Proposal of *Mycetocola* gen. nov. in the family *Microbacteriaceae* and three new species, *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasinivorans* sp. nov. and *Mycetocola lacteus* sp. nov., isolated from cultivated mushroom, *Pleurotus ostreatus*

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The taxonomic positions of 10 tolaasin-detoxifying bacteria, which were isolated from the cultivated mushroom *Pleurotus ostreatus*, were investigated. These strains are Gram-positive, obligately aerobic, non-sporulating and irregular rod-shaped bacteria. They have the following characteristics: the major menaquinone is MK-10, the DNA G+C content ranges from 64 to 65 mol%, the diamino acid in the cell wall is lysine and the muramic acid in the peptidoglycan is an acetyl type. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. On the basis of morphological, physiological and chemotaxonomic characteristics, together with DNA–DNA reassociation values and 16S rRNA gene sequence comparison data, the new genus *Mycetocola* gen. nov. is proposed for these bacteria in the family *Microbacteriaceae* and three new species are also proposed: *Mycetocola saprophilus* sp. nov. (type strain CM-01^T = IFO 16274^T = MAFF 211324^T = NRRL B-24119^T), *Mycetocola tolaasinivorans* sp. nov. (type strain CM-05^T = IFO 16277^T = MAFF 211325^T = NRRL B-24120^T) and *Mycetocola lacteus* sp. nov. (type strain CM-10^T = IFO 16278^T = MAFF 211326^T = NRRL B-24121^T). The type species of the genus is *Mycetocola saprophilus* sp. nov.

Keywords: *Mycetocola* gen. nov., *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasinivorans* sp. nov., *Mycetocola lacteus* sp. nov.

INTRODUCTION

Pseudomonas tolaasii Paine produces pathogenic extracellular toxins, tolaasins (Nutkins *et al.*, 1991; Shirata *et al.*, 1995), and causes brown blotch disease in economically important cultivated mushrooms *Pleurotus ostreatus* Kummer and *Agaricus bisporus*

Singer (Suyama & Fujii, 1993; Tolaas, 1915). In the course of the microbiological study of brown blotch disease, we isolated from *Pleurotus ostreatus* fruiting bodies some Gram-positive bacteria which detoxify the tolaasins and suppress the development of the disease (Tsukamoto *et al.*, 1998). They were obligately aerobic, non-sporulating and irregular rod-shaped bacteria. The result of phylogenetic analysis of the 16S rRNA gene showed that the tolaasins-detoxifying bacteria belong to the family *Microbacteriaceae* and bacteriological analyses also supported this result.

The family *Microbacteriaceae* (Stackebrandt *et al.*, 1997) contains 10 genera: *Agrococcus* (Groth *et al.*, 1996), *Agromyces* (Gledhill & Casida, 1979), *Clavibacter* (Davis *et al.*, 1984), *Cryobacterium* (Suzuki *et al.*, 1997), *Curtobacterium* (Yamada & Komagata,

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The DDBJ accession numbers for the 16S rRNA gene sequences of strains CM-01^T, CM-05^T and CM-10^T are AB012647, AB012646 and AB012648, respectively.

1972), *Frigoribacterium* (Kämpfer *et al.*, 2000), *Leifsonia* (Evtushenko *et al.*, 2000), *Leucobacter* (Takeuchi *et al.*, 1996), *Microbacterium* (Takeuchi & Hatano, 1998) and *Rathayibacter* (Zgurskaya *et al.*, 1993). The bacteria we isolated from *Pleurotus ostreatus* contain lysine as the diamino acid and acetyl-type muramic acid in the peptidoglycan, MK-10 as the major menaquinone, a DNA G+C content of 63.9–65.2 mol% and the isolated bacteria cannot grow at 4 °C. Species of the genus *Frigoribacterium* and some species of *Microbacterium* have lysine in the cell wall. However, the isolated bacteria are different from these organisms in that they possess a different menaquinone and have a different G+C content and growth temperature. Also, the results of phylogenetic analysis of the 16S rRNA gene revealed that the isolated bacteria form an independent cluster and that this cluster is isolated from any of the subclusters corresponding to the established genera within the family *Microbacteriaceae*.

