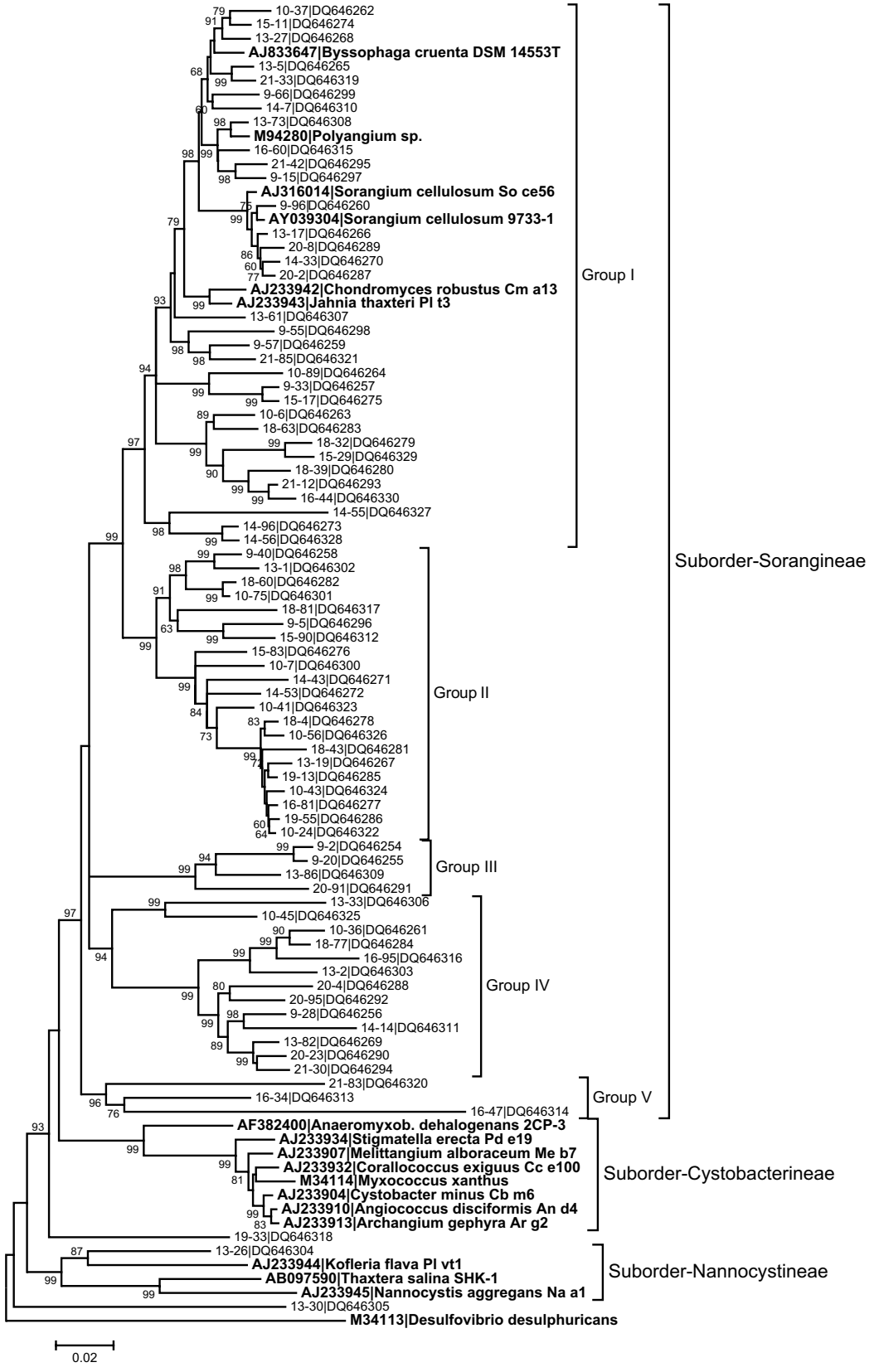

Fig. 1. A phylogenetic tree inferred from clones of 16S rRNA gene sequences related to the Sorangineae, and those from cultured myxobacteria (in boldface). Representative reference taxa were chosen following the pruning of a much larger tree using all of the cultured Sorangineae sequences available in GenBank. The bar is equivalent to two nucleotide changes per 100 bp. The numbers on branch nodes indicate bootstrap support percentages based on 1000 replicates.

