Byssovorax cruenta gen. nov., sp. nov., nom. rev., a cellulose-degrading myxobacterium: rediscovery of 'Myxococcus cruentus' Thaxter 1897

Hans Reichenbach,¹ Elke Lang,² Peter Schumann² and Cathrin Spröer²

Correspondence

Hans Reichenbach
hre@gbf.de

¹GBF – Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany

²DSMZ – German Collection of Microorganisms and Cell Cultures, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

A rare, cellulose-decomposing myxobacterium is described, and a new genus name, *Byssovorax*, is proposed for it. The organism is almost certainly identical to the species 'Myxococcus cruentus' Thaxter 1897, and that species epithet is therefore revived for the novel bacterium: the type strain of *Byssovorax cruenta* gen. nov., sp. nov., nom. rev. is strain By c2^T (=DSM 14553^T=CIP 108850^T). The G + C content of its DNA is 69.9 mol%. The 16S rRNA gene sequence shows that the species belongs to the family *Polyangiaceae*, suborder 'Sorangineae', of the *Myxococcales*. An emended description of the organism is given.

In 1897, Roland Thaxter, the discoverer of the peculiar life cycle of the myxobacteria, described in his third article on those organisms the species 'Myxococcus cruentus' (Thaxter, 1897). He could not cultivate the bacterium, but gave a clear description of its morphological characteristics from material collected in nature, including two drawings on an accompanying plate. The most striking feature of the novel organism was the intense, blood-red colour of its cell mass and fruiting bodies. The latter consisted of spherical cysts, today we would call them sporangioles, 90 to 125 µm in diameter, with 'a more or less well defined rind or wall'. The figure suggests that the sporangioles were arranged in heaps, or sori. The myxospores within the sporangioles were embedded in an amorphous matrix, were rod-shaped and measured 0.9–1 × 1.2–1.4 µm. They were 'more than usually irregular in size and form, and ... less well defined than in the other species, resembling in some respects the thickened individuals which occur in the cysts of *Chondromyces*'. The species was not cultivated, and no satisfactory material of its vegetative condition was obtained'. Yet, Thaxter gives the size of the (vegetative) rods as 0.8 × 3–8 µm. Apparently, Thaxter found the species only once, on cow dung near Burbank, TN, USA. Both the shape of the myxospores and the production of sporangioles with a wall are not compatible with the genus *Myxococcus* as defined today.

'*M. cruentus*' was apparently not seen by anybody for a whole century. It was not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1989). Yet the species does exist. Recently, after 30 years of isolating myxobacteria and accumulating a collection of more than 7000 strains, we found an organism with a most astonishing red colour unlike that of any other myxobacterium. Although it initially grew only reluctantly, we succeeded in isolating and cultivating it. It turned out to be a cellulose degrader. In addition to its colour, the organism showed several other peculiarities, as will be pointed out below. We therefore suggest that it be classified in a new genus, *Byssovorax* gen. nov. (referred to as '*Byssophaga*' by Reichenbach, 2005; however, the name *Byssophaga* would be illegitimate, having been used for a moth of the Arctiidae), and that Thaxter's species epithet, *cruenta*, be revived, for there is little doubt of the identity of our strains with Thaxter's species. The shape of the vegetative cells is typical of members of the suborder 'Sorangineae' (Reichenbach, 2005), and 16S rRNA gene sequence data show that the novel bacterium indeed belongs to the family *Polyangiaceae* of that suborder (see Fig. 6).

The type strain of *Byssovorax cruenta*, By c2^T, was isolated in April 2001 from a soil sample with rotting plant material collected in June 1996 at the roadside in a sagebrush steppe on red sandstone a few miles south of Holbrook, AZ, USA. The sample had been air-dried and stored at room temperature for almost 5 years, which shows that the organism must have produced a desiccation-resistant stage, presumably fruiting bodies and/or myxospores, for vegetative cells of myxobacteria are very sensitive to drying. The strain was obtained by both of our standard methods for isolating myxobacteria, by depositing aliquots of the sample

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain By c2^T is AJ833647.