This paper describes the unique characteristics of the strains isolated from rotting *Pleurotus ostreatus* fruiting bodies. The phylogenetic data show that they represent a new genus in the family *Microbacteriaceae*. Chemotaxonomic and physiological data support this conclusion. We propose a new genus, *Mycetocola* gen. nov., and three new species, *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasinivorans* sp. nov. and *Mycetocola lacteus* sp. nov.

METHODS

Bacterial strains. Micro-organisms isolated from rotting *Pleurotus ostreatus* fruiting bodies were cultured on PS agar medium containing 0.5 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 5 g peptone, 15 g sucrose and 15 g agar in 1 l of a decoction of 300 g potato tuber slices (pH 7.0) as described by Wakimoto (1955). Ten strains were selected as tolaasins-detoxifying strains by using the potato tuber slice method (Shirata *et al.*, 1995) (Table 1). All strains were cultured aerobically at 28 °C in a peptone/yeast extract medium (PY medium) containing 10 g peptone, 2 g yeast extract, 2 g NaCl and 2 g D-glucose in 1 l H_2O (pH 7.2). Cells used for biochemical tests were harvested by centrifugation during the stationary phase, washed with water and lyophilized.

Morphological, physiological and biochemical characteristics. Cell morphology was determined by phase-contrast microscopy following growth on PS agar. One-day-old cells in PS broth medium were negatively stained with 2.0% phosphotungstic acid (pH 6.5) and were observed with a JEM-1010 transmission electron microscope (JEOL). Physiological and biochemical characteristics were examined as described by Nishiyama (1981) and Azegami *et al.* (1987). Dye's medium C (Dye, 1962) and OY medium (Dye, 1968) were used as the basal media for acid production and utilization tests, respectively.

Chemical analyses. Cell walls were prepared from about 500 mg (dry wt) bacterial cells as described by Schleifer & Kandler (1972). Amino acids in the acid hydrolysate of the cell walls were identified by two-dimensional ascending chromatography on cellulose TLC plates (Tokyo Kasei) by the method of Harper & Davis (1979) and by HPLC as their phenylthiocarbamoyl derivatives with a model Shimadzu LC-6AD HPLC apparatus, according to the manufacturer's instructions. Glycolate tests were performed by the method of Uchida *et al.* (1999). Cellular fatty acids were extracted from dried cells (50 mg) by acid methanolysis, purified (Minnikin *et al.*, 1979) and analysed by GLC-MS with a GCMS-QP5000 spectrometer (Shimadzu) combined with a CLASS-5000 MS Workstation computer system. GLC analyses were carried out using a GC-17A gas chromatograph (Shimadzu). A BPX70 capillary column (SGE) containing 70% cyanopropyl equivalent modified siloxane (50 m $\times$ 0.25 mm) was used at 80 °C for 2 min, 80–150 °C at 15 °C min⁻¹, 150–250 °C at 8 °C min⁻¹ and then 250 °C for 5 min, with helium as carrier gas at a flow rate of 1.4 ml min⁻¹. Menaquinones were extracted from dry cells (200 mg) with chloroform/methanol (2:1, v/v), purified by silica gel TLC (Kieselgel 60F₂₅₄ plates; Merck) using hexane/diethyl ether (85:15, v/v) as solvent, extracted with acetone, dried under nitrogen stream and then analysed by HPLC with a Shimadzu model LC-5A instrument equipped with a Zorbax octyldecyl silane column (150 $\times$ 4.6 mm).

DNA base composition and DNA relatedness. Isolation and purification of chromosomal DNA and estimation of G+C content were performed by the methods of Takagi *et al.* (1993). DNA relatedness values were determined as described by Ezaki *et al.* (1989).

