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Reclassification of *Agromyces fucosus* subsp. *hippuratus* as *Agromyces hippuratus* sp. nov., comb. nov. and emended description of *Agromyces fucosus*

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The taxonomic position of *Agromyces fucosus* subsp. *hippuratus* is revised on the basis of molecular and phenotypic data. Phylogenetic analysis based on 16S rRNA gene sequences, DNA–DNA relatedness values and differences from other species in phenotypic traits revealed in this and earlier studies suggested reclassification of *A. fucosus* subsp. *hippuratus* as a separate species, *Agromyces hippuratus* sp. nov., comb. nov. The type strain is VKM Ac-1352^T (=JCM 9087^T). An emended description of *Agromyces fucosus* is given.

The genus *Agromyces*, with type species *Agromyces ramosus*, was established by Gledhill & Casida (1969) for filamentous, nutritionally fastidious, catalase- and oxidase-negative soil isolates. Zgurskaya *et al.* (1992) emended the description of the genus and described two species each including two subspecies, *Agromyces cerinus* subsp. *cerinus*, *A. cerinus* subsp. *nitratus*, *Agromyces fucosus* subsp. *fucosus* and *A. fucosus* subsp. *hippuratus*, which are characterized by rapid growth on peptone/yeast extract medium and positive catalase and oxidase reactions. Although the proposal of *A. cerinus* and its subspecies was substantiated by numerical analysis and their respective DNA–DNA relatedness values, the description of *A. fucosus* subsp. *hippuratus* was based only on phenotypic traits (Zgurskaya *et al.*, 1992). Subsequently, Suzuki *et al.* (1996) analysed DNA–DNA relatedness in representatives of the genus *Agromyces* and found that values between the type strains of *A. fucosus* subsp. *fucosus* and *A. fucosus* subsp. *hippuratus* were in the range 45–47 %, which is lower than the value of 70 % usually considered to indicate delineation of separate species (Wayne *et al.*, 1987). Recently, five additional *Agromyces* species have been described, *Agromyces bracchium*, *Agromyces luteolus*, *Agromyces rhizospherae* (Takeuchi & Hatano, 2001), *Agromyces aurantiacus* (Li *et al.*, 2003) and *Agromyces albus* (Dorofeeva *et al.*, 2003).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Agromyces fucosus* VKM Ac-1345^T is AY158025 and that of *Agromyces cerinus* subsp. *nitratus* VKM Ac-1351^T is AY277619.

During a study to identify *Agromyces* strains isolated from caves, Laiz *et al.* (2000) performed phylogenetic analyses based on the nearly complete 16S rRNA gene sequences of *A. fucosus* subsp. *fucosus* VKM Ac-1345^T (1473 bp; AY158025) and *A. cerinus* subsp. *nitratus* VKM Ac-1351^T (1465 bp; AY277619) obtained in this study and the 16S rRNA gene sequences of other *Agromyces* strains available from public DNA databases. The analysis was performed using the software package ARB (Ludwig *et al.*, 1998) with the neighbour-joining algorithm and the results showed that *A. fucosus* subsp. *fucosus* VKM Ac-1345^T and *A. fucosus* subsp. *hippuratus* VKM Ac-1352^T are significantly distant (Fig. 1). They exhibited 96·7 % 16S rRNA gene binary sequence similarity, which is lower than the threshold usually reported for members of a single bacterial species (Stackebrandt & Goebel, 1994; Roselló-Mora & Amann, 2001). The 16S rRNA gene sequence similarities between the type strains of different *Agromyces* species are in the range 96·5–98·5 % based on sequences of over 1400 bp.

A degree of DNA–DNA relatedness between *A. fucosus* subsp. *fucosus* VKM Ac-1345^T and *A. fucosus* subsp. *hippuratus* VKM Ac-1352^T was determined by measuring the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔT_m) as described by De Ley *et al.* (1970). We found a significant ΔT_m (5·6 °C) between *A. fucosus* subsp. *fucosus* and *A. fucosus* subsp. *hippuratus*, which is above the value of 5 °C used to indicate separate species (Roselló-Mora & Amann,