Further images of various growth phases of *Byssovorax cruenta* By c2^T and a *Polyangium* strain are available as supplementary material in IJSEM Online.

either on spots of living *Escherichia coli* streaked on water agar or on filter paper placed on a mineral salts agar with KNO_3 as the nitrogen source (ST21 agar) and incubating the cultures at 30°C (Reichenbach & Dworkin, 1992). Both media contained $25\text{ }\mu\text{g}$ cycloheximide ml^{-1} in order to suppress growth of fungi. A second strain, Ha r1, had been isolated from the same sample in January 1997. This strain, however, became a patent strain and is not freely available to the general public.

The organism was recognized as unusual because of its intense blood-red colour. Growth pattern and the shape of the vegetative cells suggested that it was a myxobacterium, although initially fruiting bodies were not found. It produced holes in the filter paper, which indicated that it was a cellulose degrader. The only other cellulose-decomposing myxobacteria known so far are the species of the genus '*Sorangium*'. Thus, while no blood-red '*Sorangium*' strains were ever seen by us – and we have isolated 1900 such strains – the novel bacterium has to be compared in particular with that genus.

Initially, the novel bacterium grew rather slowly, so that it took several months before pure cultures were obtained. It developed reasonably well on streaks of living *E. coli* on water agar under lysis of the food bacterium, which is in contrast to '*Sorangium*', that neither grows on nor lyses living coli bacteria. The organism also grew quite well on autoclaved *E. coli*, as does '*Sorangium*', but then the growth pattern of the two differed fundamentally. While '*Sorangium*' strains produce typical myxobacterial swarm colonies with more or less distinct radial veins (Fig. 1c), the novel organism grew in the form of scattered, small, red pseudoplasmodia with fan-like fronts and long, tapering tails (Fig. 1a, b). These pseudoplasmodia moved independently of one another over the substrate, leaving wrinkled, parchment-like slime trails behind (see Supplementary Fig. S1 in IJSEM Online). They could also penetrate the agar plate deeply and tunnel over long distances within it. Eventually, the pseudoplasmodia contracted into intensely blood-red knobs, usually $250\text{--}600\text{ }\mu\text{m}$ in size, not unlike *Myxococcus* fruiting bodies (Fig. 2a; a similar colour image is available as Supplementary Fig. S2 in IJSEM Online). Alternatively, they converted into massive, deep red rings, about $350\text{--}500\text{ }\mu\text{m}$ in diameter, with central holes of $60\text{--}120\text{ }\mu\text{m}$ (Supplementary Fig. S3). Rarely, they produced peculiar bowl- or cup-shaped structures, around $800\text{ }\mu\text{m}$ wide and $300\text{ }\mu\text{m}$ high (Supplementary Fig. S4). All this has never been seen in a '*Sorangium*' culture. *Polyangium* strains often grow in the form of isolated small, tight packs of cells (Fig. 2b), and we isolated a few strains, tentatively identified as members of *Polyangium*, that even produced pseudoplasmodia, like those described for strain By c2^T (Supplementary Fig. S5). However, these organisms were neither red nor did they attack cellulose. After many transfers, the novel bacterium sometimes changed its growth pattern, developing coherent swarm colonies with veins, as are known from other myxobacteria (Supplementary Fig. S6).

Fig. 1. Colonies of strain By c2^T (*Byssovorax cruenta* sp. nov.) (a, b) and '*Sorangium cellulosum*' So ce928 (c). While the latter myxobacterium grows as a coherent swarm, the former produces disconnected pseudoplasmodia. Slime tracks can be seen behind the pseudoplasmodia in (a); also, the pseudoplasmodia may produce a shallow depression in the agar surface (b). Bars, $110\text{ }\mu\text{m}$ (a), $260\text{ }\mu\text{m}$ (b) and $570\text{ }\mu\text{m}$ (c).

The vegetative cells from pseudoplasmodia were cylindrical rods with blunt, rounded ends, dark under the phase-contrast microscope, measuring $0.9\text{--}1.1 \times 3.5\text{--}7.5\text{ }\mu\text{m}$ (Fig. 3). Thus, they were larger and particularly fatter than the vegetative cells of '*Sorangium*' and *Polyangium*. The cells within the knobs, rings and cups were essentially of the same shape as the vegetative cells, only sometimes slightly optically refractile. These structures were clearly not the fruiting bodies of the novel organism. Real fruiting bodies were not seen initially, not even in crude cultures on filter