Amplification and sequencing of the 16S rRNA gene. The 16S rRNA gene was amplified by PCR using prokaryotic 16S rRNA gene universal primers 27f (5'-AGAGTTTGATCTGGCTCAG-3'; position 8–27 in *Escherichia coli*) and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'; 1513–

Table 1. Bacterial strains isolated from cultivated mushrooms and 16S rRNA gene accession numbers

Strain	Geographical origin of mushrooms	Proposed name	DDBJ accession no.
CM-01 ^T	Yamagata prefecture, Japan	<i>Mycetocola saprophilus</i>	AB012647
CM-02	Yamagata prefecture, Japan	<i>Mycetocola saprophilus</i>	
CM-03	Yamagata prefecture, Japan	<i>Mycetocola saprophilus</i>	
CM-05 ^T	Ibaraki prefecture, Japan	<i>Mycetocola tolaasinivorans</i>	AB012646
CM-10 ^T	Gifu prefecture, Japan	<i>Mycetocola lacteus</i>	AB012648
CM-14	Ibaraki prefecture, Japan	<i>Mycetocola lacteus</i>	
CM-15	Ibaraki prefecture, Japan	<i>Mycetocola lacteus</i>	
CM-16	Ibaraki prefecture, Japan	<i>Mycetocola lacteus</i>	
CM-20	Ibaraki prefecture, Japan	<i>Mycetocola lacteus</i>	
CM-21	Ibaraki prefecture, Japan	<i>Mycetocola lacteus</i>	

1492). The PCR products were purified by using a Sepharose Cl-2B gel (Pharmacia). The sequencing reactions were performed by using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Primers used for sequencing were 27f, 530f (5'-GTGCCAGCMGCCGCGG-3'; 515-530), 1114f (5'-GCA-ACGAGCGCAACCC-3'; 1099-1114), 515r (5'-CCGCGG-CKGCTGGCAC-3'; 530-515), 1100r (5'-GGGTTGCGC-TCGTTG-3'; 1114-1100) and 1492r. All PCR reactions were performed with a Perkin-Elmer Cetus model 9600 thermal cycler. Each extension product resulting from the sequencing reaction was purified through a Centri-Sep spin column (Applied Biosystems) and sequenced by using an ABI model 377 automated DNA sequencer.

Phylogenetic analysis and nucleotide sequence accession numbers. The multiple alignment of sequences, calculation of nucleotide substitution rates (K_{nuc} values; Kimura, 1980), construction of a neighbour-joining phylogenetic tree (Saitou & Nei, 1987) and a bootstrap analysis with 1000 replicates for evaluation of phylogenetic tree topology (Felsenstein, 1985) were carried out with the CLUSTAL W multiple sequence alignment program (Thompson *et al.*, 1994). The 16S rRNA gene sequences determined in this study have been deposited in the DDBJ database under the accession numbers shown in Table 1.

RESULTS

Morphological, biochemical and physiological characteristics

All of the strains were Gram-positive, non-sporulating, non-motile, irregular rods, 0.2–0.4 µm wide and 2.0–3.5 µm long in PS broth medium (Fig. 1). They were

Fig. 1. Electron micrograph of negatively stained cells of *Mycetocola saprophilus*, showing pleomorphic, rod-shaped cells with mesosomes. Cells are 0.2–0.4 µm wide and 2.0–3.5 µm long.

aerobic and no growth was found under anaerobic conditions. The strains formed smooth, yellowish-white colonies on PS agar. The optimal temperature for growth was 25 °C and the maximum temperature for growth was 33 °C for all strains. No strain grew at 4 °C. Ten strains were divided into three groups. The first group contains three strains, CM-01^T, CM-02 and CM-03, which did not hydrolyse Tween 80 and did not produce acid from erythritol, but did produce acid from melezitose and utilize citrate. The second group contains one strain, CM-05^T, which did not hydrolyse Tween 80, did not produce acid from erythritol and melezitose and did not utilize citrate. The third group is composed of six strains, CM-10^T, CM-14, CM-15, CM-16, CM-20 and CM-21, which hydrolysed Tween 80, produced acid from erythritol and melezitose and utilized citrate.

