Rhodanobacter spathiphylli sp. nov., a gammaproteobacterium isolated from the roots of *Spathiphyllum* plants grown in a compost-amended potting mix

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Three Gram-negative, yellow-pigmented strains were isolated from the rhizospheres of *Spathiphyllum* plants grown in a compost-amended potting mix. The strains showed biological control activity towards the root-rot plant pathogen *Cylindrocladium spathiphylli*, and were characterized to determine their taxonomic position. Cells of the strains were non-motile rods, and the strains were oxidase- and catalase-positive and unable to ferment most sugars tested. The three strains showed differences in growth temperature range, optimal growth temperature and some biochemical reactions. The majority of the fatty acids were branched, and large amounts of 15:0 iso and 17:1 iso ω 9c were present. The 16S rRNA gene sequence (1497 bp) of strain B39^T showed the highest level of similarity (98.5 %) to that of *Rhodanobacter fulvus* IAM 15025^T, followed by *Rhodanobacter lindaniclasticus* LMG 18385^T (96.0 %; strain no longer extant), *Dyella koreensis* CCUG 50883^T (96.4 %), *Dyella japonica* DSM 16301^T (96.3 %), *Frateriella aurantia* LMG 1558^T (96.2 %) and *Fulvimonas soli* LMG 19981^T (95.9 %). Less than 90 % 16S rRNA gene sequence similarity was observed for other members of the *Gammaproteobacteria*. The mean DNA–DNA reassociation value for the three strains was 100 % and was 25 % when the strains were compared with DNA from *R. fulvus* LMG 23003^T. The strains had a mean DNA G + C content of 67.6 mol%. On the basis of their phylogenetic, genomic and phenotypic properties, the three strains represent a novel species within the genus *Rhodanobacter*, for which the name *Rhodanobacter spathiphylli* sp. nov. is proposed. The type strain is strain B39^T (=LMG 23181^T=DSM 17631^T).

Soil-borne fungal diseases are an important threat to many agricultural and horticultural crops. In the last 20 years, the use of soil fumigants and fungicides for the control of these diseases has been severely restricted or even prohibited. This situation has sparked great interest in the development of alternative control measures against soil-borne plant diseases. One approach is the use of compost as a substrate able to suppress soil-borne plant pathogens. Since the late 1970s, there have been numerous reports showing the disease-suppressive properties that can be obtained from compost amendment (reviewed by De Clercq *et al.*, 2004). The pathogen-suppressive properties of compost are mainly attributable to biological factors, although chemical and physical factors are inextricably involved. In addition to

containing the numerous micro-organisms usually observed in compost (reviewed by Ryckeboer *et al.*, 2003), a disease-suppressing compost should also harbour micro-organisms that are beneficial in terms of disease suppression or should be capable of stimulating beneficial edaphic microbial populations through nutritional amendment. However, because of the enormous microbial variability inherent in the composting process, composts are not consistently colonized by the antagonistic micro-organisms (biocontrol agents) necessary for disease suppression (Hoitink & Boehm, 1999). Therefore composts are sometimes artificially amended with one or more of these biological control agents, to increase the overall disease suppressiveness of the material (Hoitink & Boehm, 1999). The organisms described herein were isolated during a screening process carefully designed to select for appropriate biocontrol agents.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B39^T is AM087226.

A polyphasic taxonomic study was performed on three strains isolated from the rhizosphere of *Spathiphyllum*. These plants were previously grown in a compost-amended substrate with disease-suppressive properties directed against the soil-borne fungal pathogen *Cylindrocladium spathiphylli*. The three strains exhibit *in vivo* and *in vitro* biological control activity towards this pathogen. The results of genotypic and phenotypic analyses showed that the three isolates belong to a novel *Rhodanobacter* species.

The novel strains, B35, B39^T and B42, were isolated at 25 °C on nutrient agar (LAB M) amended with 100 p.p.m. cycloheximide (A.G. Scientific) from the roots and rhizospheres of *Spathiphyllum* 'Alpha' plants. The strains were maintained at -80 °C on beads from Microbank (Pro-Lab Diagnostics). The reference strain *Rhodanobacter fulvus* LMG 23003^T was included in some experiments. The type strain of the type species of the genus, *Rhodanobacter lindaniclasticus* LMG 18385^T, could not be included, as this strain, which is the only known strain of this species, no longer exists (Mergaert *et al.*, 2002).