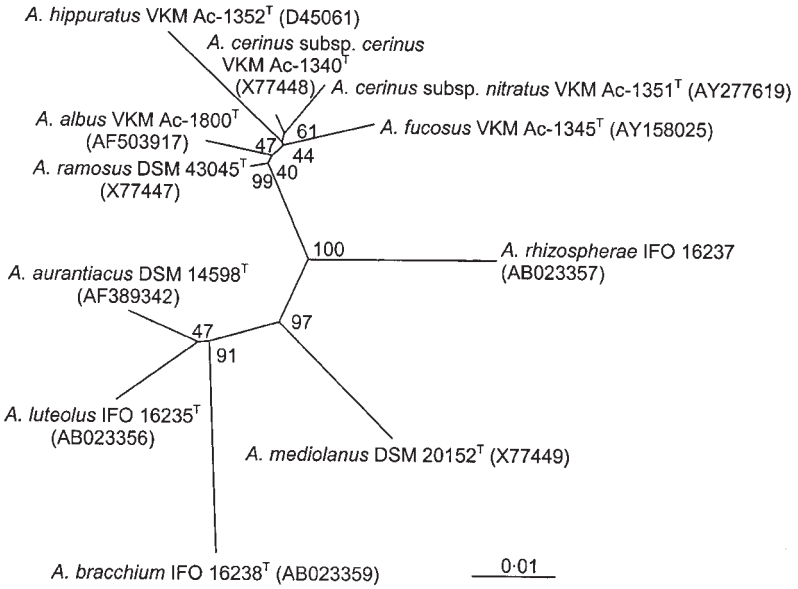

Fig. 1. Phylogenetic tree showing relationships between the 16S rRNA gene sequences of *Agromyces* type species. Accession numbers for the sequences are also given. Bootstrap numbers are percentages obtained from 1000 trials. Bar, 0·01 nucleotide substitutions per site.

2001). This result is in agreement with DNA–DNA relatedness values (45–47 %) reported for these strains by Suzuki *et al.* (1996). The above data demonstrate that *A. fucosus* subsp. *fucosus* and *A. fucosus* subsp. *hippuratus* belong to different genomic species (Wayne *et al.*, 1987).

On the basis of previously published data, it was not possible unambiguously to conclude whether *A. fucosus* subsp. *hippuratus* represents a separate genomic species within the genus *Agromyces*, because its relationship with *A. cerinus* subsp. *nitratus* is vague. Our phylogenetic analysis based on 16S rRNA gene sequences showed that *A. fucosus* subsp. *hippuratus* VKM Ac-1352^T is different from both *A. cerinus* subsp. *nitratus* VKM Ac-1351^T and *A. cerinus* subsp. *cerinus* VKM Ac-1340^T (Fig. 1), exhibiting 97·8 and 98·4 % 16S rRNA gene sequence similarities to these strains, respectively. Investigation of DNA–DNA relatedness between *A. fucosus* subsp. *hippuratus* VKM Ac-1345^T and *A. cerinus* subsp. *nitratus* VKM Ac-1352^T revealed a ΔT_m of 9·6 °C, suggesting these strains belong to different species (Roselló-Mora & Amann, 2001). Similar analyses performed on the type strains of *A. cerinus* subsp. *cerinus* and *A. cerinus* subsp. *nitratus* revealed a small ΔT_m (4·1 °C) and confirmed their close DNA–DNA relatedness, as reported by Zgurskaya *et al.* (1992) and Suzuki *et al.* (1996).

