Oryzihumus leptocrescens gen. nov., sp. nov.

Akiko Kageyama,¹ Yoko Takahashi,¹ Tamae Seki,¹ Hiroshi Tomoda¹
and Satoshi Ōmura^{1,2}

Correspondence

Yoko Takahashi
ytakaha@lisci.kitasato-u.ac.jp

Kitasato Institute for Life Sciences, Kitasato University¹, and The Kitasato Institute², 5-9-1
Shirokane, Minato-ku, Tokyo 108-8641, Japan

Three novel strains were isolated from a soil sample collected in Japan using GPM agar plates supplemented with superoxide dismutase and/or catalase. The strains were Gram-positive, catalase-positive, irregular rod-shaped bacteria with *meso*-diaminopimelic acid as a peptidoglycan diagnostic diamino acid, and the acyl type of the peptidoglycan was acetyl. The major menaquinone was MK-8(H₄). Mycolic acids were not detected. The G + C content of the DNA was 72–73 mol%. On the basis of morphological and chemotaxonomic properties and a phylogenetic analysis using 16S rRNA gene sequences, these strains were classified as a novel genus and species, *Oryzihumus leptocrescens* gen. nov., sp. nov., in the family *Intrasporangiaceae* of the order *Actinomycetales*. The type strain is KV-628^T (=NRRL B-24347^T = JCM 12835^T = NBRC 100762^T).

Various techniques for the isolation of novel microbial strains have been reported in order to facilitate the discovery of novel bioactive compounds from micro-organisms. An efficient method of discovering novel bioactive metabolites is through the isolation of new micro-organisms, and numerous approaches have been attempted to date (Huck *et al.*, 1991; Iwai & Takahashi, 1992; Jiang & Xu, 1996; Nioh *et al.*, 1995; Nonomura & Hayakawa, 1988; Suzuki *et al.*, 1998; Takeuchi & Hatano, 1999). In a previous report, a new isolation method using agar medium containing oxidant scavengers, such as superoxide dismutase (SOD) or SOD plus catalase, was described (Takahashi *et al.*, 2003). Using this method, we succeeded in increasing the number of bacterial strains isolated from soil samples.

Among 45 strains isolated from paddy soil in Saitama Prefecture, Japan, using this new isolation method, 20 strains belonged to the class *Actinobacteria*. Three strains were irregular rods, found to have *meso*-diaminopimelic acid in the peptidoglycan; in the phylogenetic tree based on 16S rRNA gene sequences, they were loosely associated with *Ornithinococcus hortensis*. *Ornithinococcus hortensis* cells are cocci containing ornithine in their peptidoglycan.

In this paper, we report on the morphological, physiological and biochemical characteristics, cell composition, DNA–DNA hybridization and 16S rRNA gene sequences of the

three isolates in comparison with those of *Ornithinococcus hortensis*. On the basis of the characteristics studied, these isolates represent a novel genus and species, *Oryzihumus leptocrescens* gen. nov., sp. nov.

Strains KV-628^T, KV-641 and KV-656 were isolated from soil samples collected from a paddy field in Saitama Prefecture, Japan. Soil samples (2 g) were suspended in 18 ml sterile water and then mixed. Soil particles were allowed to sediment, the liquid phase was diluted 10⁵ times and 100 µl was spread onto the surface of four kinds of GPM agar (1.0 % glucose, 0.5 % peptone, 0.5 % meat extract, 0.3 % NaCl, 1.2 % agar; pH 7.0) plate (Takahashi *et al.*, 2003), as follows: (1) unsupplemented; (2) supplemented with SOD (300 U per plate); (3) supplemented with catalase (2100 U per plate); or supplemented with SOD plus catalase. Each plate was cultured at 27 °C. Biomass for biochemical and chemotaxonomic testing was prepared by culture in Todd–Hewitt broth (Difco) or nutrient broth (Difco) at 27 °C for 3 or 4 days, followed by harvesting by centrifugation.

