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Taxonomic Note

Designation of type strains for seven species of the order *Myxococcales* and proposal for neotype strains of *Cystobacter ferrugineus*, *Cystobacter minus* and *Polyangium fumosum*

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Running Title: (Neo-)Type strains for species of myxobacteria

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Summary

Ten species of the order *Myxococcales* with validly published names are devoid of living type strains. Four species of the genus *Chondromyces* are represented by dead herbarium samples as the type material. For a *Melittangium* species and two *Polyangium* species, no physical type material was assigned at the time of validation of the names or later on. According to rule 18f of the International Code of Nomenclature of Bacteria the following type strains are designated for these species: strain Cm a14^T (= DSM 14605^T = JCM 12615^T) as the type strain of *Chondromyces apiculatus*, strain Cm c5^T (= DSM 14714^T = JCM 12616^T) as the type strain of *Chondromyces crocatus*, strain Sy t2^T (= DSM 14631^T = JCM 12617^T) as the type strain of *Chondromyces lanuginosus*, strain Cm p51^T (= DSM 14607^T = JCM 12618^T) as the type strain of *Chondromyces pediculatus*, strain Me b8^T (= DSM 14713^T = JCM 12633^T) as the type strain of *Melittangium boletus*, strain Pl s12^T (= DSM 14670^T = JCM 12637^T) as the type strain of *Polyangium sorediatum* and strain Pl sm5^T (= DSM 14734^T = JCM 12638^T) as the type strain of *Polyangium spumosum*. Furthermore, the type strains given for three species of the genera *Cystobacter* and *Polyangium* had been kept at one university institute and have been lost according to our investigations. According to Rule 18c of the Bacteriological Code, we propose the following neotype strains: strain Cb fe18 (= DSM 14716 = JCM 12624) as the neotype strain of *Cystobacter ferrugineus*, strain Cb m2 (= DSM 14751 = JCM 12627) as the neotype strain of *Cystobacter minus*, and strain Pl fu5 (= DSM 14668 = JCM 12636) as the neotype strain of *Polyangium fumosum*. The proposals of the strains is based on the descriptions and strain proposals given in the respective chapters of Bergey's Manual of Systematic Bacteriology (2005).

An exceptionally high number of myxobacterial species descriptions is not supported by the availability of formally acknowledged living type strain material. Because of this lack of type material, the species could not be included, for example, in species-representing 16S rRNA gene sequence databases. These are the most frequently used guides in taxonomy in our days, and for that reason, great efforts are taken to fill the sequencing gaps (Yarza *et al.*, 2013). The International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) allows for the designation of **type strains** in cases where descriptions or dead specimens represent the type of described and validly published species names, and for the proposal of **neotype** strains if a strain on which the original description was based cannot be found. These measures have been installed in order to clear the way for inclusion of such species in future examinations, in particular in studies including “new “ methods which have not been applied at the time of the species description. In this communication, we formally designate type strains for seven and formally propose neotype strains for three species of the order *Myxococcales*.

The present wording of Rule 18f of the International Code is: “ If a description or illustration constitutes, or a dead preserved specimen has been designated as the type of a species [Rule 18a(1)] and a later strain of this species is cultivated, then the type strain may be designated by the person who isolated the strain or by a subsequent author. This type strain shall then replace the description, illustration or preserved specimen as the nomenclatural type. The designation of a type strain in this manner must be published in the IJSB/IJSEM, the authorship and date of priority of publication being determined by the effective and valid publication of the name by the original authors (Rule 24b).”

The presently designated type strains of the species *Chondromyces apiculatus* (Thaxter, 1897), *Chondromyces crocatus* (Berkeley, 1874), *Chondromyces lanuginosus* (Kofler, 1913) and *Chondromyces pediculatus* (Thaxter 1904) are dead herbarium specimens in the Thaxter collection (TC), housed in the Farlow Herbarium, Harvard University, Cambridge, U.S.A. (Table 1). Howard McCurdy studied myxobacteria at the University of Windsor, Ontario, Canada about 1960-1970. He assigned specific samples of the Thaxter collection as the types of these species (McCurdy, 1971). The species names were included in the Approved Lists (Skerman *et al.*, 1980).