Small-scale DNA extracts were prepared by using the method of Niemann *et al.* (1997), and the 16S rRNA gene sequences of strains B35 and B39^T were amplified by using a PCR with conserved primers (Coenye *et al.*, 1999). PCR products were purified using the NucleoFast 96PCR Clean-up kit (Macherey-Nagel) according to the instructions of the manufacturer. Sequence analysis was performed as described previously (Van Trappen *et al.*, 2004). Evolutionary distances were calculated using the algorithm of Jukes & Cantor (1969), and a phylogenetic tree was constructed using the neighbour-joining method with the TREECON program (Van de Peer & De Wachter, 1994).

Partial (810 bp) and complete (1497 bp) 16S rRNA gene sequences were determined for strains B35 and B39^T, respectively. Comparative sequence analysis revealed that the sequences of these strains were identical over a stretch of 810 bp, except for two unidentified nucleotide positions in

the sequence of strain B35. The 16S rRNA gene sequence from B39^T revealed 98.5 % similarity with that of *R. fulvus* IAM 15025^T, 96.0 % sequence similarity with that of *R. lindaniclasticus* LMG 18385^T, 96.4 % with that of *Dyella koreensis* CCUG 50883^T, 96.3 % with that of *Dyella japonica* DSM 16301^T, 96.2 % with that of *Frateuria aurantia* LMG 1558^T and 95.9 % with that of *Fulvimonas soli* LMG 19981^T. Less than 90.0 % sequence similarity was observed with respect to other members of the *Gammaproteobacteria*, such as *Xanthomonas sacchari* LMG 471^T (89.8 %), *Stenotrophomonas maltophilia* LMG 958^T (89.8 %), *Lysobacter antibioticus* DSM 2044^T (89.8 %), *Pseudoxanthomonas broegbernensis* ATCC BAA-10^T (89.7 %), *Lysobacter enzymogenes* DSM 2043^T (89.6 %), *Xylella fastidiosa* ATCC 35879^T (89.6 %), *Xanthomonas melonis* LMG 8670^T (89.3 %), *Xanthomonas campestris* LMG 568^T (89.3 %) and *Luteimonas mephitis* DSM 12574^T (89.2 %). The estimated phylogenetic relationships of strain B39^T and the nearest members of the *Gammaproteobacteria* are shown in a neighbour-joining dendrogram with bootstrap percentages (Fig. 1). The tree obtained with the maximum-likelihood method (TREE-PUZZLE, default parameters with 50 000 puzzling steps) showed essentially the same topography as the neighbour-joining tree (data not shown).

The genomic relatedness between strains B39^T, B35, B42 and the most closely related strain, *R. fulvus* LMG 23003^T, was determined by means of DNA-DNA hybridization. DNA was prepared according to the method of Wilson (1987) and DNA-DNA hybridizations were carried out with photo-biotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements (mean standard deviation ± 7 %; Goris *et al.*, 1998). The hybridization temperature was 50 °C and reciprocal experiments were performed for every pair of strains. The mean hybridization level between strains B39^T, B35 and B42 was 100 %, indicating that they belong to a single species (Wayne *et al.*, 1987), whereas the values for DNA-DNA binding with *R.*

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain B39^T. Bootstrap percentages (of 500 replicates) above 70 % are shown. GenBank accession numbers are shown in parentheses. Bar, 1 nucleotide substitution per 10 nucleotides.

fulvus LMG 23003^T were low (26 % with B39^T, 24 % with B35 and 26 % with B42). These results demonstrate that the three isolates are genotypically distinct from *R. fulvus* (their nearest neighbour phylogenetically) and constitute a novel species within the genus *Rhodanobacter*.

The DNA G + C contents of the isolates were determined using an HPLC method, as described by Van Trappen *et al.* (2003). The G + C contents of strains B39^T, B35 and B42 were 67.6, 67.8 and 67.5 mol%, respectively. These values are higher than the G + C contents of the other *Rhodanobacter* species, which range from 63 to 65.3 mol% (Im *et al.*, 2004).

The cellular fatty acid patterns of the strains were determined as described by Mergaert *et al.* (2001). The three strains possessed very similar fatty acid profiles: the major fatty acids were 11:0 iso (5.6 %), 11:0 iso 3-OH (8.2 %), 13:0 iso 3-OH (5.1 %), 15:0 iso (24.2 %), 17:0 iso (9.0 %), 17:1 iso ω 9c (26.9 %) and summed feature 3 (5.7 %), comprising 15:0 iso 2-OH, 16:1 ω 7c or both. In Table 1, the fatty acid compositions of the novel strains and *R. fulvus* are compared: the profiles are very similar but differ in terms of the amounts of 11:0 iso, 11:0 iso 3-OH, 13:0 iso 3-OH, 15:0 anteiso and 16:0 present.