Morphological observation under a scanning electron microscope (model JSM-5600; JEOL) was performed on cultures grown on GPM medium at 27 °C for 6 days. The ability of the strain to grow on a range of sole carbon sources at 1 % (w/v) was determined in carbon-utilization media (Pridham & Gottlieb, 1948) with yeast nitrogen base agar without amino acids (Nihon Pharmaceutical). NaCl tolerance and pH and temperature ranges for growth were determined on 1/5 nutrient agar. The three isolates were characterized biochemically using API ZYM (bioMérieux) according to the manufacturer's instructions. Susceptibility to antibiotics was tested by culture in YD agar (1.0 % glucose, 1.0 % yeast extract, 1.5 % agar; pH 7.0) medium

Published online ahead of print on 2 September 2005 as DOI 10.1099/ij.s.0.63799-0.

Abbreviation: SOD, superoxide dismutase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Oryzihumus leptocrescens* KV-628^T, KV-641 and KV-656 are AB193172, AB193173 and AB193174, respectively.

supplemented with the following antibiotics: erythromycin, aztreonam, rifampicin, novobiocin, streptomycin and chloramphenicol.

Whole-cell hydrolysates were analysed for diaminopimelic acid isomers using TLC (Becker *et al.*, 1965; Hasegawa *et al.*, 1983); the *N*-acyl types of the muramic acid were determined by using the method of Uchida & Aida (1977). Purified cell wall was obtained by following the method of Kawamoto *et al.* (1981), and the amino acid composition of the hydrolysed cell wall was determined using an automatic amino acid analyser (model L-8500A; Hitachi). Whole-cell sugars were analysed by using the method of Becker *et al.* (1965), the presence of mycolic acid was examined by using the TLC method of Tomiyasu (1982), and phospholipids were extracted and identified by following the method of Minnikin *et al.* (1977). Menaquinones were extracted and purified according to the method of Collins *et al.* (1977) and were then analysed by HPLC (model 802-SC; Jasco) on a chromatograph equipped with a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). The cellular fatty acids were determined. The strains were cultured on 1/5 nutrient agar plates. Methyl esters of cellular fatty acids were prepared by direct transmethylation with methanolic hydrochloride and were analysed by GLC (model GC-17A; Shimadzu) with a DB-23 capillary column (0.25 mm $\times$ 30 m; J&W Scientific) (Suzuki & Komagata, 1983).

PCR amplification for selection of *Actinobacteria* was performed. DNA was prepared by sonication (Yu *et al.*, 2002). A part of domain III of the 23S rRNA gene was amplified by using the method of Yu *et al.* (2002).

The DNA G + C content and DNA–DNA relatedness values were determined. DNA was isolated as described by Saito & Miura (1983), with some modifications. The G + C content was estimated by using HPLC (Tamaoka & Komagata, 1984). Levels of DNA–DNA relatedness were determined by the method of Ezaki *et al.* (1989), using photobiotin and a microplate format.

DNA was prepared by sonication (Yu *et al.*, 2002). The 16S rRNA gene sequence was amplified by PCR (Takahashi *et al.*, 2002) and sequenced with an automatic sequence analyser (ABI PRISM 377A; PE Applied Biosystems) using a PRISM Ready Reaction dye primer cycle sequencing kit (PE Applied Biosystems).

Species related to the novel isolate were determined by performing sequence database searches using BLAST. The sequence data of related species were retrieved from GenBank. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987). The statistical significance of the tree topology was evaluated by bootstrap analysis of sequence data with CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were determined by visual comparison and manual calculation.

Strains KV-628^T, KV-641 and KV-656 were isolated from isolation GPM agar medium supplemented with SOD, catalase or SOD plus catalase. PCR amplification was performed to determine if these strains were members of the *Actinobacteria*. This test was established to distinguish strains of the *Actinobacteria* from both Gram-negative bacteria and Gram-positive bacteria with low-G + C DNA contents by taking advantage of the specific insertion of a 100 bp sequence in domain III of the 23S rRNA gene of the *Actinobacteria*. The PCR was designed to amplify a $\sim$ 380 bp fragment from the *Actinobacteria* and a $\sim$ 270 bp fragment from other bacteria (Roller *et al.*, 1992; Yu *et al.*, 2002). Amplicons of these strains were all $\sim$ 350 bp in length, and the results showed that all three of the isolated strains were members of the *Actinobacteria*.