According to a curator of the herbarium, the specimen for *C. lanuginosus* seems to be lost whereas the other three specimens are still there, dried on the original substrates, accompanied by some slides.

For the species *Melittangium boletus* (Jahn 1924), *Polyangium sorediatum* (Brockman, 1989) and *Polyangium spumosum* (Brockman, 1989) no physical type strains were assigned in the Approved Lists (Skerman *et al.*, 1980) or in Validation List No. 31, respectively. Instead, the descriptions of Brockman (1989) or simply the statement “not cultivated” are given.

Bergey’s Manual of Systematic Bacteriology, second edition, includes comprehensive chapters about the members of the order *Myxococcales*. Reichenbach 2005a –e are the chapters relevant for the taxa mentioned in this paper. These chapters are based on the experience and knowledge accumulated during 40 years of intense investigations on myxobacteria and were written after having isolated more than 3000 myxobacterial strains. Based on the original species descriptions, appropriate strains were selected and described as the type strains of the respective species (Table 1). However, it was not formally proposed in the IJSEM until now to accept these strains as the type strains.

On the reason that presently dead preserved material constitutes, or a description has been designated as, the type strain of the mentioned species, or no type strain has been assigned, it is formally proposed that the strains selected by Reichenbach shall be designated as the type strains of the respective species according to Rule 18f. The proposed type strains listed in Table 1 shall replace the dead specimen or descriptions. These are *Chondromyces apiculatus* Cm a14^T, *Chondromyces crocatus* Cm c5^T, *Chondromyces lanuginosus* Sy t2^T, *Chondromyces pediculatus* Cm p51^T, *Melittangium boletus* Me b8^T, *Polyangium sorediatum* Pl s12^T and *Polyangium spumosum* Pl sm5^T. The prerequisite for the acceptance of type strains, their deposit and availability in two culture collections is achieved. The designation of the type strains is based on the descriptions given in the respective chapters of Bergey’s Manual (Reichenbach 2005a,c,d). In order to facilitate the comparison of these recent descriptions with those of the authors who originally proposed, revived or emended the species these original descriptions are assembled in Supplementary Table 1. The fatty acid composition of the proposed type strains are given in Supplementary Table 2. The figures of the original descriptions and of the proposed type strains are shown face to face with figures of the proposed type strains in Figure 1.

The Bacteriological Code also allows for the proposal of **neotype** strains according to Rule 18c: “If a strain on which the original description was based cannot be found, a neotype strain may be proposed. A neotype strain

must be proposed (proposed neotype) in the IJSB, together with citation of the author(s) of the name, a description or reference to an effectively published description, and a record of the permanently established culture collection(s) where the strain is deposited (see also Note 1 to Rule 24a).”

The species *Cystobacter ferrugineus*, *Cystobacter minus* and *Polyangium fumosum* were first described by Krzemieniewska & Krzemieniewski (Krzemieniewska & Krzemieniewski, 1926, 1927, 1930). McCurdy assigned three of his isolates as the type strains for the above mentioned three species, respectively (McCurdy, 1970; Table 1). The species names and type strains were included in the Approved Lists (Skerman *et al.*, 1980) but they have never been deposited in a culture collection to the best of our knowledge. In 2007, we wrote a letter to the head of the microbiology laboratory of the University Windsor with the request for subcultures of the strains *C. ferrugineus* M-203^T, *C. minus* M-307^T and *P. fumosum* M257^T. Even though the impact on microbial taxonomy was stressed there was no response. In 2012, another attempt to contact the department at Windsor University was more successful in the respect that we received answers from two colleagues at Windsor and from H. McCurdy who retired several years ago. However, they informed us that they cannot find the samples. Since 1981, there were no scientific papers originating from the University of Windsor dealing with myxobacteria (PubMed), a fact additionally suggesting that nobody at the University had a research interest to keep the cultures alive or, at least, under surveillance. For that reasons we conclude that these cultures must have been lost.