At the phenotypic level, *A. fucosus* subsp. *hippuratus* differs from *A. fucosus* subsp. *fucosus* and from both subspecies of *A. cerinus* with regard to cell wall teichoic acid composition, which are water-soluble carbohydrate-containing polymers covalently linked to peptidoglycan by phosphodiester bridges and which occur in many Gram-positive bacteria (Baddiley, 1972; Naumova *et al.*, 2001). Two strains of *A. fucosus* subsp. *hippuratus* (VKM Ac-1352^T and VKM Ac-1353) contained 1,5-poly(ribitol phosphate) with tetrasaccharide substituents (Gnilozub *et al.*, 1994; Naumova *et al.*, 2001), whereas four strains of *A. fucosus* subsp. *fucosus* (VKM Ac-1345^T, VKM Ac-1346, VKM Ac-1347 and VKM Ac-1349) contained 1,3-poly(glycerol phosphate) with *β*-*N*-acetylglucosamine substituents (Malysheva, 1994). Five analysed strains of *A. cerinus* subsp. *cerinus* (VKM Ac-1340^T, VKM Ac-1342, VKM Ac-1343, VKM Ac-1344 and VKM Ac-1350) contained poly(arabitol phosphate) teichoic acid, and *A. cerinus* subsp. *nitratus* VKM Ac-1351^T, the only known strain of this subspecies, contained poly(ribofuranosylribitol phosphate) polymer in the cell wall (Shashkov *et al.*, 1993, 1995; Malysheva, 1994; Naumova *et al.*, 2001). The structures and combinations of cell wall teichoic acids are considered of high taxonomic value and were shown to be usually species-specific in all actinomycete genera in which they were analysed (for references, see Naumova *et al.*, 2001). These data are consistent with a clear physiological discrimination between *A. fucosus* subsp. *hippuratus* and other recognized species (Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Takeuchi & Hatano, 2001; Li *et al.*, 2003; Dorofeeva *et al.*, 2003). In addition, representatives of the *A. fucosus* subspecies were reported to differ in polyamine composition (Altenburger *et al.*, 1997); for instance, *A. fucosus* subsp. *fucosus* contained putrescine and 1,3-diaminopropane whereas *A. fucosus* subsp. *hippuratus* contained putrescine and spermidine.

Fatty acid profiles of the type strains of *A. fucosus* and *A. hippuratus* were analysed using the Sherlock Microbial Identification System (MIDI) (Sasser, 1991). They showed only slight differences in their percentages of iso- and anteiso-15 : 0 to 17 : 0 fatty acids, which appeared to be of little value for species differentiation. The cellular fatty acid compositions determined in *A. fucosus* subsp. *hippuratus* and *A. fucosus* subsp. *fucosus* are, respectively, as follows (%): iso-$C_{14:0}$, 0·9 and 0·6; *n*-$C_{14:0}$, 0·2 and 0·4; iso-$C_{15:0}$, 10·5 and 6·4; anteiso-$C_{15:0}$, 51·7 and 47·2; *n*-$C_{15:0}$, 0·1 and 0·1; iso-$C_{16:0}$, 14·3 and 14·6; *n*-$C_{16:0}$, 1·5 and 1·0; iso-$C_{17:0}$, 1·9 and 2·3; anteiso-$C_{17:0}$, 18·1 and 27·5; *n*-$C_{18:0}$, 0·1 and 0·2.

Thus, based on the 16S rRNA gene sequence analysis, DNA–DNA relatedness values and differences in phenotypic traits obtained in this and earlier studies (Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Takeuchi & Hatano, 2001; Dorofeeva *et al.*, 2003), we propose to reclassify *A. fucosus* subsp. *hippuratus* as a separate species, *Agromyces hippuratus* sp. nov., comb. nov. (type strain VKM Ac-1352^T), and to emend the description of *A. fucosus*. The latter is restricted to strains assigned previously to *A. fucosus* subsp. *fucosus* (Zgurskaya *et al.*, 1992).

Description of *Agromyces hippuratus* sp. nov., comb. nov.

Agromyces hippuratus (hip.pu.ra′tus. N.L. n. *hippuratum* hippurate; N.L. masc. adj. *hippuratus* pertaining to hippurate, relating to the ability to decompose hippurate).

Basonym: *Agromyces fucosus* subsp. *hippuratus* Zgurskaya *et al.* 1992.