sequences. Group II was composed of 21 clones (28.4% of the total 74 sequences) with sequence similarity values of 89 to 98%. This group was more closely related to Group I than the other groups. Groups III and IV were composed of 4 and 13 clones, respectively (5.4% and 17.6%), and the three clones in Group V represented 4.1% of the total 74 sequences derived from soil. This last group was distantly related (less than 85% sequence similarity) to Group I and may represent a newly discovered suborder. The number of cloned sequences affiliated with each group in Groups II–V was inversely proportional to the evolutionary distance between any one of them and Group I, which may reflect the fact that the Sorangineae specific primer (W4) and probe (W5) were designed from the available sequenced data of the cultured Sorangineae strains, which were, after all, only present in Group I.

3.2. Molecular taxonomy of cultured and uncultured myxobacteria

An earlier phylogenetic analysis of 12 representative myxobacteria strains revealed three distinct clades: *Myxococcus*, *Chondromyces*, and *Nannocystis* (Shimkets and Woese, 1992). A later study using more myxobacterial strains also supported this conclusion (Spröer et al., 1999). After that time, many myxobacteria strains with halophilic or slightly halophilic (Iizuka et al., 1998, 2003a,b; Fudou et al., 2002), halotolerant (Li et al., 2002; Zhang et al., 2002a,b), psychrophilic (Dawid, 2000), and anaerobic (Sanford et al., 2002) characteristics have been discovered. Hundreds of 16S rRNA gene sequences of cultured myxobacteria are now available in GenBank, which enables detailed phylogenetic analyses of the myxobacteria. Fig. 2a is a comprehensive phylogenetic tree inferred by using all the available cultured and uncultured myxobacteria 16S rRNA gene sequences. Almost all cultured myxobacteria, including the common and more unusual, were found to members of one of the three clades, which are now known as the three myxobacterial suborders, Cystobacterineae, Sorangineae and Nannocystineae (Reichenbach, 2004). NUST 6 and NU-2, the only two strains not included in the three suborders, were described as salt-tolerant myxobacterial isolates, but no further information is available (Zhang et al., 2002a,b).

The most significant characteristic of the tree in Fig. 2a is that the cultured myxobacteria represent only a small portion of the group's diversity with respect to 16S rRNA gene sequences. Despite this diversity, most of the myxobacterial genera, which were originally classified by their fruiting body morphology, constituted monophyletic clades in this molecular systematic analysis. Within-genus sequence similarity values were generally higher than 98%.

The expansion of the tree in Fig. 2b illustrates the phylogenetic relationships of members of the genus *Myxococcus*. The study by Stackebrandt and Päuker (2005) of 33 strains of *Corallococcus coralloides* isolated from 14 countries and five continents supported that the strains with this type of fruiting body structure also had highly similar 16S rRNA gene sequences. Thus our study, and that of Stackebrandt and Päuker (2005), suggest that shape and structure of the fruiting body is a good taxonomic character, although there are some exceptions. For example, the morphologically distinct genus *Melittangium* appears to be polyphyletic in that the 16S rRNA gene sequences of its different members are 99% or more similar to that of either *Corallococcus* or *Cystobacter*, each of which is also polyphyletic in Fig. 2. Although the suborder Nannocystineae was not explored, a few uncultured Nannocystineae sequences discovered during our survey of the diversity of Cystobacterineae and Sorangineae were also characterized in this study.

Comparing the phylogenies of the cultured and uncultured myxobacteria, we can see that there are many uncultured myxobacteria in nature, which are often significantly distinct from the cultured myxobacteria in their 16S rRNA gene sequences. Even in the Mx-Fruiting group, the *Myxococcus*-related sequences derived from soil were not only distributed in the cultured *Myxococcus* branches, but also formed separate uncultured branches (Fig. 2b).