Chemotaxonomic characteristics

The amino acid composition of the cell walls of the representative strains of each group was glutamic acid, glycine, alanine, lysine and an unknown amino acid in the molar ratio of 0.60:1.50:1.00:2.14:1.03 for strain CM-01^T, 2.11:2.06:1.00:1.74:0.94 for strain CM-05^T and 1.69:1.39:1.00:1.11:0.80 for strain CM-10^T. Despite further analysis using two-dimensional paper chromatography, the identification of the unknown amino acid was unsuccessful. The glycan moiety of the cell walls of all strains contained acetyl residues. Cellular fatty acids of all the strains were composed mainly of anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}, and the composition ratios were approximately 56, 39 and 5 % in strains CM-01^T, CM-02 and CM-03, 63, 33 and 4 % in strain CM-05^T and 51, 46 and 3 % in strains CM-10^T, CM-14, CM-15, CM-16, CM-20 and CM-21, respectively. These classifications agree with the results derived from the biochemical and physiological tests (see above). The menaquinones of strain CM-01^T were MK-10 (53 %), MK-9 (23 %), MK-11 (12 %) and MK-8 (8 %), those of strain CM-05^T were MK-10 (58 %), MK-9 (21 %), MK-11 (14 %) and MK-8 (4 %), and those of strain CM-10^T were MK-10 (54 %), MK-9 (21 %), MK-11 (14 %) and MK-8 (4 %).

DNA relatedness

The levels of DNA relatedness were analysed and the results are presented in Table 2. Three strains CM-01^T, CM-02 and CM-03 were related with 81–96 % homology and the levels among the other seven strains ranged from 12 to 54 %. Strain CM-05^T did not show a high level of DNA relatedness with any other strains (18–28 %). Six strains, CM-10^T, CM-14, CM-15, CM-16, CM-20 and CM-21, showed a high level of DNA relatedness with each other (79–84 %), but did not show a high level of relatedness with the other four strains (16–52 %). On the basis of these results, the three groups into which the 10 strains have been divided can be classified at the species level.

Table 2. DNA base composition and DNA relatedness of the strains

Strain	G + C content (mol %)	DNA relatedness (%) with:		
		CM-01 ^T	CM-05 ^T	CM-10 ^T
CM-01 ^T	65.2	100	26	45
CM-02	65.2	81	23	42
CM-03	65.2	96	26	52
CM-05 ^T	65.1	12	100	16
CM-10 ^T	63.9	42	18	100
CM-14	64.6	46	28	79
CM-15	64.6	46	25	84
CM-16	64.6	46	23	80
CM-20	64.6	54	25	84
CM-21	64.7	47	26	79

Phylogenetic analysis of 16S rRNA gene

Almost complete 16S rRNA gene sequences of 1444 or 1445 bases of the 10 strains were determined. Strains CM-01^T, CM-02 and CM-03 had identical 16S rRNA gene sequences as did strains CM-10^T, CM-14, CM-15,

CM-16, CM-20 and CM-21. The phylogenetic tree based on calculated K_{nuc} values clearly shows that the representative strains CM-01^T, CM-05^T and CM-10^T formed an independent cluster at the base of the *Microbacterium* cluster in the family *Microbacteriaceae* with a 100% bootstrap confidence level (Fig. 2).

DISCUSSION

We isolated from rotting *Pleurotus ostreatus* fruiting bodies 10 micro-organisms that markedly reduce the level of extracellular toxins, tolaasins, produced by *Pseudomonas tolaasii*, the most destructive pathogen of cultivated mushrooms. They were saprophytic, but not parasitic nor pathogenic to *Pleurotus ostreatus* (Tsukamoto *et al.*, 1998). To clarify the taxonomic positions of these bacteria, we examined their morphological, physiological and chemotaxonomic characteristics, together with DNA–DNA re-association values and 16S rRNA gene sequence comparisons. The strains are Gram-positive, obligately aerobic, non-sporulating and irregular rod-shaped bacteria with high G + C contents (64–65 mol%) and they also have the following characteristics: the

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences of *Mycetocola* species and some close relatives. The tree was created by the neighbour-joining method and the numerals indicate the percentages of bootstrap samplings, derived from 1000 replications.

