Reclassification of *Myxococcus flavescens* Yamanaka *et al.* 1990^{VP} as a later synonym of *Myxococcus virescens* Thaxter 1892^{AL}

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The taxonomic relatedness between the species *Myxococcus flavescens* and *Myxococcus virescens* was investigated. Literature data had already indicated the synonymy between the two species but this observation had not been formalized. Additional evidence that the two taxa represent a single species was provided by comparison of metabolic properties, cellular fatty acid profiles and from a DNA–DNA reassociation value of >80 %. Data from this study led to the proposal that *M. flavescens* should be reclassified as a later synonym of *M. virescens*.

The description of *Myxococcus flavescens* was effectively published in 1987 (Yamanaka *et al.*, 1987) and the name was validated in Validation List 33 (Yamanaka *et al.*, 1990). In the original description the high phenotypic similarity between *M. flavescens* and *Myxococcus virescens* Thaxter 1892 was already obvious, as the type strains of both species differed only in the colour of diffusible pigments. While *M. flavescens* showed deliquescent fruiting bodies on modified SP agar (see below for composition) and formed a diffusible light-yellow pigment, *M. virescens* showed only rudimentary fruiting bodies on this medium and excreted a diffusible greenish pigment (Yamanaka *et al.*, 1987). As pointed out by Reichenbach (2005), high 16S rRNA gene sequence similarities (Spröer *et al.*, 1999), and ‘the size of its myxospores [of *M. flavescens*], its yellow colour and the myxovirescin type antibiotics produced by it ... are in perfect fit with the description of *Myxococcus virescens*’. As a consequence, Reichenbach (2005) did not include *M. flavescens* in the list of *Myxococcus* species though, according to the List of Prokaryotic names with Standing in Nomenclature (<http://www.bacterio.cict.fr/>), both names are valid. In spite of arguments supporting the synonymy between *M. virescens* and *M. flavescens*, no formal reclassification has been proposed to date.

Morphologically characterized isolates of *M. flavescens* and *M. virescens*, but not the type strains, were included in the phylogenetic study of Spröer *et al.* (1999). In the meantime, the 16S rRNA gene sequences of *M. virescens* DSM 2260^T (GenBank no. DQ768119) and *M. flavescens* DSM 4946^T

(DQ768130) are available in public databases. These organisms share 100 % 16S rRNA gene sequence identity and both show 99.9 % sequence similarity with the type strain of *Myxococcus xanthus* NBRC 13542^T (AB218205).

In the present study, we provide additional physiological, chemotaxonomic and genomic evidence that the two taxa represent a single species. Physiological tests were performed with *M. virescens* DSM 2260^T and DSM 52256 and *M. flavescens* DSM 4946^T and DSM 52472. Media were inoculated with cells suspended in MD1 after growth on CY agar for 2 days. Pigmentation was observed on SP agar supplemented with 3 g (wet wt) bakers’ yeast cells per litre. All media used are described in Reichenbach & Dworkin (1981). Hydrolysis of starch and xylan was tested using overlay agar. The first layer consisted of water agar (1⁻¹: 1 g CaCl₂·2H₂O and 15 g agar) and the second of starch or xylan agar (1⁻¹: 2 g native potato starch, 0.5 g casitone, 1 g CaCl₂·2H₂O, 1 g MgSO₄·7H₂O and 10 g agar). For xylan hydrolysis, starch was replaced by 5 g xylan 1⁻¹ from oat spelt. The plates were evaluated after being covered with Lugol’s solution after 5 days (starch) or 4 days (xylan) incubation. Clear halos with diameters larger than that of the swarms and lasting for longer than 5 min were scored positive. Salt tolerance was measured as swarm growth on CY agar plates containing 15 g NaCl 1⁻¹, incubated for 3 weeks. Aesculin hydrolysis was tested on slants with the composition (1⁻¹): 3 g casitone, 1 g yeast extract, 1 g aesculin, 1 g sodium citrate and 0.05 g ground ferric citrate. Slants were read after 8 days. Tolerance to antibiotics was determined using VY/2 agar plates containing 250 mg kanamycin sulphate or 50 mg streptomycin sulphate per litre, respectively. Growth was observed for up to 5 days.

All four strains showed negative reactions towards degradation of xylan and hydrolysis of starch and aesculin. All

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSM 2260^T is DQ768119.

A table showing the major whole-cell fatty acids of the type strains of *Myxococcus virescens* and *Myxococcus flavescens* is available as supplementary material with the online version of this paper.

strains were sensitive to kanamycin and resistant to streptomycin. Intra-species reactions varied between positive and weak towards growth at 1.5 % NaCl. Negative hydrolysis of starch contradicts the information given by Yamanaka *et al.* (1987), who listed all strains of *Myxococcus* as being positive. This discrepancy may be due to the different incubation periods used. While Yamanaka *et al.* (1987) evaluated the test after 1, 2 and 3 weeks, our cultures were incubated for 5 days before being read. The finding that, in a comparative study, all strains of *Corallococcus* did hydrolyse starch (Stackebrandt *et al.*, 2007) indicates the proper set-up of the test.