Based on the tree topologies of the cultured and uncultured myxobacteria, we suggest that the sequences recovered directly from soil by using a cultivation-independent approach cultured may represent myxobacteria which are incapable of forming fruiting bodies. There are several lines of evidence which led us to this hypothesis:

1. Since the first report by Thaxter (1892), myxobacteria have been isolated from many different samples and places by many different research groups. The isolation techniques have been modified many times, but all involved the collection and/or growth promotion of fruiting bodies. Most recently obtained new isolates have been shown to be members of previously described genera, not new and divergent taxa. It is therefore reasonable to suggest that the morphology-based isolation techniques have allowed the discovery of most types of the fruiting myxobacteria. That is to say the myxobacteria that have been isolated to date represent most of the fruiting myxobacteria which exist in nature, and these have been used in the construction of the present myxobacterial taxonomy. By carefully comparing the 16S rRNA gene sequences of the uncultured and cultured myxobacteria, we can see that most of the sequences obtained directly from soil were not similar to those from cultivated taxa (Fig. 1; Wu et al., 2005). The uncultured myxobacteria may represent groups of myxobacteria that are genetically unable to form fruiting bodies. Alternatively, these myxobacteria might have been previously overlooked because their growth requirements were not met by the laboratory cultivation methods. In fact, we examined the soil sample (about 0.5 g) carefully under a dissecting microscope to establish if myxobacterial fruiting bodies existed, but found no visible fruiting bodies. However, we found many uncultured