Table 3. Distinguishing characteristics between *Mycetocola* gen. nov. and other genera in the family *Microbacteriaceae*

Data from Evtushenko *et al.* (2000), Kämpfer *et al.* (2000) and Takeuchi & Hatano (1998). DAB, 2,4-diaminobutyric acid; Lys, lysine; Orn, ornithine; ND, not determined.

Genus	Peptidoglycan		Major menaquinone	G + C content (mol %)
	Diamino acid	Acyl type		
<i>Mycetocola</i>	Lys	Acetyl	MK-10	64–65
<i>Agrococcus</i>	L-DAB	Acetyl	MK-11, 12	74
<i>Agromyces</i>	L-DAB	Acetyl	MK-11, 12	71–76
<i>Clavibacter</i>	DL-DAB	Acetyl	MK-9, 10	67–78
<i>Cryobacterium</i>	L-DAB	ND	MK-10	65
<i>Curtobacterium</i>	Orn	Acetyl	MK-9	68–75
<i>Frigoribacterium</i>	Lys	Acetyl	MK-9	71.7
<i>Leifsonia</i>	DL-DAB	ND	MK-10, 11	66–73
<i>Leucobacter</i>	L-DAB	Acetyl	MK-11	66.2
<i>Microbacterium</i>	Lys/Orn	Glycolyl	MK-11, 12, 13, 14	65–72
<i>Rathayibacter</i>	L-DAB	ND	MK-10	63–72

major menaquinone is MK-10, the diamino acid in the cell wall is lysine and the muramic acid in the peptidoglycan is an acetyl type.

The result of phylogenetic analysis of the 16S rRNA gene sequences revealed that these strains form a monophyletic and distinct cluster, and that this cluster was isolated from any of the subclusters corresponding to the established genera within the family *Microbacteriaceae*. On the basis of the data described above, we concluded that these strains should belong to a new genus in the family *Microbacteriaceae*. Therefore, we propose a new genus, *Mycetocola* gen. nov., for these bacteria isolated from mushrooms. This conclusion was supported by the results of chemotaxonomic studies. Table 3 shows some of the characteristics that distinguish the new genus and other genera of *Microbacteriaceae*. In the family *Microbacteriaceae*, the genus *Frigoribacterium* is the only genus that has lysine as a diamino acid and acetyl-type muramic acid in the peptidoglycan. However, the genus *Mycetocola* can be distinguished from the genus *Frigoribacterium* by motility, growth temperature, major menaquinone content, major fatty acid content and DNA G+C content. *Frigoribacterium* is motile, grows at 2 °C (optimal temperature for growth is 4–10 °C), the major menaquinone is MK-9, the major fatty acids are anteiso-C_{15:0} and iso-C_{16:0}, and the G+C content is 71.7 mol %. *Mycetocola* is non-motile, does not grow at 4 °C, the major menaquinone is MK-10, the major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}, and the G+C content is 63.9–65.2 mol %.

The 10 strains in the genus *Mycetocola* can be divided into three species according to the results of DNA–DNA hybridization tests (Table 2). This conclusion was supported by the results of phenotypic characterization, hydrolysis of Tween 80, acid production from erythritol or melezitose, utilization of citrate and patterns of cellular fatty acids. 16S rRNA gene

sequence analysis of the representative strains of these three groups reinforced their status as novel species in the genus *Mycetocola*. On the basis of these physiological and chemotaxonomic characteristics, and the result of phylogenetic analysis together with DNA–DNA reassociation, we propose the new species *Mycetocola saprophilus* sp. nov., *Mycetocola tolaasini-vorans* sp. nov. and *Mycetocola lacteus* sp. nov. The type species of the genus is *Mycetocola saprophilus*.

Description of *Mycetocola* gen. nov.

Mycetocola (my.ce'to.co.la. L. n. *myceto* fungus; Gr. adj. *-cola* inhabitant; M.L. masc. n. *mycetocola* fungus-dweller).