Fig. 2. (a) Pseudoplasmodia of *Byssovorax cruenta* By c2^T may eventually contract into bright-red knobs resembling *Myxococcus* fruiting bodies. Bar, 200 μ m. A similar image in colour is available as Supplementary Fig. S2 in IJSEM Online. (b) In contrast to *Byssovorax*, *Polyangium* strains as a rule produce compact pseudoplasmodia, and only late in development as an initiation of fruiting body formation. Note short slime tails at the rear ends of the cell masses and depressions in the agar surface. Bar, 380 μ m.

paper, in which ‘*Sorangium*’ strains almost always produce copious numbers of fruiting bodies in the form of dense packets, or sori, of tiny, angular sporangioles (Fig. 4a, b). Only after years of cultivation, and for unknown reasons, did we finally obtain some true fruiting bodies of the novel organism on filter paper on yeast (VY/2) agar, the usual growth medium. They were sori consisting of large, red sporangioles with a clearly distinguishable wall (Fig. 4c–f). The sori were more or less roundish and very variable in size, 50 to more than 1000 μ m wide, usually measuring between 190 $\times$ 250 and 400 $\times$ 550 μ m. The sporangioles were spherical to angular and varied, too, substantially in size, measuring 70 $\times$ 90 to 160 $\times$ 190 μ m, mostly between 80 and 140 μ m in diameter. Thus, they were much larger than the sporangioles of ‘*Sorangium*’. Even within the same sorus, the sporangioles were often vastly different in size, but smaller

Fig. 3. Vegetative cells of *Byssovorax cruenta* By c2^T from yeast agar, in phase-contrast. Bar, 10 μ m.

sori also tended to contain smaller sporangioles. Immature fruiting bodies produced on a minimal medium (MM20 agar; see below) were much more delicate, with sori around 50–60 μ m, and the sporangioles that arose were 9–27 μ m in diameter (Fig. 4c). The cells from the sori, presumably myxospores, were similar to vegetative cells in shape, only stouter and somewhat optically refractile, 1.5–1.7 $\times$ 3.3–5 μ m in size (Fig. 5). Occasionally, scattered single, spherical sporangioles were produced, 60–80 μ m in diameter, sometimes with a second outer wall at some distance (Supplementary Fig. S7). A smaller, secondary sporangiole had obviously arisen within the primary sporangiole, a phenomenon known from many myxobacteria. In cultures on filter paper, roundish crystals were often seen within the macerated cellulose, singly or in clusters, which could be mistaken for sporangioles; they are, however, smaller than those, and colourless (Supplementary Fig. S8). Further, in old, degenerating cultures, numerous bright red, spherical droplets of an oily material, 9–14 μ m in diameter, appeared in the swarm area (Supplementary Fig. S9).

The pure strain grew well on agar containing whole cells of bakers’ yeast (VY/2 agar; Reichenbach & Dworkin, 1992) with production of clear lysis zones. Transfers to fresh plates were not always successful, however, especially when the parent plate was more than 2 weeks old (30 °C). Also, for unknown reasons, the colonies sometimes spread only a little beyond the inoculum. Growth was more reliable and vigorous on filter paper placed on VY/2 agar. The filter paper became completely decomposed in the course of 2 to 3 weeks (30 °C), and the organism produced deeply blood-red cell masses, knobs and veins on it. It also passed on to the agar surface and spread considerably beyond the filter paper. Again, the yeast cells were degraded. After several weeks, the bacterial cells disintegrated completely, the colonies became carmine-red to purplish and lots of the red oil droplets mentioned above appeared. The organism also grew on filter paper placed on mineral salts agar with nitrate as the

Fig. 4. (a–b) Fruiting bodies of '*Sorangium cellulosum*' strains So ce1595 (a) and So ce1385 (b) from macerated filter paper, slightly pressed into the slide mount (a) and *in situ* on the agar surface (b). Bars, 130 μ m (a) and 100 μ m (b). (c–f) Fruiting bodies of *Byssovorax cruenta* By c2^T *in situ* on the agar surface, sori consisting of sporangioles of varying size without cover (c, d) and under coverslips (e, f). Bars, 80 μ m (c), 200 μ m (d) and 105 μ m (e, f).