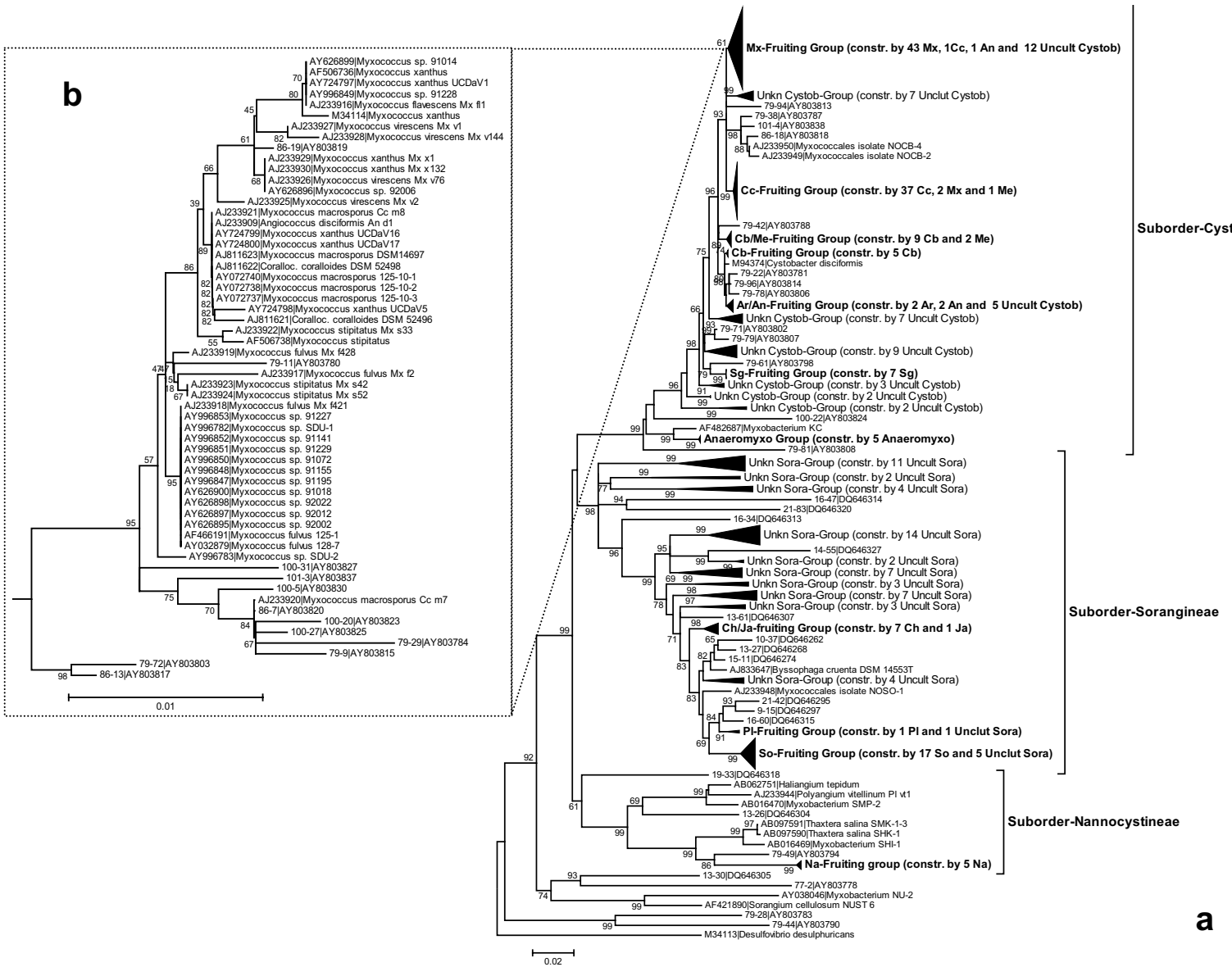

Fig. 2. Phylogeny of cultured and uncultured myxobacteria, inferred from 16S rRNA gene sequences. All of the available cultured myxobacterial 16S rRNA gene sequences were used in this analysis. The bar in (a) is equivalent to two, and in (b) to one nucleotide changes per 100 bp. The numbers on branch nodes indicate bootstrap support percentages based on 1000 replicates. The homologous sequences are grouped and labeled with the numbers of members included (in bigger letter size). The fruiting myxobacteria groups are in boldface. The detailed construction of the Mx-Fruiting group is shown for demonstration. *Abbreviations:* constr., constructed; unkn, unknown; uncult, uncultured; Cystob, Cystobacterineae; Sora, Sorangineae; Mx, *Myxococcus*; Cc, *Corallococcus*; An, *Angiococcus*; Me, *Melittangium*; Cb, *Cystobacter*; Ar, *Archangium*; Sg, *Stigmatella*; Anaeromyxo, *Anaeromyxobacter*; Ch, *Chondromyces*; Ja, *Jahnia*; Pl, *Polyangium*; So, *Sorangium*; Na, *Nannocystis*.

myxobacterial sequences and seven cultured myxobacterial strains in the soil sample. According to Dawid's studies on the isolation of myxobacteria from 1398 soil samples from 64 countries or states in all continents, one to ten fruiting myxobacterial species can be found in a soil sample (Dawid, 2000). Reichenbach's experiences in the recent decades also indicate that 0.5–1 g of the richest myxobacterial biota may yield 5–10 myxobacterial species, defined by their morphological characteristics (Reichenbach, 1993). Taken together with results of the current study, it appears that some myxobacteria survive in nature without forming fruiting bodies.

2. Cultured myxobacteria are able to produce fruiting bodies with a variety of morphological features. Phylogenetic analysis of cultured myxobacteria indicates that the taxa described in part using fruiting body morphology are mostly monophyletic. Morphogenesis of a fruiting body is a complicated developmental process which is genetically regulated and controlled. A small change in the morphogenesis-related genes may result in deficiencies in fruiting body formation (Guo et al., 1996; Lu et al., 2005), which then renders the myxobacteria undetectable with classical isolation techniques. They may, however, still survive in nature, together with their fruiting ancestors, and thus form the basis for our sequences of uncultured myxobacteria. The uncultured myxobacteria that have high sequence similarity to the cultured, fruiting myxobacteria may be those cells that are deficient in the formation of fruiting bodies; whereas the uncultured myxobacteria that are distinctly different from the cultured fruiting myxobacteria and located in unique phylogenetic linaeges may be those cells that are truly the non-fruiting myxobacteria. The uncultured myxobacteria may be truly uncultivable, or the optimum culture conditions have not yet been established. As for the taxonomy of myxobacteria, the isolated fruiting myxobacteria represent only a small fraction of the diversity of this phylogenetic group. To isolate the uncultured but cultivable myxobacteria will require the development of new morphogenesis-independent isolation techniques.
3. Myxobacteria are phylogenetically placed in the δ-division of the proteobacteria. Compared with the other members of the this division, myxobacteria show complicated social behavior, at least among those species that have been isolated and cultured. However, sociality is not a consistent characteristic. The sociality of *Myxococcus xanthus*, and particularly the ability to form fruiting bodies can rapidly degrade under asocial growth conditions (Velicer et al., 1998). Velicer et al. (2002) suggested that there is a direct trade-off between competitive fitness in one particular environment and the maintenance of a social trait. The phylogenetic analysis of the uncultured soil myxobacteria 16S rRNA gene sequences described in this paper not only supports the suggestions of Velicer et al. (1998, 2002), but also may indicate that the myxobacteria include non-fruiting types.