Since the presently assigned type strains of the mentioned species are no more available as living cultures it is formally proposed that the strains selected by Reichenbach shall be proposed as the neotype strains of the respective species according to Rule 18c, as given in Table 1. The deposit and availability of the neotype strains from two culture collections is achieved. The proposals of the neotype strains are based on the suggestions in Reichenbach 2005b and 2005d. In these chapters, the strains *Cystobacter ferrugineus* Cb fe18, *Cystobacter minus* Cb m2 and *Polyangium fumosum* Pl fu5 were proposed as the type strains according to the species descriptions given in the respective chapters which rely on the original species descriptions by Krzemieniewska and Krzemieniewski and Mc Curdy (Reichenbach 2005b,d). However, since type strains have already be assigned these strains have to be proposed as the neotype strains of the respective species according to rule 18c.

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Table 1: Myxobacterial species for which a cultivable type strain or neotype strain is formally proposed, and the 16S rRNA sequences of the proposed neotype strains. ^aAL: type strain as given in Approved Lists (Skerman *et al.*, 1989). VL: types as given in Validation List No 31.

Species name and authors of the species description	Type strain in Approved Lists	Designated type strain	DSM number of the type strain	JCM number of the type strain	Figure No.	Accession number of the 16S rRNA gene sequence	Reference
<i>Chondromyces apiculatus</i> Thaxter 1897	TC 4481 ^T AL ^a	Cm a14 ^T	14605 ^T	12615 ^T	1	AJ233938	Reichenbach 2005a
<i>Chondromyces crocatus</i> Berkeley and Curtis 1874; type species of the genus	TC 601 ^T AL	Cm c5 ^T	14714 ^T	12616 ^T	2	GU207874	Reichenbach 2005a
<i>Chondromyces lanuginosus</i> Kofler 1913	TC 4494 ^T AL	Sy t2 ^T	14631 ^T	12617 ^T	3	AJ233939	Reichenbach 2005a
<i>Chondromyces pediculatus</i> Thaxter 1904	TC 4524 ^T AL	Cm p51 ^T	14607 ^T	12618 ^T	4	GU207875	Reichenbach 2005a
<i>Melittangium boletus</i> Jahn 1924; type species of the genus	“not cultivated” AL	Me b8 ^T	14713 ^T	12633 ^T	5	AJ233908	Reichenbach 2005c
<i>Polyangium solediatum</i> (ex Thaxter 1904) Brockman 1989	description in Brockman 1989 VL	Pl s12 ^T	14670 ^T	12637 ^T	6	GU207880	Reichenbach 2005d
<i>Polyangium spumosum</i> (ex Krzemieniewska and Krzemieniewski 1927) Brockman 1989	description in Brockman 1989 VL	Pl sm5 ^T	14734 ^T	12638 ^T	7	GU207881	Reichenbach 2005d
		Proposed neotype strain	DSM number of the neotype strain	JCM number of the neotype strain			
<i>Cystobacter ferrugineus</i> (Krzemieniewska and Krzemieniewski 1927) McCurdy 1970	Windsor M-203 ^T AL	Cb fe18	14716	12624	8	NR_025343<- AJ233901; DQ768112	Reichenbach 2005b
<i>Cystobacter minus</i> (Krzemieniewska and Krzemieniewski 1926) McCurdy 1970	Windsor M-307 ^T AL	Cb m2	14751	12627	9	AJ233903; DQ768113	Reichenbach 2005b
<i>Polyangium fumosum</i> Krzemieniewska and Krzemieniewski 1930	Windsor M257 ^T AL	Pl fu5	14668	12636	10	GU207879	Reichenbach 2005d

a

b

Figure 1: *Chondromyces apiculatus*. a, Drawing by Thaxter (1897), plate XXX on pages 405-406; b, fruiting body and vegetative cells (insert) of Cm a14^T, bars 100 μ m (fruiting body) and 10 μ m (cells).