The description is based on phenotypical data of Zgurskaya *et al.* (1992), Gnilozub *et al.* (1994), Malysheva (1994), Groth *et al.* (1996), Suzuki *et al.* (1996), Altenburger *et al.* (1997) and Dorofeeva *et al.* (2003). Colonies on nutrient media are opaque, entire, convex and usually penetrate into the agar media. Produces a yellow carotenoid pigment. Branching hyphae (width, 0·2–0·6 mm) break into diphtheroid and rod-like, irregular, non-motile fragments. Aerobic, catalase- and oxidase-positive. Mesophilic; optimum growth is at 26–30 °C. Strains are able to grow at 7 °C; weak or no growth occurs at 37 °C. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, melibiose, L-rhamnose, salicin, sucrose, trehalose and D-xylose are used for growth as sole carbon sources in a salt medium supplemented with 0·1 % (w/v) yeast extract (Zgurskaya *et al.*, 1992). Adonitol, dulcitol, *meso*-inositol, lyxose, D-mannitol, methyl β-D-arabinopyranoside and L-sorbose are not used as sole carbon sources in the same medium. Acids are produced from D-arabinose, glycerol and L-rhamnose; no acid production from cellobiose, inulin, lactose, maltose, raffinose, D-ribose, salicin or trehalose. Acid production from L-arabinose and sucrose is variable. Fumarate, hippurate, malate and pyruvate are utilized. No alkaline reactions with ascorbate, citrate, gluconate, oxalate, propionate, salicylate, succinate and tartrate are observed; some strains show positive reaction with *trans*-aconitate. Nitrate is reduced to nitrite and tyrosinase is produced. Aesculin, hypoxanthine and starch are hydrolysed. Adenine, elastin, guanine, pectin, testosterone, urea and xanthine are not decomposed or hydrolysed. Casein is usually not hydrolysed. Indole test is negative. No growth occurs on media supplemented with 5 % NaCl, 0·01 % sodium azide or 0·0175 % potassium tellurite. The major menaquinone is MK-12 with a minor amount of MK-13. Polyamine content is low; putrescine and spermidine are predominant compounds (data for the type strain only). Cell wall sugars are rhamnose, galactose and trace mannose. Cell wall contains 1,5-poly(ribitol phosphate) teichoic acid. The G+C content of the DNA is about 71 mol%. Isolated from soil.

The type strain is VKM Ac-1352^T (=JCM 9087^T). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of this strain is D45061.

Emended description of *Agromyces fucosus*

The description is based on phenotypical data of Zgurskaya *et al.* (1992), Malysheva (1994), Groth *et al.* (1996), Suzuki *et al.* (1996), Altenburger *et al.* (1997) and Dorofeeva *et al.* (2003).

Colonies on nutrient media are opaque, entire and convex, and occasionally penetrate into the agar media. Produces a yellow carotenoid pigment. Branching hyphae (width, 0·2–0·6 mm) break into diphtheroid and rod-like, irregular, non-motile fragments. Catalase- and oxidase-positive. Mesophilic; optimum growth is at 26–30 °C. Strains are able to grow at 7 °C; no or weak growth occurs at 37 °C. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, methyl D-glucoside, L-rhamnose, salicin, sucrose, trehalose and D-xylose are used for growth as sole carbon sources in a salt medium supplemented with 0·1 % (w/v) yeast extract (Zgurskaya *et al.*, 1992). Dulcitol, erythritol, lyxose, D-mannitol, methyl-β-D-arabinopyranoside and L-sorbose are not used as sole carbon sources in the same medium. Some strains, including the type strain, use melibiose for growth, whereas adonitol and *meso*-inositol are not used by most of the strains, including the type strain. Acids are usually produced from L-arabinose, cellobiose, glycerol, inulin, lactose, maltose, L-rhamnose, salicin, sucrose and D-xylose by most strains (reactions of the type strain are positive or variable). Acetate, malate and pyruvate are utilized. Alkaline reactions with fumarate and propionate are variable (type strain is positive). Some strains utilize citrate and succinate (type strain is negative). Ascorbate, *trans*-aconitate, gluconate, oxalate, salicylate and tartrate are not utilized. Production of H_2S is variable; the type strain shows a positive reaction. Arbutin, aesculin and starch are hydrolysed; adenine, elastin, guanine, pectin, testosterone, urea and xanthine are not decomposed or hydrolysed. Hippurate, hypoxanthine, Tween 40 and tyrosine are decomposed by some strains, including the type strain. Casein is usually not hydrolysed. Indole test is negative. Nitrate reduction test is negative or weak reaction is observed. No growth occurs on media supplemented with 5 % NaCl, 0·01 % sodium azide or 0·0175 % potassium tellurite. The major menaquinone is MK-12; the second most common component is MK-13. Polyamine content is low; putrescine and 1,3-diaminopropane are the predominant compounds (data for the type strain only). Cell wall sugars are galactose, rhamnose, fucose and minor mannose (type strain); glucose may occur but fucose is lacking in other strains. Cell wall contains 1,3-poly(glycerol phosphate) teichoic acid. The G+C content of the DNA is 70–72 mol%. Isolated from soil.

The type strain is VKM Ac-1345^T (=DSM 8597^T). The GenBank accession number for the 16S rRNA gene sequence of this strain is AY158025.

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