Our studies of uncultured myxobacteria in soil greatly expand the phylogenetic breadth of this group. The appearance of the fruiting ability in myxobacteria is relatively recently derived trait that is found only in the myxobacteria. Whether fruiting body formation is an ancestral trait for the group that, along with gliding motility, defines the myxobacteria, or whether it has evolved independently in different clades is an interesting question that the current study has not resolved. The isolation and culture of the non-fruiting types myxobacteria would not only offer the opportunity to confirm our hypothesis, but it would lead to a better understand of myxobacteria and their evolution.

Acknowledgments

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References

Brosius, J., Palmer, M.L., Kennedy, P.J., Noller, H.F., 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 75, 4801–4805.

Dawid, W., 2000. Biology and global distribution of myxobacteria in soils. FEMS Microbiol. Rev. 24, 403–427.

Dworkin, M., 1996. Recent advances in the social and developmental biology of the myxobacteria. Microbiol. Rev. 60, 70–102.

Fudou, R., Jojima, Y., Iizuka, T., Yamanaka, S., 2002. *Haliangium ochraceum* gen. nov., sp. nov. and *Haliangium tepidum* sp. nov.: novel moderately halophilic myxobacteria isolated from coastal saline environments. J. Gen. Appl. Microbiol. 48, 109–116.

Guo, D., Bowden, M.G., Pershad, R., Kaplan, H.B., 1996. The *Myxococcus xanthus* rfbABC operon encodes an ATP-binding cassette transporter homolog required for O-antigen biosynthesis and multicellular development. J. Bacteriol. 178, 1631–1639.

Iizuka, T., Jojima, Y., Fudou, R., Hiraishi, A., Ahn, J.W., Yamanaka, S., 2003a. *Plesiocystis pacifica* gen. nov., sp. nov., a marine myxobacterium that contains dihydrogenated menaquinone, isolated from the Pacific coasts of Japan. Int. J. Syst. Evol. Microbiol. 53, 189–195.

Iizuka, T., Jojima, Y., Fudou, R., Tokura, M., Hiraishi, A., Yamanaka, S., 2003b. *Enhygromyxa salina* gen. nov., sp. nov., a slightly halophilic myxobacterium isolated from the coastal areas of Japan. Syst. Appl. Microbiol. 26, 189–196.

Iizuka, T., Jojima, Y., Fudou, R., Yamanaka, S., 1998. Isolation of myxobacteria from the marine environment. FEMS Microbiol. Lett. 169, 317–322.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings Bioinformat. 5, 150–163.

Li, Y.Z., Li, J., Zhou, L., Zhang, Y., Hu, W., Chen, Q., 2000. Isolation and identification of myxobacterial sources in China. Acta Microbiol. Sin. (in Chinese) 40, 652–656.

Li, Y.Z., Hu, W., Zhang, Y.Q., Qiu, Z., Zhang, Y., Wu, B.H., 2002. A simple method to isolate salt-tolerant myxobacteria from marine samples. J. Microbiol. Methods 50, 205–209.

Lu, A., Cho, K., Black, W.P., Duan, X.Y., Lux, R., Yang, Z., Kaplan, H.B., Zusman, D.R., Shi, W., 2005. Exopolysaccharide biosynthesis

genes required for social motility in *Myxococcus xanthus*. Mol. Microbiol. 55, 206–220.

