Intrasporangium chromatireducens sp. nov., a chromate-reducing actinobacterium isolated from manganese mining soil, and emended description of the genus *Intrasporangium*

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A Gram-positive, aerobic actinobacterium with high chromate [Cr(VI)]-reducing ability, designated strain Q5-1^T, was isolated from manganese mining soil in Hunan Province, central-south China. The organism formed branching hyphae and contained LL-diaminopimelic acid in the cell-wall peptidoglycan. The polar lipid profile was characterized by diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and an unidentified phospholipid. The major fatty acids were iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0}, and the predominant menaquinone was MK-8(H₄). The genomic DNA G + C content was 71.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Q5-1^T was closely related to *Intrasporangium calvum* DSM 43043^T (=NRRL B-3866^T) and *Humihabitans oryzae* KV-657^T (=NRRL B-24470^T) with similarities of 96.6 and 96.4 %, respectively. Comparison of phenotypic, biochemical and chemotaxonomic characters of strain Q5-1^T and phylogenetically related strains revealed that the isolate represents a novel species of the genus *Intrasporangium*, for which the name *Intrasporangium chromatireducens* sp. nov. is proposed. The type strain is Q5-1^T (=KCTC 19811^T=CCTCC AA 2010019^T=CGMCC 1.10750^T=NRRL B-59521^T). An emended description of the genus *Intrasporangium* is also proposed.

The type genus *Intrasporangium*, belonging to the family *Intrasporangiaceae*, was proposed in 1967 on the basis of the type species, *Intrasporangium calvum*, which was isolated from air in a school dining room (Kalakoutskii *et al.*, 1967). At the time of writing, the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997; Stackebrandt & Schumann 2000, 2006; Zhi *et al.*, 2009) contained 19 genera with validly published names, the members of which were isolated from air, soil, sludge, sediment and mining waste. All of the genera in the family contain MK-8(H₄) as the predominant menaquinone (Stackebrandt & Schumann, 2006), except for *I. calvum*, which has MK-8 as its major quinone (Collins *et al.*, 1984; Kalakoutskii, 1989; Kageyama *et al.*, 2007). The genus *Humihabitans* (Kageyama *et al.*, 2007), like *Intrasporangium*, is also monospecific and was proposed mainly based on the different menaquinone profile from its closest phylogenetic neighbour, *Intrasporangium*.

Chromium (Cr) is widely used in many industries and large quantities of Cr(VI)-containing wastes pose a great threat to environmental safety and human health. Micro-organisms with the ability to reduce highly toxic chromate [Cr(VI)] into less hazardous and insoluble trivalent chromium [Cr(III)] have great potential for Cr(VI) bioremediation (Eccles, 1995). During the course of an investigation of culturable bacteria from manganese mine soil in Huayuan County (28° 30' N 109° 24' E), China's second-largest manganese minerals area, Hunan Province, central-south China, several Cr(VI)-resistant bacteria were isolated that were identified as *Acinetobacter* (two strains), *Bacillus* (two strains), *Rhodococcus* (two strains), *Klebsiella* (two strains) and *Intrasporangium* (one strain, named Q5-1^T; Yang *et al.*, 2009) based on 16S rRNA gene sequence analysis. Strain Q5-1^T, which showed both high Cr(VI) resistance and high reduction ability, was chosen for this study due to its potential novel status.

Phenotypic, physiological/biochemical and chemotaxonomic analyses were performed following the proposed minimal standards for describing new genera and species of the suborder *Micrococccineae* (Schumann *et al.*, 2009), and using *I. calvum* NRRL B-3866^T and *Humihabitans oryzae*

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Q5-1^T is FJ487951.

Three supplementary figures are available with the online version of this paper.

NRRL B-24470^T as reference strains (Kalakoutskii *et al.*, 1967; Schumann *et al.*, 1997; Kageyama *et al.*, 2007). The actinobacteria were cultured on Luria–Bertani medium (LB; 1 % tryptone, 0.5 % yeast extract, 1 % NaCl and 1.5 % agar; pH 8), trypticase soy agar (TSA, pH 8; Bacto), nutrient agar (NA; 0.5 % tryptone, 0.3 % yeast extract and 1.5 % agar; pH 8) and GPM agar plates (1 % D-glucose, 0.5 % peptone, 0.5 % meat extract, 0.3 % NaCl and 1.5 % agar; pH 8) to observe colony morphologies. Growth was tested at 4, 10, 15, 28, 37, 45 and 50 °C using 1/5-strength NA plates (Kageyama *et al.*, 2007) incubated for 7 days. Growth conditions were determined at pH 3–11 and NaCl concentrations of 0, 1, 3, 5 and 7 % (w/v) by incubating strains in shake-flasks at 170 r.p.m. at 37 °C with 1/5-strength nutrient broth for 4 days. Growth under anaerobic conditions was determined by incubating strains in an anaerobic chamber with an O₂-absorbing and CO₂-generating agent (Anaero-Pack; Mitsubishi Gas Chemical). Gram staining was performed using the standard Gram reaction combined with the KOH lysis test method (Ryu, 1938). Morphological observations with and without potassium chromate (K₂CrO₄) in LB medium were carried out using a scanning electron microscope (JSM-6390; JEOL). Cr(VI) reduction tests were performed as described by Yang *et al.* (2009). Catalase activity was detected using the standard method employing 3 % (v/v) H₂O₂. The ability of the strain to grow on a range of sole carbon sources was determined using carbon utilization media (Pridham & Gottlieb, 1948), and API 20NE and ID 32GN test kits (bioMérieux). Acid production tests were performed under aerobic conditions (Smibert & Krieg, 1994; Gordon & Mihm, 1957). Other physiological properties, including enzyme activities, were examined using API 20NE, ID 32GN and API ZYM systems (bioMérieux) according to the manufacturer's instructions, unless otherwise indicated.

