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-1352T (=JCM 9087T). 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 brachium*, *Agromyces luteolus*, *Agromyces rhizophorae* (Takeuchi & Hatano, 2001), *Agromyces aurantius* (Li et al., 2003) and *Agromyces albus* (Dorofeeva et al., 2003).

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-1345T (1473 bp; AY158025) and *A. cerinus* subsp. *nitratus* VKM Ac-1351T (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-1345T and *A. fucosus* subsp. *hippuratus* VKM Ac-1352T 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-1345T and *A. fucosus* subsp. *hippuratus* VKM Ac-1352T was determined by measuring the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔTm) as described by De Ley et al. (1970). We found a significant ΔTm (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,
On the basis of previously published data, it was not possible unambiguously to conclude whether A. fucosus subsp. hippocratus represents a separate genomic species within the genus Agromyces, because its relationship with A. cerinus subsp. nitratīus is vague. Our phylogenetic analysis based on 16S rRNA gene sequences showed that A. fucosus subsp. hippocratus VKM Ac-1352T is different from both A. cerinus subsp. nitratīus VKM Ac-1351T and A. cerinus subsp. cerinus VKM Ac-1340T (Fig. 1), exhibiting 97-8 and 98-4 % 16S rRNA gene sequence similarities to these strains, respectively. Investigation of DNA–DNA relatedness between the type strains of A. cerinus subsp. hippocratus and other recognized species (Naumova et al., 2001), whereas four strains of A. fucosus subsp. fucosus (VKM Ac-1345T, 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-1340T, VKM Ac-1342, VKM Ac-1343, VKM Ac-1344 and VKM Ac-1350) contained poly(arabitol phosphate) teichoic acid, and A. cerinus subsp. nitratīus VKM Ac-1351T, the only known strain of this subspecies, contained poly(ribofuranosyribitol 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. hippocratus and A. cerinus subsp. nitratīus, whereas A. fucosus subsp. hippocratus contained putrescine and 1,3-diaminopropane whereas A. fucosus subsp. hippocratus contained putrescine and spermidine.

Fatty acid profiles of the type strains of A. fucosus and A. hippocratus 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. hippocratus and A. fucosus subsp. fucosus are, respectively, as follows (%) iso-C15:0: 7.2, 3 and 0.2; anteiso-C15:0: 5.3; iso-C16:0: 6.6; anteiso-C15:0; 0.1; iso-C17:0: 0.1; anteiso-C17:0: 14; 16-6; n-C16:0: 1.5; n-C17:0: 1.4; n-C18:0: 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.rat.us. N.L. n. *hippuratum* hippurate; N.L. masc. adj. *hippuratus* pertaining to hippurate, relating to the ability to decompose hippurate).


The description is based on phenotypical data of Zgurskaya et al. (1992), Gnilozub et al. (1994), Malyshova (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. 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.

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<th>Carbon Source</th>
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<td>D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, melibiose, L-rhamnose, salicin, sucrose, trehalose and D-xylose</td>
<td>Used for growth as sole carbon sources in a salt medium supplemented with 0.1% (w/v) yeast extract (Zgurskaya et al., 1992).</td>
<td>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, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, melibiose, L-rhamnose, salicin, sucrose, trehalose and D-xylose. Some strains utilize citrate and succinate (type strain is negative). Ascorbate, trans-aconitate, gluconate, oxalate, salicylate and tartrate are not utilized. Production of H2S 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. There is no growth 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.</td>
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The type strain is VKM Ac-1345T ( = DSM 8597T). The GenBank accession number for the 16S rRNA gene sequence of this strain isAY158025.

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References