nitrogen source (ST-21 agar; Reichenbach & Dworkin, 1992). On this medium, growth was much more reluctant, but the filter paper was clearly decomposed (Supplementary Fig. S10). No growth occurred on pure peptone agar, such as CY agar [0.3 % Casitone (Difco), 0.1 % yeast extract], and even as little as 0.1 % Casitone in VY/2 agar prevented growth; peptones therefore seemed to be inhibitory. However, on filter paper on CY agar, there was reasonable growth with destruction of the filter paper. Obviously, the organism required a utilizable carbon source, and a good

carbon source could overcome inhibition by peptone. There was also growth on chitin agar with slow lysis of the chitin, but only if the chitin top layer was on water agar (CHIT7 agar) and not when it was on peptone agar (CHIT6 agar; Reichenbach & Dworkin, 1992). This behaviour differs in several respects from that of '*Sorangium*'. Thus, many '*Sorangium*' strains will also grow on CHIT6 agar with degradation of chitin, quite a few even on CY agar, and almost all strains grow well on filter paper on ST21 agar, where they decompose the cellulose and produce enormous numbers of fruiting bodies (Fig. 4a). Most of them grow very well on VY/2 agar, often with production of fruiting bodies, but lysis of the yeast cells is usually moderate or even absent. The novel organism grew well at 30 °C and at around pH 7. It was fully aerobic. There was delayed growth at 26 and 38 °C on VY/2 agar and on filter paper on VY/2 agar, with cellulose decomposition only at 38 °C. Growth was always relatively slow. On suitable media, large swarm colonies developed within 1 week; on poor media, results could not be evaluated before 2–3 weeks.

Fig. 5. Optically refractile cells of *Byssovorax cruenta* By c2^T, presumably myxospores, in phase-contrast. Bar, 13 μ m.

The nutritional requirements of the bacterium were studied on agar plates. There was no growth on plain water agar or on filter paper on water agar. For further studies, minimal media were applied. MM20 agar contained 0.05 %

MgSO₄·7H₂O, 0.02 % CaCl₂·2H₂O, 0.01 % K₂HPO₄, 0.01 % KNO₃ and 1.2 % agar; the pH was adjusted to 7.2 after autoclaving. GR33 medium contained 0.15 % MgSO₄·7H₂O, 0.02 % CaCl₂·2H₂O, 0.01 % K₂HPO₄, 0.01 % KNO₃, 8 mg ferric sodium EDTA l⁻¹, a standard trace element solution, 20 mM HEPES and, instead of agar, 0.75 % Gelrite; the pH was again adjusted to 7.2 after autoclaving.

There was only minimal growth on these media, probably because of nutrient transfer with the inoculum. Clearly, agar did not serve as a carbon source. However, with filter paper, these media supported good growth under cellulose degradation, even after several transfers on the same medium. Instead of filter paper, pads of regenerated cellulose could be used, which were broken down completely, whereas filters of cellulose nitrate or cellulose acetate were not decomposed and did not allow growth. Clearly, cellulose was a sufficient carbon source for the novel organism, and nitrate was a useful nitrogen source.

A study of alternative nitrogen sources was performed on GR33 plates with filter paper as the carbon source and nitrate replaced by other nitrogen compounds. In no case was there any growth on these media without filter paper. Urea at 0.1 % supported good growth. So did sodium glutamate at 0.01 and 0.05 %, but at 0.1 % it prevented all growth. There was also excellent growth with vitamin-free Casamino acids (Difco) at 0.01 %, while at 0.1 % all growth was blocked. Apparently, glutamate and Casamino acids could serve only as sources of nitrogen and not carbon, and both became inhibitory at slightly elevated concentrations even in the presence of a good carbon source, such as cellulose. The same was true with Casitone (Difco), a tryptically digested casein peptone, which by itself supports growth of almost all myxobacteria, including many strains of '*Sorangium*'.

An investigation of potential carbon sources revealed a strong inhibitory effect of glucose. When 0.1 % or even 0.05 % glucose (filter-sterilized, as were all carbon compounds in this study) was added to yeast (VY/2) agar, all growth stopped, but when filter paper was placed on these media, the bacterium grew well, and the filter paper was broken down completely. At a glucose concentration of 0.2 % in yeast agar, the organism could not even grow on filter paper.

No growth was seen on minimal media with galactose, lactose, sucrose, sodium acetate or sodium lactate (each at 0.05 %). With the exception of acetate, good growth under destruction of cellulose was obtained when filter paper was placed on these media. Slight growth occurred with 0.05 % fructose, with good growth after the addition of filter paper. An increase of fructose to 0.3 % in MM20 agar allowed good growth with or without filter paper; the latter was degraded. Cellobiose at 0.05 % in minimal media allowed some growth, even good growth with filter paper; cellulose was degraded only reluctantly. At 0.2 % cellobiose, however, growth was poor or absent with or without filter paper.