Nearly complete 16S rRNA gene sequences were determined for the three isolated strains. A database search demonstrated that these strains belonged to the suborder *Micrococcineae* of the family *Intrasporangiaceae*. It was clear from the phylogenetic tree (Fig. 1) that the three strains formed a monophyletic clade loosely associated with *Ornithinococcus hortensis*. The sequence similarity values among KV-628^T, KV-641 and KV-656 were above 99.9%, whereas the sequence similarity values between these three strains and *Ornithinococcus hortensis* were below 95.6%. Sequence similarity values for KV-628^T and other *Intrasporangiaceae* family members were as follows: *Terrabacter tumescens*, 95.2%; *Knoellia sinensis*, 95.2%; *Intrasporangium calvum*, 94.8%; *Janibacter limosus*, 94.8%; *Tetrasphaera japonica*, 94.8%; *Arsenicicoccus bolidensis*, 94.8%; *Terracoccus luteus*, 94.4%; and *Ornithinimicrobium humiphilum*, 93.7%.

The three strains were also examined for a set of phenotypic and chemotaxonomic characteristics. Strains KV-628^T, KV-641 and KV-656 were very similar in terms of morphological and chemotaxonomic characteristics. The three strains were irregular rods and the cell diameters varied from 0.4 to 0.9 μ m by 0.9 to 1.9 μ m (Fig. 2). The cells of the three strains were Gram-positive, catalase-positive and showed no motility. The strains were able to grow at 15–37 °C and at pH 4–9, but at pH 9 only trace growth was observed. NaCl in 1/5 nutrient agar medium was tolerated up to 4%, while only trace growth was observed at 5% NaCl.

The physiological characteristics are given in the species description. The three isolated strains were very similar in terms of carbon-source assimilation and enzyme content.

The DNA G + C content of KV-628^T, KV-641 and KV-656 was 72–73 mol% (with the HPLC nucleoside method). The cell-wall peptidoglycan of KV-628^T, KV-641 and KV-656 contained *meso*-diminopimelic acid, alanine and glutamic acid at molar ratios of 1.0:1.6:1.0, 1.0:1.7:1.0 and 1.0:1.7:1.0, respectively. The three isolated strains contained a peptidoglycan of type A1 γ (Schleifer & Kandler, 1972). The predominant menaquinone was MK-8(H₄). The acyl type was acetyl. Mycolic acids were not detected. The

Fig. 1. Phylogenetic tree, derived from 16S rRNA gene sequences, created using the neighbour-joining method and K_{nuc} values. Only values above 50 % significance are indicated. The solid circles indicate that the corresponding nodes are also recovered in the maximum-likelihood tree. The tree was unrooted and *Rarobacter faecitabidus* was used as an outgroup.

predominant cellular fatty acids were iso- $C_{14:0}$, iso- $C_{15:0}$, anteiso- $C_{15:0}$ and iso- $C_{16:0}$ (Table 1).

To confirm that these strains belong to a novel species, DNA–DNA hybridization relatedness was determined. The levels of DNA–DNA relatedness among the three isolated strains and *Ornithinicoccus hortensis* were determined. The values among the three isolated strains were 90–101 %, whereas those between the three strains and *Ornithinicoccus hortensis* were below 25 %. The values were well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for species classification.

At present, the family *Intrasporangiaceae* contains nine genera: *Arsenicococcus* (Collins *et al.*, 2004), *Intrasporangium* (Kalakoutskii *et al.*, 1967), *Janibacter* (Martin *et al.*, 1997), *Knoellia* (Groth *et al.*, 2002), *Terrabacter* (Collins *et al.*, 1989), *Terracoccus* (Prauser *et al.*, 1997), *Tetrasphaera* (Maszenan *et al.*, 2000), *Ornithinicoccus* (Groth *et al.*,

1999) and *Ornithinimicrobium* (Groth *et al.*, 2001). Table 2 shows the differential characteristics of the members of the family *Intrasporangiaceae*. The genera *Janibacter*, *Knoellia* and *Tetrasphaera* also have meso-diaminopimelic acid in their cell walls, but *Janibacter*, *Knoellia* (iso- $C_{15:0}$, iso- $C_{17:0}$, iso- $C_{16:0}$ and anteiso- $C_{17:0}$) and *Tetrasphaera* differ in terms of cellular fatty acids.