The following morphological, physiological and biochemical tests were performed. Gram staining, catalase activity and oxidase activity were investigated using conventional

media and methods (MacFaddin, 1980). The growth of the strains was investigated under aerobic conditions on nutrient agar, Luria–Bertani agar (Sigma), MacConkey agar no. 3 (Oxoid) and tryptone soy agar (TSA) [tryptone soy broth (TSB; Oxoid) amended with 15 g agar no. 1 l⁻¹ (LAB M)] for 48 h at 25 °C. The temperature range and optimum for growth were assessed through dilution plating and the counting of colony-forming units on TSA incubated at 7, 15, 20, 25, 30, 35, 40 and 45 °C for 48 h (with three replicates for each strain). Strains B35 and B39^T were unable to grow at ≤ 7 °C or ≥ 45 °C; strain B42 was unable to grow at ≤ 20 °C or ≥ 40 °C. The optimum temperature for growth was 30 °C for strains B35 and B42 and 35 °C for strain B39^T. Biochemical tests were performed on 2-day-old cultures of the three isolates (grown in TSB at 29 °C) by using the API 20NE (OD₅₅₀ = 0.15), API ZYM (OD₅₅₀ = 1.5), API 50 CHE (OD₅₅₀ = 1) and API ID 32 systems (bioMérieux). API ZYM tests were read after 4 h incubation at 35 °C; all other tests were read after 48 h at 28–29 °C. The API ID 32 system was also applied to *R. fulvus* LMG 23003^T as a positive control. API tests were performed according to the instructions of the manufacturer, with up to three repetitions. The biochemical and physiological characteristics of the three novel strains are detailed in the species description and in Table 2. The three novel strains seem to grow on a narrow range of substrates as sole carbon sources, a property that they seem to have in common with members of the genus *Rhodanobacter*. The carbon sources utilized by the novel isolates and the *Rhodanobacter* species differ, however: the isolates use D-sorbitol and glycogen, *R. fulvus* uses D-glucose, mannose, salicin, D-melibiose, D-sucrose, N-acetylglucosamine and maltose (Im *et al.*, 2004) and the substrates used by *R. lindaniclasticus* include D-glucose, lactate, caprate, malate and citrate (Nalin *et al.*, 1999). The novel isolates, *R. fulvus* and *R. lindaniclasticus* cannot grow on (amongst others) adipic acid, phenylacetic acid, mannitol, L-fucose, L-arabinose, 2-ketogluconate, 5-ketogluconate, D-ribose, inositol or malonate. The *Rhodanobacter* species and the novel isolates also share the following properties: the presence of oxidase and catalase activities, the absence of indole production and arginine dihydrolase and urease activities and the inability to ferment D-glucose. The three novel strains responded differently from the *Rhodanobacter* species in some biochemical tests (Table 2), however. Strain B42 shows a positive response for β -D-glucosidase activity and for the degradation of gelatin, whereas strains B35 and B39^T gave negative responses. On the other hand, strains B35 and B39^T were positive for N-acetyl- β -D-glucosaminidase activity and strain B42 was negative. The reactions for the production of acids from D-maltose and starch were negative for strain B42 and weakly positive for strains B35 and B39^T.

On the basis of this polyphasic taxonomic analysis, the three novel strains can be clearly differentiated from species of the genus *Rhodanobacter* and represent a novel species in this genus, for which the name *Rhodanobacter spathiphylli* sp. nov. is proposed.

Table 1. Fatty acid composition of the novel strains (*R. spathiphylli* sp. nov.) and *R. fulvus* Jip2^T

Mean percentages of total fatty acids (with standard deviations where appropriate) are given. Other fatty acids each accounted for less than 1 % of total fatty acids. Summed feature 3 comprises 15:0 iso 2-OH and/or 16:1 ω 7c. Unknown fatty acids are designated by their equivalent chain lengths (ECL) relative to the chain lengths of known straight-chain, saturated fatty acids. Data for *R. fulvus* Jip2^T are from Im *et al.* (2004). —, Not detected.

Fatty acid	<i>R. spathiphylli</i> (n = 3)	<i>R. fulvus</i> Jip2 ^T
11:0 iso	5.6 $\pm$ 0.3	3.5
11:0 iso 3-OH	8.2 $\pm$ 0.6	4.4
13:0 iso 3-OH	5.1 $\pm$ 0.3	2.9
15:0 anteiso	3.1 $\pm$ 0.3	6.7
15:0 iso	24.2 $\pm$ 0.3	21.3
15:1 iso F	—	1.2
16:0	1.9 $\pm$ 0.1	6.3
16:0 iso	3.1 $\pm$ 0.2	2.2
17:0 iso	9.0 $\pm$ 1.0	10.9
17:0 cyclo	—	2.0
17:1 iso ω 9c	26.9 $\pm$ 1.1	24.6
Summed feature 3	5.7 $\pm$ 0.5	5.9
Unknown (ECL 11.799)	3.6 $\pm$ 0.3	2.2

Table 2. Phenotypic characteristics that differentiate the novel strains (*R. spathiphylli* sp. nov.) from the type strains of *Rhodanobacter* species

Data for reference strains are from Nalin *et al.* (1999) (*R. lindaniclasticus*) and Im *et al.* (2004) (*R. fulvus*). +, Positive; –, negative; w, weak reaction; ND, not determined.