Pigmentation of *M. flavescens* DSM 4946^T and *M. virescens* DSM 2260^T was observed on modified medium SP. After 4 days incubation, the culture of *M. virescens* was greenish yellow and that of *M. flavescens* was orange–yellow. However, after 8 days incubation, the colour of both agar plates was orange–yellow. The fruiting bodies of strain DSM 2260^T were orange. Strain DSM 4946^T did not produce fruiting bodies.

Biomass of *M. flavescens* DSM 4946^T and *M. virescens* DSM 2260^T for whole-cell fatty acid analysis was obtained from CY agar plates that had been incubated for 2 days at 28 °C. A suspension of cells grown on VY/2 agar was used as the inoculum. Fatty acid methyl esters were obtained as described previously (Kämpfer & Kroppenstedt, 1996) and were separated using a gas chromatograph (model 5898A; Hewlett Packard). Peaks were computed automatically using the Microbial Identification standard software package (Sasser, 1990).

The two type strains showed basically the same fatty acid profile. Small variations (above 1 % of total) occurred at the quantitative level: whereas strain DSM 2260^T showed slightly higher amounts of the fatty acids C_{14:0} (4.0 %), iso-C_{17:1}ω10c (2.2 %), C_{16:1}ω5c (3.2 %) and C_{16:1}ω11c (1.2 %) than strain DSM 4946^T, the latter strain showed higher amounts of C_{15:0} (4.0 %), iso-C_{14:0} 3-OH (6.6 %) and an unknown fatty acid with an ECL (equivalent chain-length) of 14.959 (6.2 %). The major components, iso-C_{15:0} (41–51 %), iso-C_{17:0} (7.2–7.7 %) and C_{16:1}ω5c (>10 %) agree with the description of Yamanaka *et al.* (1987) for *M. flavescens* (see Supplementary Table S1, available in IJSEM Online).

For DNA–DNA reassociation experiments, DNA from *M. virescens* DSM 2260^T and *M. flavescens* DSM 4946^T was isolated using a French pressure cell (Thermo Spectronic) and purified by using chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out in duplicate, according to the methods described by De Ley *et al.* (1970) and Huß *et al.* (1983) at 70 °C in 2 × SSC (standard saline citrate buffer) containing 10 % formamide, with a model Cary 100 Bio UV/VIS-spectrophotometer (Varian, Australia) equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with an *in situ* temperature probe (Varian). The high value of 82.3 % DNA–DNA relatedness (mean of two measures: 81.7 and 82.9 %) supports the finding that the

two type strains represent the same species. The type strain of another phylogenetically closely related species, *M. xanthus* DSM 16526^T, had a value that was slightly less than 70 % [mean 69.1 % (68.3 and 71.0)] with *M. virescens* DSM 2260^T. The moderately high DNA–DNA relatedness values support the overall phenotypic (Yamanaka *et al.*, 1987), morphological (Reichenbach, 2005) and 16S rRNA gene sequence similarities (Spröer *et al.*, 1999). The most reliable difference between *M. virescens* and *M. xanthus* is the presence, in *M. virescens*, of a thick capsule surrounding the myxospores.

On the basis of the evidence presented, it is proposed that the two species *M. virescens* and *M. flavescens* be united under the same name. As a rule of priority (Rules 38 and 42 of the *Bacteriological Code*; Lapage *et al.*, 1992), the name *M. virescens* should be retained and strains of *M. flavescens* should be reclassified as such. The emendation of the species description of *M. virescens* is based on that given in *Bergey's Manual of Systematic Bacteriology* (Reichenbach, 2005).

Emended description of *Myxococcus virescens* Thaxter 1892 (Approved Lists 1980)

Myxococcus virescens (vi.res'cens. L. part. adj. *virescens* becoming green).

Vegetative cells are slender rods with short tapering ends, cigar-shaped, 0.7–0.8 × 4–8 µm. Fruiting bodies are globular or oval, and rarely even finger-like; on agar media plump ridges and, very often, a deliquescent mound are observed; typically greenish yellow in colour, but often yellow–orange, and even non-pigmented (grey). Fruiting bodies can be large, 150–500 µm. Myxospores are spherical, optically refractile and very large [1.9–2.4 µm in diameter (range 1.7–2.6 µm)], with a thick capsule. Their large size is the most reliable distinguishing characteristic for the species. Characteristic of the family, swarm colonies are non-pigmented or greenish yellow, or sometimes orange. Temperature and pH ranges and nutritional requirement are as for the other species of the genus. Catalase-positive. Positive for hydrolysis of casein, DNA and starch. Negative for hydrolysis of starch, aesculin and xylan, when tested on overlay agar after 4–5 days. Tolerates 1.5 % NaCl. Kanamycin-sensitive, streptomycin-resistant. Major fatty acids are iso-C_{15:0}, C_{16:1}ω5c and iso-C_{17:0} whereas C_{14:0}, C_{15:0}, iso-C_{14:0} 3-OH, anteiso-C_{17:1} and/or iso-C_{17:1} and an unknown fatty acid with ECL 14.959 occur in smaller amounts. Common organisms in soil, in rotting plant material and in samples from the littoral region; easily obtained when rabbit or sheep dung is incubated in a moist chamber. Phylogenetic neighbour of *M. xanthus*. The DNA G + C content is 68–71 % (buoyant density; T_m).

The type strain is M22^T (=ATCC 25203^T=DSM 2260^T).

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