On the basis of the distinct phylogenetic position of the three novel isolates within the family *Intrasporangiaceae*, as well as their differential characteristics in terms of cell morphology and cell-wall murein type, a novel genus and species, *Oryzihumus leptocrescens* gen. nov., sp. nov., is proposed.

Description of *Oryzihumus* gen. nov.

Oryzihumus (Ory.zi.hu'mus. L. fem. n. *oryza* rice; L. masc. n. *humus* soil; N.L. masc. n. *Oryzihumus* rice soil).

Fig. 2. Scanning electron micrograph of cells from a 6-day-old culture of strain KV-628^T grown on GPM agar medium at 27 °C. Bar, 2 μm.

Table 1. Cellular fatty acid composition (%) of strains KV-628^T, KV-641 and KV-656

Abbreviations for fatty acids are as follows: $C_{16:0}$, hexadecanoic acid; i- $C_{15:0}$, 13-methyl tetradecanoic acid; ai- $C_{15:0}$, 12-methyl tetradecanoic acid.

Fatty acid	KV-628 ^T	KV-641	KV-656
i- $C_{13:0}$	—	0.5	—
i- $C_{14:0}$	12.9	7.7	4.8
$C_{14:0}$	—	1.0	2.8
i- $C_{15:0}$	23.1	40.1	27.3
ai- $C_{15:0}$	3.1	10.7	14.5
i- $C_{16:0}$	35.8	13.7	9.4
$C_{16:0}$	0.6	0.7	5.2
i- $C_{17:0}$	0.5	0.9	0.6
ai- $C_{17:0}$	1.4	5.0	5.0

Table 2. Differential characteristics of strain KV-628^T and related taxa

L-Orn, L-ornithine; meso-A₂pm, meso-diaminopimelic acid; LL-A₂pm, LL-diaminopimelic acid; A, anteiso methyl-branched; I, iso methyl-branched; S, straight-chain saturated; U, monounsaturated.

Taxon	Cell morphology	Wall diamino acid	DNA G + C content (mol%)	Murein type	Major menaquinone	Fatty acid type
KV-628 ^T	Irregular rods	meso-A ₂ pm	72–73	A1 γ	MK-8(H ₄)	SAI
<i>Arsenicicoccus</i> *	Cocci	LL-A ₂ pm	72	A3 γ	MK-8(H ₄)	SAIU
<i>Intrasporangium</i> †	Hyphae	LL-A ₂ pm	68	A3 γ	MK-8	SAI
<i>Janibacter</i> ‡	Coccoid to rod-shaped	meso-A ₂ pm	70	A1 γ	MK-8(H ₄)	SIU
<i>Knoellia</i> §	Coccoid to rod-shaped	meso-A ₂ pm	68–69	A1 γ	MK-8(H ₄)	SAI
<i>Terrabacter</i> ‡	Cocci to rods	LL-A ₂ pm	70–73	A3 γ	MK-8(H ₄)	SAI
<i>Terracoccus</i>	Cocci	LL-A ₂ pm	73	A3 γ	MK-8(H ₄)	SAI
<i>Tetrasphaera</i> ¶	Cocci	meso-A ₂ pm	68–71	A1 γ	MK-8(H ₄)	SAIU
<i>Ornithinicoccus</i> #	Cocci	L-Orn	72	A4 β	MK-8(H ₄)	SAI
<i>Ornithinimicrobium</i> **	Cocci	L-Orn	70	A4 β	MK-8(H ₄)	SAI

*Data from Collins *et al.* (2004).

†Data from Schumann *et al.* (1997).

‡Data from Martin *et al.* (1997).

§Data from Groth *et al.* (2002).

||Data from Prauser *et al.* (1997).

¶Data from Maszenan *et al.* (2000).

#Data from Groth *et al.* (1999).