The nearly full-length 16S rRNA gene sequence of strain Q5-1^T was amplified by PCR with universal primers Uni-27F and Uni-1492R (Wilson *et al.*, 1990), and the PCR product was ligated into a pGEM-T vector (Promega). DNA sequencing was performed by the Beijing Genomics Institute (Beijing, China). Sequence similarities were calculated using NCBI alignment and the EzTaxon program (Chun *et al.*, 2007). Multiple alignments of sequences were carried out using CLUSTAL_X, followed by manual correction (Thompson *et al.*, 1997; Tindall *et al.*, 2010). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) trees were constructed using MEGA4.0 (Tamura *et al.*, 2007), and a maximum-likelihood tree was generated using the PHYML package (Guindon & Gascuel, 2003) with 1000 replicates in a non-parametric bootstrap analysis, the general time-reversible model of nucleotide substitution and four substitution-rate categories.

Whole-cell hydrolysates were analysed for diaminopimelic acid (A₂pm) isomers by means of TLC (Hasegawa *et al.*, 1983). Purified cell walls were obtained by the method of Kawamoto *et al.* (1981) and peptidoglycan types were

analysed according to Schleifer & Kandler (1972). Menaquinones were extracted and purified by the method of Collins *et al.* (1977) and analysed via HPLC as described by Tamaoka *et al.* (1983). For whole-cell fatty acid analysis, strains were grown in TSB medium at 28 °C until they reached exponential phase and analysed by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10). Polar lipids were extracted as described by Minnikin *et al.* (1979) and identified by two-dimensional TLC sprayed with specific reagents (Collins & Jones, 1980). The G + C content of the genomic DNA was determined by HPLC according to the method of Mesbah *et al.* (1989). Levels of DNA–DNA relatedness were determined using the thermal denaturation and renaturation method of Huß *et al.* (1983).

Cell morphologies of strain Q5-1^T grown in the presence and absence of chromate are shown in Supplementary Fig. S1 (available in IJSEM Online). Detailed results of the polyphasic taxonomic characterization of strain Q5-1^T are given in the species description. Characteristics of strain Q5-1^T were typical of those of *Intrasporangium* and *Humihabitans* species, but there were some clear differences. The main differential phenotypic characters of strain Q5-1^T, *I. calvum* NRRL B-3866^T and *H. oryzae* NRRL B-24470^T are shown in Table 1. In addition, strain Q5-1^T was able to reduce 0.5 mM Cr(VI) aerobically in LB medium within 16 h, but *I. calvum* NRRL B-3866^T and *H. oryzae* NRRL B-24470^T did not show Cr(VI) reduction. After 48 h incubation with K₂CrO₄, cells of strain Q5-1^T became longer (Supplementary Fig. S1).

The 1449 bp 16S rRNA gene sequence of strain Q5-1^T showed similarities of <96.7 % to the type strains of species in the family *Intrasporangiaceae*. The two highest similarities were to *I. calvum* DSM 43043^T (=NRRL B-3866^T) (96.6 %) and *H. oryzae* KV-657^T (=NRRL B-24470^T) (96.4 %). Phylogenetic analyses using the neighbour-joining, maximum-parsimony and maximum-likelihood methods all showed that strain Q5-1^T fell into the same cluster with *I. calvum* DSM 43043^T and *H. oryzae* KV-657^T with high bootstrap values (Fig. 1).

The cell-wall peptidoglycan of strain Q5-1^T was of type A3_γ and contained LL-A₂pm, glycine, alanine and glutamic acid. MK-8(H₄) was the predominant menaquinone. Major fatty acids (>10 %) were iso-C_{14:0} (22.5 %), iso-C_{15:0} (23.9 %), anteiso-C_{15:0} (10.6 %) and iso-C_{16:0} (19.7 %). The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannoside and an unknown phospholipid (Supplementary Fig. S2, available in IJSEM Online). The DNA G + C content was 71.5 mol% (Table 1). DNA–DNA hybridization values of strain Q5-1^T with *I. calvum* NRRL B-3866^T and *H. oryzae* NRRL B-24470^T were 48.6 ± 1.14 % and 50.1 ± 0.95 %, respectively. These values were well below the 70 % cut-off point for species classification recommended by Wayne *et al.* (1987).