In contrast to glucose, the addition of 0.05–0.15 % *N*-acetylglucosamine to yeast (VY/2) agar stimulated growth substantially, with or without filter paper. The yeast cells and, if present, the filter paper were decomposed. However, on minimal media with up to 0.3 % *N*-acetylglucosamine as the carbon source, there was only growth when filter paper was added; the latter was attacked.

One sugar that clearly and invariably supported growth, including on minimal media, was maltose. At concentrations between 0.05 and 0.4 % in minimal media, the organism grew quite well without filter paper, although even better with it. There also was good growth when nitrate was replaced by ammonium (sulfate). While the higher maltose concentrations allowed somewhat better growth, the difference between 0.1 and 0.4 % was not substantial. Cellulose degradation, however, was delayed at 0.2 % and blocked completely at 0.4 %. Good growth was also obtained with 0.1 % L-arabinose or 0.1 % D-xylose in nitrate agar with and without filter paper; in both cases cellulose was decomposed. Starch (0.2 % in nitrate minimal media) was hydrolysed, but allowed good growth only in combination with filter paper; the cellulose was degraded. The same was the case with xylan (from oat husks, 0.2 %).

'*Sorangium*' strains, in contrast, are not inhibited by glucose, and may in fact be cultivated on glucose and nitrate, and grow very well on starch as the only carbon source.

Efforts to cultivate the novel bacterium in a defined liquid medium were only moderately successful. Cultures of 20 ml medium in 100 ml Erlenmeyer flasks were shaken on a rotary shaker at 30 °C and 160 r.p.m. in constant, artificial light. A mineral salts medium, MM32LM, was used (KNO₃, 0.05 %; MgSO₄·7H₂O, 0.02 %; CaCl₂·2H₂O, 0.02 %; K₂HPO₄, 0.01 %; ferric sodium EDTA, 1 mg per 100 ml; standard trace element solution; HEPES, 50 mM). When cellulose powder (around 0.5 %) was added to this medium, growth was moderate and slow, in the form of large flakes and bright red nodules. The medium remained turbid even after 35 days. Good and sustained growth was obtained with maltose in the concentration range between 0.05 and 0.5 %. At higher concentrations (1–4 %), the inoculum tended to degenerate quickly. Without maltose, there was no growth at all, which excludes feeding effects. The organism grew as small (0.05–3 mm diameter), intensely red nodules, and could be transferred to the same medium consecutively several times. Yet growth was slow, and the results of experiments could be analysed only after 8–14 days.

The response to various antibiotics was tested on yeast (VY/2) agar. Filter-sterilized solutions of the compounds were added after autoclaving to give 50 µg ml⁻¹ each. There was good growth in the presence of carbenicillin (sodium salt) and trimethoprim, moderate growth with polymyxin B sulfate and poor growth with bacitracin A. No growth was possible with chlortetracycline hydrochloride, oxytetracycline hydrochloride, cephalothin (sodium salt),

phosphomycin (disodium salt) or rifampicin. Kanamycin sulfate, which is still often tolerated by '*Sorangium*' strains at 1000 µg ml⁻¹, allowed growth of the novel bacterium only up to 10 µg ml⁻¹. Acriflavin hydrochloride was completely inhibitory at 0.5 µg ml⁻¹, but there was some growth at 0.2 µg ml⁻¹.

The G + C content of the DNA of the novel organism was determined by HPLC (Mesbah *et al.*, 1989) and was found to be 69.9 mol%.

The complete sequence of the 16S rRNA gene of the type strain was elucidated, and phylogenetic analysis was performed as described by Margesin *et al.* (2004) and Rainey *et al.* (1996). The highest similarity values were found with '*Sorangium cellulosum*' (= *Polyangium cellulosum*) ATCC 25531 (95.7%), *Chondromyces apiculatus* Cm a2 (95.0%) and *Chondromyces pediculatus* Cm p17 (94.6%). Thus, the bacterium clearly belongs to the family *Polyangiaceae*, suborder '*Sorangineae*', of the *Myxococcales* (Fig. 6).