Cells are Gram-positive, obligately aerobic, irregular rods, 0.2–0.4 × 2.0–3.5 µm. Endospores are not produced. Non-motile. Colonies are circular, convex, smooth and yellowish-white on PS agar. Growth optimal at 25 °C, but no growth above 33 °C nor at 4 °C in PS broth medium. Catalase is produced, but oxidase is not produced. The following tests are positive: aesculin hydrolysis, growth in peptone water, acid production from D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, salicin, D-sorbitol, sucrose, trehalose and D-xylose, and utilization of fumarate. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, liquefaction of gelatin, Voges–Proskauer reaction, methyl red reaction, lecithinase, tyrosinase, urease, hydrolysis of arbutin, arginine and casein, production of diffusible and fluorescent pigment, hydrogen sulfide, indole, 3-ketolactose, 2-ketogluconate and levan from sucrose, growth in Corn's solution, Fermi's solution and Uschinsky's solution, utilization of asparagine as sole source of carbon and nitrogen, maceration of potato tubers, hypersensitive reaction in tobacco leaves, acid production from D-arabinose, L-arabinose,

D-dulcitol, *m*-inositol, inulin, D-raffinose, L-rhamnose and starch, and utilization of benzoate, butyrate, *m*-hydroxybenzoate, malonate, oxalate, propionate, D- and L-tartarate. All strains of the genus have the ability to detoxify tolaasins produced by *Pseudomonas tolaasii*. Cell wall peptidoglycan contains lysine as a diamino acid and the muramic acid of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-10 and small amounts of MK-9 and MK-11 are present. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The G + C content is 63.9–65.2 mol%. Forms an independent phylogenetic cluster at the base of the *Microbacterium* cluster in the family *Microbacteriaceae*. Type species is *Mycetocola saprophilus*. Isolated from cultivated mushroom *Pleurotus ostreatus*.

Description of *Mycetocola saprophilus* sp. nov.

Mycetocola saprophilus (sa.pro.ph'il.us. Gr. adj. *sapros* putrid; Gr. adj. *philus* loving; M.L. adj. *saprophilus* putrid-loving).

Cells are Gram-positive, obligately aerobic, irregular rods, 0.2–0.4 × 2.0–3.5 µm, non-sporulating and non-motile. Smooth, yellowish-white colonies are found on PS agar. The maximum temperature for growth is 33 °C and the optimal temperature for growth is 25 °C, but no growth at 4 °C in PS broth medium. Catalase is produced, but oxidase is not produced. The following tests are positive: aesculin hydrolysis, growth in peptone water, acid production from D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, salicin, D-sorbitol, sucrose, trehalose and D-xylose, utilization of citrate and fumarate, and detoxification of tolaasins produced by *Pseudomonas tolaasii*. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, liquefaction of gelatin, Voges–Proskauer reaction, methyl red reaction, lecithinase, tyrosinase, urease, hydrolysis of arbutin, arginine, casein and Tween 80, production of diffusible and fluorescent pigment, hydrogen sulfide, indole, 3-ketolactose, 2-ketogluconate and levan from sucrose, growth in Corn's solution, Fermi's solution and Uschinsky's solution, utilization of asparagine as sole source of carbon and nitrogen, maceration of potato tubers, hypersensitive reaction in tobacco leaves, acid production from D-arabinose, L-arabinose, D-dulcitol, erythritol, *m*-inositol, inulin, D-raffinose, L-rhamnose and starch, and utilization of benzoate, butyrate, *m*-hydroxybenzoate, malonate, oxalate, propionate, D- and L-tartarate. Cell wall peptidoglycan contains lysine as a diamino acid and the muramic acid of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-10 and small amounts of MK-9 and MK-11 are present. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The G + C content is 65.2 mol%. Isolated from cultivated mushroom *Pleurotus ostreatus*. Type strain is CM-01^T (= IFO 16274^T = MAFF 211324^T = NRRL B-24119^T).

Description of *Mycetocola tolaasinivorans* sp. nov.

Mycetocola tolaasinivorans (to.laa.si.ni.vo'rans. N.L. neut. n. *tolaa sinum* tolaasin, type of toxin produced by *Pseudomonas tolaasii*; L. part. adj. *vorans* devouring, decomposing; N.L. part. adj. *tolaa sinivorans* tolaasin-decomposing).