Fig. 70.

a. *Chondromyces crocatus*, Berk. and Curt., with its spores. Both magnified. From specimens on a decayed gourd from South Carolina.

b. *Stigmatella aurantiaca*, Berk. and Curt. From specimens on *Sphaeria Hibisci*, South Carolina. Magnified.

c. *Cheiromyces stellatus*, Berk. and Curt.

d. Spores of ditto. Both magnified. From specimens on *Scirpus eriophorus*, sent from Pennsylvania by Dr. Michener.

a

b

Figure 2: *Chondromyces crocatus*. a, Drawing by Berkeley (1857), page 313; b, fruiting bodies of Cm c5, bar 500 μ m

a

b

Figure 3: *Chondromyces lanuginosus*. a, Figures by Kofler (1913), page 877, courtesy Österreichische Akademie der Wissenschaften. No 1-3 depict *C. lanuginosus*; b, fruiting body of Sy t2^T, bar 100 µm.

a

b

Figure 4: *Chondromyces pediculatus*. a, Drawing by Thaxter (1904), plate XXVI on page 411. No 7-13 depict *C. pediculatus*; b, fruiting body of Cm p51^T, bar 100 μ m.

a

B

c

Figure 5: *Melittangium boletus*. a, Drawing by Jahn (1924), plate II, figure 17 on page 78, courtesy www.borntraeger-cramer.de; b and c, fruiting body of Me b8^T, bar in b= 120 μ m, in c= 80 μ m.

THAXTER on MYXOBACTERIACEAE.

a

b, c

Figure 6: *Polyangium sorediatum*. a, Drawing by Thaxter (1904), plate XXVII, no 22-30 depict *P. sorediatum*; b and c, fruiting bodies of PI s12^T, bars 200 μ m. Insert: crushed sporangium releasing the single sporangioles.

a

b, c and d

Figure 7: *Polyangium spumosum*. a, Figures by Krzemieniewska & Krzemieniewski. (1926b), courtesy Polish Botanical Society, plate V, no 19 depicts *P. spumosum*, and Krzemieniewska & Krzemieniewski. (1930), courtesy Polish Botanical Society, plate XVI, no 10-12 depict *P. spumosum*; b, c and d, degenerated fruiting bodies of PI sm5^T, bars b= 500 μ m, c= 100 μ m, d= 250 μ m.

1. *Cystobacter ferrugineus*, vegetative cells 900x.
2. *Cystobacter ferrugineus*, resting cells 800x.
3. *Cystobacter ferrugineus*, membrane with microcyst imprints 800x.
4. *Cystobacter fuscus*, sporangia 185x.
5. *Cystobacter fuscus*, vegetative cells 800x.
6. *Cystobacter fuscus*, microcysts 800x.
7. *Polyangium aureum*, sporangia 900x.
8. *Polyangium rugiseptum*, myxospores 800.

a

b, c, d

Figure 8: *Cystobacter ferrugineus*. a, Figures by McCurdy. (1970), page 290; b, c, d, Cb fe18, myxospores (b) and fruiting bodies on *E. coli* as food bacteria (c) and on a cellulose plate (d), bars b= 10 μ m, c = 1 mm and d= 10 mm.

9. *Cystobacter minus*, vegetative cells 700x.
10. *Cystobacter minus*, microcysts and membrane 700x.
11. *Cystobacter minus*, sporangium formed with old sporangial wall 600x.
12. *Polyangium fumosum*, myxospores 750x.
13. *Polyangium fumosum*, xporangia 750x.
14. *Polyangium fumosum*, myxospores 700x.
- 15a. *Polyangium vitellinum*, vegetative cells 700x.
- 15b. *Polyangium vitellinum*, myxospores 700x.
16. *Polyangium vitellinum*, xporangia 50x.
17. *Polyangium vitellinum*, portion of sporangial membrane 700x.

a

b, c

Figure 9: *Cystobacter minus*. a, Figures by McCurdy. (1970), page 291; b and c, fruiting bodies of Cb m2, bar 500 μ m.

Figure 10: *Polyangium fumosum*. a, Drawing by Krzemieniewska & Krzemieniewski. (1930), courtesy Polish Botanical Society, plate XVI, figures 6 – 9 depict *P. fumosum*; b swarm and single sporangium (insert) of Pl fu5, c, fruiting bodies of Pl fu5, bars b= 2000 μm , insert = 100 μm , c = 300 μm .