Presently, all cellulose-decomposing myxobacteria are classified in the genus '*Sorangium*' and in just two species, '*S. cellulosum*' (formerly *Polyangium cellulosum*) and '*Sorangium nigrum*'. The organism described above, while being a cellulose degrader, differs in so many essential morphological and physiological characteristics from '*Sorangium*' that creation of a new genus for it appears justified, and it is suggested to name it *Byssovorax*. It may be mentioned in this connection that, in the older literature, cellulolytic myxobacteria have been described belonging to other genera, such as '*Myxococcus cellulosus*' and '*Podangium cellulosum*' (now '*Stigmatella cellulosum*') (Pronina, 1962). We have been isolating cellulolytic myxobacteria now for more than 25 years, yet we have never encountered these species. While this does not

disprove their existence – the isolation of *Byssovorax* may be considered a warning – we are rather inclined to assume that these organisms were in fact bacteriolytic strains simply growing in company with genuine cellulose degraders, which is indeed often observed. '*Angiococcus cellulosa*', described by Mishustin (1938), later turned out to be the chytridiomycete *Rhizophlyctis rosea* [Mishustin, 1968 (transliterated as Michoustine in the latter article)]. It should also be understood that cellulolytic *Cytophaga* and *Sporocytophaga*, which were classified for some time as myxobacteria, are now recognized to belong to a different phylum.

As pointed out above, the bacterium described here is not really novel but was known before as '*Myxococcus cruentus*' Thaxter 1897. Therefore, the species epithet will be retained for the present organism. It appears that '*M. cruentus*' has been reported in the literature only once more after Thaxter (1897). In 1930, H. and S. Krzemieniewscy gave a somewhat confusing description of a myxobacterium that they isolated twice from soil collected in beech forests of the Tatra mountains, Poland (Krzemieniewscy & Krzemieniewscy, 1930). They regarded it as identical to Thaxter's species, but renamed it '*Chondrococcus cruentus*' (now '*Corallococcus cruentus*'), because its firm fruiting bodies did not disintegrate when suspended in water and therefore would rather fit Jahn's genus '*Chondrococcus*' (which is, however, not supposed to have sporangioles). Judging from the (rather unsatisfactory) figures that accompany the text, the vegetative cells of that organism appear to have tapering ends and the myxospores appear to be spherical. The authors talk about cysts embedded in a colourless slime envelope, but do not mention specifically a true wall. The vegetative cells measured 0.4–1.0 × 3.6–5.4 µm and the myxospores 1.0–1.3 × 1.3–1.5 µm. The text as well as the figures rule out the idea that the bacterium was really '*M. cruentus*', and it is certainly not identical with the myxobacterium described in this article.

Fig. 6. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of *Byssovorax cruenta* By c2^T. Bar, 5% sequence divergence.

Description of *Byssovorax* gen. nov. Reichenbach 2006

Byssovorax [Bys.so.vo'rax. Gr. fem. n. *byssos* cotton, fine linen (for cellulose); L. adj. *vorax* voracious, devouring; N.L. fem. n. *Byssovorax* devourer of cellulose].

Vegetative cells are cylindrical with rounded ends and move by gliding. Swarm colonies consist of many small pseudoplasmodia with a fan-like anterior and a tapering posterior end, migrating independently on and within the (agar) substrate. The pseudoplasmodia may contract after some time into knob-, ring- or cup-like masses that are, however, not fruiting bodies. Fruiting bodies consist of more or less spherical sporangioles with a definite wall, arranged in dense clusters, or sori. The genus belongs to the family *Polyangiaceae*, suborder '*Sorangineae*', order *Myxococcales*. The type (and so far only) species is *Byssovorax cruenta*.

Description of *Byssovorax cruenta* sp. nov., nom. rev. (ex Thaxter 1897, 409) Reichenbach 2006

Byssovorax cruenta (cru.en'ta. L. fem. adj. *cruenta* blood-red).