Characteristic	<i>R. spathiphylli</i> sp. nov.			<i>R. fulvus</i> IAM 15025 ^T	<i>R. lindaniclasticus</i> LMG 18385 ^T
	B35	B39 ^T	B42		
DNA G+C content (mol%)	67.8	67.6	67.5	65.3	63
Motility	–	–	–	+	–
β-D-Galactosidase	–	–	–	+	–
α-D-Glucosidase	–	–	–	+	ND
α-D-Mannosidase	–	–	–	+	–
β-D-Glucosidase	–	–	+	ND	ND
N-Acetyl-β-D-glucosaminidase	+	+	–	+	ND
Protease (gelatin)	–	–	+	–	ND
Growth on:					
D-Glucose	–	–	–	+	+
D-Mannose	–	–	–	+	–
N-Acetyl-β-D-glucosamine	–	–	–	+	–
D-Maltose	–	–	–	+	–
Capric acid	–	–	–	–	+
Malic acid	–	–	–	–	+
Trisodium citrate	–	–	–	–	+
Salicin	–	–	–	+	–
D-Melibiose	–	–	–	+	–
Sorbitol	w	w	w	+	–
Glycogen	w	w	w	–	–
Acids from D-maltose	w	w	–	ND	ND
Acids from starch	w	w	–	ND	ND

Description of *Rhodanobacter spathiphylli* sp. nov.

Rhodanobacter spathiphylli (spa.thi.phyl'li. N.L. gen. n. *spathiphylli* of *Spathiphyllum*, referring to the isolation source of the first strains, the rhizosphere of *Spathiphyllum* plants).

Cells are Gram-negative, rod-shaped, 0.8 × 1.5–4.0 µm in size and non-motile. They form smooth, circular, yellow colonies as large as 2–3 mm in diameter on nutrient agar and TSA after 48 h incubation at 30 °C. The strains grow well on nutrient agar, TSA and other common bacteriological media at ≥25 °C and ≤35 °C for all strains. Strains B35 and B39^T are unable to grow at ≤7 °C or ≥45 °C. Strain B42 is unable to grow at ≤20 °C or ≥40 °C. The optimum temperature for growth is 30 °C for strains B35 and B42 and 35 °C for strain B39^T. All strains give positive results in tests for the following: oxidase, catalase, alkaline phosphatase, C₄ esterase, C₈ esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, aesculin, *p*-nitrophenyl-β-D-galactopyranosidase, growth on sorbitol and growth on glycogen; weak acid production from aesculin, citrate, salicin and potassium 5-ketogluconate is observed. All strains give negative results in tests for the following:

C₁₄ lipase, trypsin, α-chymotrypsin, α-D-galactosidase, β-D-galactosidase, β-D-glucuronidase, α-D-glucosidase, α-D-mannosidase, α-fucosidase, nitrate reduction, indole production, fermentation of D-glucose, arginine dihydrolyase, urease, growth on D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-β-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, salicin, D-melibiose, L-fucose, propionate, valerate, histidine, 2- and 5-ketogluconate, 3- and 4-hydroxybutyrate, L-proline, rhamnose, D-ribose, inositol, D-sucrose, itaconate, suberate, malonate, acetate, D- and L-lactate, L-alanine, 3-hydroxybenzoate and L-serine and acid production from glycerol, erythritol, D- and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, D-cellobiose, D-lactose (bovine origin), D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate and potassium 2-ketogluconate. The three strains respond differently in tests for some biochemical characteristics. Strain B42 gives a positive response for

β -D-glucosidase activity and for the degradation of gelatin, whereas strains B35 and B39^T give negative responses. Strains B35 and B39^T give positive results for *N*-acetyl- β -D-glucosaminidase activity whereas strain B42 gives a negative response. The reactions for the production of acids from D-maltose and starch are negative for strain B42 and weakly positive for strains B35 and B39^T. The cells contain the fatty acids 15:0 iso and 17:1 iso ω 9c as the main constituents. The mean DNA G+C content is 67.6 mol%.

The type strain, B39^T (=LMG 23181^T=DSM 17631^T), was isolated from the rhizospheres of *Spathiphyllum* plants grown in a compost-amended potting mix.

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