Cells are Gram-positive, obligately aerobic, irregular rods, 0.2–0.4 × 2.0–3.5 µm, non-sporulating and non-motile. Smooth, yellowish-white colonies are found on PS agar. The maximum temperature for growth is 33 °C and the optimal temperature for growth is 25 °C, but no growth at 4 °C in PS broth medium. Catalase is produced, but oxidase is not produced. The following tests are positive: aesculin hydrolysis, growth in peptone water, acid production from D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, salicin, D-sorbitol, sucrose, trehalose and D-xylose, utilization of fumarate, and detoxification of tolaasins produced by *Pseudomonas tolaasii*. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, liquefaction of gelatin, Voges–Proskauer reaction, methyl red reaction, lecithinase, tyrosinase, urease, hydrolysis of arbutin, arginine, casein and Tween 80, production of diffusible and fluorescent pigment, hydrogen sulfide, indole, 3-ketolactose, 2-ketogluconate and levan from sucrose, growth in Corn's solution, Fermi's solution and Uschinsky's solution, utilization of asparagine as sole source of carbon and nitrogen, maceration of potato tubers, hypersensitive reaction in tobacco leaves, acid production from D-arabinose, L-arabinose, D-dulcitol, erythritol, *m*-inositol, inulin, melibiose, D-raffinose, L-rhamnose and starch, and utilization of benzoate, butyrate, citrate, *m*-hydroxybenzoate, malonate, oxalate, propionate, D- and L-tartarate. Cell wall peptidoglycan contains lysine as a diamino acid and the muramic acid of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-10 and small amounts of MK-9 and MK-11 are present. The G + C content is 65.1 mol%. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. Isolated from cultivated mushroom *Pleurotus ostreatus*. Type strain is CM-05^T (= IFO 16277^T = MAFF 211325^T = NRRL B-24120^T = strain 9405^T, described in Tsukamoto *et al.*, 1998).

Description of *Mycetocola lacteus* sp. nov.

Mycetocola lacteus (lac.te'us. L. adj. *lacteus* milky).

Cells are Gram-positive, obligately aerobic, irregular rods, 0.2–0.4 × 2.0–3.5 µm, non-sporulating and non-motile. Smooth, yellowish-white colonies are found on PS agar. The maximum temperature for growth is 33 °C and the optimal temperature for growth is 25 °C, but no growth at 4 °C in PS broth medium. Catalase is produced, but oxidase is not produced. The following tests are positive: hydrolysis of aesculin and Tween 80, growth in peptone water, acid production from D-

cellobiose, dextrin, erythritol, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, D-ribose, salicin, D-sorbitol, sucrose, trehalose and D-xylose, utilization of citrate and fumarate, and detoxification of tolaasins produced by *Pseudomonas tolaasii*. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, liquefaction of gelatin, Voges–Proskauer reaction, methyl red reaction, lecithinase, tyrosinase, urease, hydrolysis of arbutin, arginine and casein, production of diffusible and fluorescent pigment, hydrogen sulfide, indole, 3-ketolactose, 2-keto-gluconate and levan from sucrose, growth in Corn's solution, Fermi's solution and Uschinsky's solution, utilization of asparagine as sole source of carbon and nitrogen, maceration of potato tubers, hypersensitive reaction in tobacco leaves, acid production from D-arabinose, L-arabinose, D-dulcitol, *m*-inositol, inulin, D-raffinose, L-rhamnose and starch, and utilization of benzoate, butyrate, *m*-hydroxybenzoate, malonate, oxalate, propionate, D- and L-tartarate. Cell wall peptidoglycan contains lysine as a diamino acid and the muramic acid of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-10 and small amounts of MK-9 and MK-11 are present. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The G + C content is 63.9–64.7 mol%. Isolated from cultivated mushroom *Pleurotus ostreatus*. Type strain is CM-10^T (= IFO 16278^T = MAFF 211326^T = NRRL B-24121^T).

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