Displays the following attributes in addition to the characteristics of the genus. Vegetative cells are rather stout, $0.9\text{--}1.1 \times 3.5\text{--}7.5\text{ }\mu\text{m}$, phase dark. Pseudoplasmodia are deeply blood-red, of very variable size: fan-like anterior ends are $200\text{--}2800\text{ }\mu\text{m}$ wide, tails are $2600\text{--}8000\text{ }\mu\text{m}$ long, often branched with several fans. The migrating pseudoplasmodia deposit slime trails of a parchment-like texture. After many transfers, true swarm colonies with veins may arise. The pseudoplasmodia eventually contract into $350\text{--}500\text{ }\mu\text{m}$ wide, blood-red rings, $250\text{--}600\text{ }\mu\text{m}$ large knobs or cup-shaped structures, around $800\text{ }\mu\text{m}$ wide and $300\text{ }\mu\text{m}$ high. Fruiting bodies are produced rarely and consist of large red sporangioles, $80\text{--}140\text{ }\mu\text{m}$ in diameter (between 70×90 and $160 \times 190\text{ }\mu\text{m}$), in flat, sheet-like sori, $200\text{--}600\text{ }\mu\text{m}$ in diameter (sometimes more than $1000\text{ }\mu\text{m}$ wide). Myxospores resemble vegetative cells in shape, but are stouter, $1.5\text{--}1.7 \times 3.3\text{--}5\text{ }\mu\text{m}$, and optically refractile. Crystalline cellulose (filter paper) is broken down completely. The organism requires a carbohydrate for growth; suitable substrates are cellulose, maltose, L-arabinose, D-xylose, fructose and cellobiose, in order of decreasing suitability. Glucose prevents all growth at the low concentration of 0.1% , even in the presence of cellulose. Good nitrogen sources are nitrate, ammonium, urea, Casitone (Difco), Casamino acids and glutamate; slightly elevated concentrations of the organic nitrogen source, namely 0.2% , may become totally inhibitory. *N*-Acetylglucosamine seems to serve (mainly) as an excellent nitrogen source. Chitin and starch are degraded, but only chitin allows growth and seems to be used as a carbon and nitrogen source. Cellobiose at somewhat higher concentrations (0.2%) blocks cellulose degradation, as does glucose. The organism can be cultivated on yeast (VY/2) agar; the β -glucan of the bakers' yeast cell wall apparently supplies the required carbohydrate. Better growth is obtained on filter paper placed on VY/2 agar. Grows between 26 and $38\text{ }^{\circ}\text{C}$; optimum temperature around $30\text{ }^{\circ}\text{C}$; optimum pH about 7.2 . Strictly aerobic. The G+C content of the DNA is $69.9\text{ mol}\%$ (by HPLC).

The type strain, By c2^T (= DSM 14553^T = CIP 108850^T), was isolated from soil with decaying plant material collected

south of Holbrook, AZ, USA. It appears to be a very rare organism, or at least to live in places that have only rarely been investigated. So far, only two strains, obtained from the same sample, have been isolated in pure culture.

Acknowledgements

We wish to thank Professor Dr E. Stackebrandt for valuable comments, Mrs Rosemarie Penkert, Mrs Heike Petrat and Mrs Sabine Welnitz for excellent technical assistance and Dr Jean Euzéby, Toulouse, for his help in finding a correct name.

References

- Krzemieniewscy, H. & Krzemieniewscy, S. (1930). Miksobakterje Polski. Czesc trzezia (Die Myxobakterien von Polen. III. Teil). *Acta Soc Bot Pol* 7, 250–273 (in Polish with German summary).
- Margesin, R., Schumann, P., Spröer, C. & Gounot, A. M. (2004). *Arthrobacter psychrophenicus* sp. nov., isolated from an alpine ice cave. *Int J Syst Evol Microbiol* 54, 2067–2072.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 39, 159–167.
- Mishustin, E. N. (1938). Cellulose-decomposing myxobacteria. *Mikrobiologiya* 7, 427–444 (in Russian).
- Mishustin, E. (1968). Microorganismes cellulolytiques des sols de l'U.R.S.S. *Ann Inst Pasteur* 115, 596–603 (in French).
- Pronina, N. I. (1962). Description of new species and varieties of cellulose-decomposing myxobacteria. *Microbiology* (English translation of *Mikrobiologiya*) 31, 384–390.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal for *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* 46, 1088–1092.
- Reichenbach, H. (2005). Order VIII. *Myxococcales* Tchan, Pochon and Prévot 1948, 398^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 1059–1072. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Reichenbach, H. & Dworkin, M. (1992). The myxobacteria. In *The Prokaryotes*, 2nd edn, pp. 3416–3487. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1989). *Approved Lists of Bacterial Names (amended edition)*. Washington, DC: American Society for Microbiology.
- Thaxter, R. (1897). Further observations on the *Myxobacteriaceae*. *Bot Gaz* 23, 395–411.