**Data from Groth *et al.* (2001).

Gram-positive, catalase-positive, aerobic, non-motile irregular rods. The peptidoglycan is of the A type of direct cross-linkage and contains meso-diaminopimelic acid, alanine and glutamic acid. The acyl type of the glycan chain of peptidoglycan is acetyl. Mycolic acids are absent. The major menaquinone is MK-8(H₄). The DNA G + C content is 72–73 mol%. Phylogenetically, this genus is a member of the family *Intrasporangiaceae*, suborder *Micrococcineae*. The type species is *Oryzihumus leptocrescens*.

Description of *Oryzihumus leptocrescens* sp. nov.

Oryzihumus leptocrescens (lep.to.cre'scens. Gr. adj. *leptos* thin, fine, delicate, slender; L. part. adj. *crescens* growing; N.L. part. adj. *leptocrescens* slender growing).

Cells are irregular rods 0.4–0.9 μ m by 0.9–1.9 μ m. Colonies are pale yellow. Aerobic to microaerophilic. Growth occurs between pH 4 and 9 and at 15 and 37 °C. In 1/5 nutrient agar medium, NaCl is tolerated up to 5 %. D-Glucose, maltose, sucrose and trehalose are assimilated, but L-rhamnose and D-xylose are not. Susceptible to erythromycin (15 μ g ml⁻¹), rifampicin (30 μ g ml⁻¹), novobiocin (30 μ g ml⁻¹), streptomycin (10 μ g ml⁻¹) and chloramphenicol (30 μ g ml⁻¹). Not susceptible to aztreonam (100 μ g ml⁻¹). Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase and β -glucosidase are detected by the API ZYM enzyme assay; alkaline phosphatase, cystine arylamidase, trypsin, chymotrypsin, β -glucuronidase,

N-acetyl- β -glucosaminase, α -mannosidase and α -fucosidase are not detected. A weak reaction for lipase (C14) is detected. Variable reactions for α -galactosidase are detected. The DNA G + C content is 72–73 mol%.

Habitat: paddy soil in Japan. The type strain is KV-628^T (=NRRL B-24347^T=JCM 12835^T=NBRC 100762^T).

Acknowledgements

This study was supported, in part, by a grant from the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

References

Becker, B., Lechevalier, M. P. & Lechevalier, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl Microbiol* **13**, 236–243.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Collins, M. D., Dorsch, M. & Stackebrandt, E. (1989). Transfer of *Pimelobacter tumescens* to *Terrabacter* gen. nov. as *Terrabacter tumescens* comb. nov. and of *Pimelobacter jensenii* to *Nocardioides* as *Nocardioides jensenii* comb. nov. *Int J Syst Bacteriol* **39**, 1–6.

Collins, M. D., Routh, J., Saraswathy, A., Lawson, P. A., Schumann, P., Welinder-Olsson, C. & Falsen, E. (2004). *Arsenicicoccus bolidensis* gen. nov., sp. nov., a novel actinomycete isolated from contaminated lake sediment. *Int J Syst Evol Microbiol* **54**, 605–608.

- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Groth, I., Schumann, P., Martin, K., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinicoccus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.
- Groth, I., Schumann, P., Weiss, N., Schuetze, B., Augsten, K. & Stackebrandt, E. (2001). *Ornithinimicrobium humiphilum* gen. nov., sp. nov., a novel soil actinomycete with L-ornithine in the peptidoglycan. *Int J Syst Evol Microbiol* **51**, 81–87.
- Groth, I., Schumann, P., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (2002). *Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov., two novel actinobacteria isolated from a cave. *Int J Syst Evol Microbiol* **52**, 77–84.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Huck, T. A., Porter, N. & Bushell, M. E. (1991). Positive selection of antibiotic-producing soil isolates. *J Gen Microbiol* **137**, 2321–2329.
- Iwai, Y. & Takahashi, Y. (1992). Selection of microbial sources of bioactive compounds. In *The Search for Bioactive Compounds from Microorganisms*, pp. 281–302. Edited by S. Ōmura. New York: Springer.
- Jiang, C.-L. & Xu, L.-H. (1996). Diversity of aquatic actinomycetes in lakes of the middle plateau, Yunnan, China. *Appl Environ Microbiol* **62**, 249–253.
- Kalakoutskii, L. V., Kirillova, I. P. & Krasil'nikov, N. A. (1967). A new genus of the Actinomycetales, *Intrasporangium* gen. nov. *J Gen Microbiol* **48**, 79–85.
- Kawamoto, I., Oka, T. & Nara, T. (1981). Cell wall composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensis*, and related organisms. *J Bacteriol* **146**, 527–534.
- Kimura, M. & Ohta, T. (1972). On the stochastic model for estimation of mutation distance between homologous proteins. *J Mol Evol* **2**, 87–90.
- Martin, K., Schumann, P., Rainey, F. A., Schuetze, B. & Groth, I. (1997). *Janibacter limosus* gen. nov., sp. nov., a new actinomycete with meso-diaminopimelic acid in the cell wall. *Int J Syst Bacteriol* **47**, 529–534.
- Maszenan, A. M., Seviour, R. J., Patel, B. K. C., Schumann, P., Burghardt, J., Tokiwa, Y. & Stratton, H. M. (2000). Three isolates of novel polyphosphate-accumulating Gram-positive cocci, obtained from activated sludge, belong to a new genus, *Tetrasphaera* gen. nov., and description of two new species, *Tetrasphaera japonica* sp. nov. and *Tetrasphaera australiensis* sp. nov. *Int J Syst Evol Microbiol* **50**, 593–603.
- Minnikin, D. E., Patel, P. V., Alshamony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Nioh, I., Osada, M., Yamamura, T. & Muramatsu, K. (1995). Acidophilic and acid-tolerant actinomycetes in an acid tea field soil. *J Gen Appl Microbiol* **41**, 175–180.
- Nonomura, H. & Hayakawa, M. (1988). New methods for the selective isolation of soil Actinomycetes. In *Biology of Actinomycetes* '88, pp. 288–293. Edited by Y. Okami, T. Beppu & H. Ogawara. Tokyo: Japan Scientific Societies.
- Prauser, H., Schumann, P., Rainey, F. A., Kropppenstedt, R. M. & Stackebrandt, E. (1997). *Terracoccus luteus* gen. nov., sp. nov., an LL-diaminopimelic acid-containing coccoid actinomycete from soil. *Int J Syst Bacteriol* **47**, 1218–1224.
- Pridham, T. G. & Gottlieb, D. (1948). The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. *J Bacteriol* **56**, 107–114.
- Roller, C., Ludwig, W. & Schleifer, K. H. (1992). Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes. *J Gen Microbiol* **138**, 1167–1175.
- Saito, H. & Miura, K. (1983). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Suzuki, K. & Komagata, K. (1983). Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. *Int J Syst Bacteriol* **33**, 188–200.
- Suzuki, S., Takahashi, K., Okuda, T. & Komatsubara, S. (1998). Selective isolation of *Actinobispora* on gellan gum plate. *Can J Microbiol* **44**, 1–5.
- Takahashi, Y., Matsumoto, A., Seino, A., Iwai, Y. & Ōmura, S. (2002). *Streptomyces avermectinus* sp. nov., an avermectin-producing strain. *Int J Syst Evol Microbiol* **52**, 2163–2168.
- Takahashi, Y., Katoh, S., Shikura, N., Tomoda, H. & Ōmura, S. (2003). Superoxide dismutase produced by soil bacteria increases bacterial colony growth from soil samples. *J Gen Appl Microbiol* **49**, 263–266.
- Takeuchi, M. & Hatano, K. (1999). Phylogenetic analysis of *Actinobacteria* in the mangrove rhizosphere. *IFO Res Commun* **19**, 47–62.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tomiyasu, I. (1982). Mycolic acid composition and thermally adaptative changes in *Nocardia asteroides*. *J Bacteriol* **151**, 828–837.
- Uchida, K. & Aida, K. (1977). Acyl type of bacterial cell wall: its simple identification by a colorimetric method. *J Gen Appl Microbiol* **23**, 249–260.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yu, L., Takahashi, Y., Matsumoto, A., Seino, A., Iwai, Y. & Ōmura, S. (2002). Application of PCR for selection of gram-positive bacteria with high DNA G+C content among new isolates. *Actinomycetologica* **16**, 1–5.