McCurdy, H.D., 1989. Order Myxococcales. In: Staley, J.T., Bryant, M.P., Pfennig, N., Holt, J.G. (Eds.), Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, pp. 2139–2170.

Reichenbach, H., 1993. Biology of the myxobacteria: ecology and taxonomy. In: Dworkin, M., Kaiser, D. (Eds.), Myxobacteria II. American Society for Microbiology, Washington, DC, pp. 13–62.

Reichenbach, H., 1999. The ecology of the myxobacteria. Environ. Microbiol. 1, 15–21.

Reichenbach, H., 2004. The Myxococcales. In: Garrity, G.M. (Ed.), Bergey's Manual of Systematic Bacteriology. Springer-Verlag, New York, pp. 1059–1143.

Reichenbach, H., Dworkin, M., 1992. The myxobacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds.), The Prokaryotes, second ed. Springer-Verlag, New York, pp. 3416–3487.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, third ed. Cold Spring Harbor, NY, USA.

Sanford, R.A., Cole, J.R., Tiedje, J.M., 2002. Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halorespiring facultative anaerobic myxobacterium. Appl. Environ. Microbiol. 68, 893–900.

Shimkets, L.J., 1990. Social and developmental biology of myxobacteria. Microbiol. Rev. 54, 473–501.

Shimkets, L.J., Dworkin, M., Reichbach, H., 2005. The myxobacteria. In: The Prokaryotes, third ed. Springer-Verlag, New York, NY, release 3.19.

Shimkets, L., Woese, C.R., 1992. A phylogenetic analysis of the myxobacteria: basis for their classification. Proc. Natl. Acad. Sci. USA 89, 9459–9463.

Spröer, C., Reichenbach, H., Stackebrandt, E., 1999. The correlation between morphogenetic classification of myxobacteria. Int. J. Syst. Bacteriol. 49, 1255–1262.

Stackebrandt, E., Päuker, O., 2005. Gene sequence heterogeneity of *Corallococcus coralloides* strains isolated from geographically diverse locations. Environ. Microbiol. 7, 1017–1023.

Thaxter, R., 1892. Contributions from the cryptogamic laboratory of Harvard University. XVI-II. On the Myxobacteriaceae, a new order of Schizomycetes. Bot. Gaz. 14, 389–406.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Velicer, G.J., Kroos, L., Lenski, R.E., 1998. Loss of social behaviors by *Myxococcus xanthus* during evolution in an unstructured habitat. Proc. Natl. Acad. Sci. USA 95, 12376–12380.

Velicer, G.J., Lenski, R.E., Kroos, L., 2002. Rescue of social motility lost during evolution of *Myxococcus xanthus* in an asocial environment. J. Bacteriol. 184, 2719–2727.

Watve, M.G., Shete, A.M., Jadhave, N., Wagh, S.A., Shelar, S.P., Chakraborti, S.S., Botre, A.P., Kulkarni, A.A., 1999. Myxobacterial diversity in Indian soils—how many species do we have? Curr. Sci. 77, 1089–1095.

Wu, Z.H., Jiang, D.M., Li, P., Li, Y.Z., 2005. Exploring the diversity of myxobacteria in a soil niche by myxobacteria-specific primers and probes. Environ. Microbiol. 7, 1602–1610.

Yan, Z.C., Wang, B., Li, Y.Z., Gong, X., Zhang, H.Q., Gao, P.J., 2003. Morphologies and phylogenetic classification of cellulolytic Myxobacteria. Syst. Appl. Microbiol. 26, 104–109.

Zhang, J., Wang, R., Jiang, P., Liu, Z., 2002a. Production of an exopolysaccharide bioflocculant by *Sorangium cellulosum*. Lett. Appl. Microbiol. 34, 178–181.

Zhang, J., Liu, Z., Wang, S., Jiang, P., 2002b. Characterization of a bioflocculant produced by the marine myxobacterium *Nannocystis* sp. NU-2. Appl. Microbiol. Biotechnol. 59, 517–522.