Table 1. Comparison of the phenotypic characters of strain Q5-1^T with the two phylogenetically most closely related reference strains

Strains: 1, Q5-1^T; 2, *I. calvum* NRRL B-3866^T; 3, *H. oryzae* NRRL B-24470^T. Data were taken from this study unless otherwise mentioned. +, Positive; –, negative; w, weak reaction.

Characteristic	1	2	3
Colour of colonies*	Pale yellow to cream-whitish	Whitish to cream-whitish	Pale yellow to yellow
pH range	5–10	6–8	5–11
Temperature range (°C)	10–45	20–40	8–40
Acid production from:			
Maltose	+	–	+
Sucrose	+	w	+
Lactose	–	–	+
Galactose	+	–	w
D-Xylose	+	–	+
D-Glucose	+	–	–
D-Mannitol	w	–	–
D-Mannose	+	w	w
Growth on compounds as sole carbon sources:			
DL-Lactate	+	–	–
Inositol	–	–	+
Adipate	+	–	–
Acetate	+	–	–
Valerate	+	–	–
D-Ribose	+	–	–
D-Sorbitol	–	–	+
L-Alanine	+	–	–
Glycogen	–	–	+
L-Rhamnose	–	+	+
D-Glucose	+	–	+
Enzyme activities (API ZYM)			
Alkaline phosphatase	–	w	–
α-Galactosidase	–	–	+
β-Galactosidase	–	–	+
β-Glucuronidase	+	–	–
Chemotaxonomic characters			
Menaquinone	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)
Polar lipids†	DPG, PG, PE, PI, PIM, PL	DPG, PG, PE, PI, PIM, PL	DPG, PE, PI, PIM, PL
DNA G + C content (mol%)	71.5	68.2‡	70§
Whole-cell fatty acids (%)			
iso-C _{14:0}	22.5	12.6 (5.0¶)	9.1 (7.3§)
iso-C _{15:0}	23.9	20.9 (37.8¶)	41.3 (39.6§)
anteiso-C _{15:0}	10.6	6.27 (12.6¶)	5.4 (5.8§)
iso-C _{16:0}	19.7	22.5 (12.3¶)	8.1 (13.3§)
iso-C _{16:1} H	9.5	17.2	6.5
C _{15:0}	1.4	0.9 (2.7¶)	3.9 (4.8§)
C _{16:0}	1.8	– (1.2¶)	0.9
10-methyl C _{16:0}	2.4	–	1.8
anteiso-C _{17:1} ω9c	1.4	4.2	0.5
anteiso-C _{17:0}	1.0	1.5	0.7
C _{17:1} ω8c	0.6	1.7	4.6 (6.4§)

*Determined on LB agar and NA plates.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid(s).

‡Data from Kalakoutsii (1989).

§Data from Kageyama *et al.* (2007).

||Fatty acids that represent <0.5 % were omitted.

¶Data from Schumann *et al.* (1997).

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences (1390 bp) after multiple sequence alignment and manual adjustment showing the phylogenetic positions of strain Q5-1^T and strains of selected type species of the family *Intrasporangiaceae*. Open stars indicate that the corresponding nodes were also found in trees generated with both the maximum-parsimony and maximum-likelihood algorithms; filled stars indicate that the corresponding node was also found in the maximum-likelihood tree. Bootstrap percentages (based on 1000 replications) >50 % are shown at branching points. Bar, 0.005 substitutions per nucleotide position. *I. calvum* DSM 43043^T and *H. oryzae* KV-657^T correspond to NRRL strain collection numbers NRRL B-3866^T and B-24470^T, respectively.

Results from the polyphasic taxonomic analysis revealed that strain Q5-1^T, *I. calvum* NRRL B-3866^T and *H. oryzae* NRRL B-24470^T all formed branching and fragmenting mycelia. They were positive for catalase activity and nitrate reduction, and negative for oxidase activity and H₂S production. In the API ZYM enzyme assay, they all produced esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α -glucosidase and naphthol-AS-BI-phosphohydrolase. The cellular fatty acid contents for the three strains consisted predominantly of branched fatty acids, including (>5 % of total fatty acids) iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{16:1} H, but there were differences in the proportions (Table 1). They all contained LL-A₂pm in the cell-wall peptidoglycan. MK-8(H₄) was the predominant quinone for *I. calvum* NRRL B-3866^T and for *I. calvum* DSM 43043^T (W. Li, personal communication), which differed from the original chemotaxonomic characterization of the genus *Intrasporangium* (MK-8; Collins *et al.*, 1984; Kalakoutskii, 1989; Kageyama *et al.*, 2007). Based on the fact that the genus *Humihabitans* (*H. oryzae* NRRL B-24470^T) contained the same major menaquinone as *Intrasporangium* (*I. calvum* NRRL B-3866^T) (Supplementary Fig. S3, available in IJSEM Online), similar fatty acid and polar lipid profiles (Table 1), and high 16S rRNA gene sequence similarities [98.7 % using EzTaxon server, 97.6 % using NCBI alignment (Kageyama *et al.*, 2007)], it may be appropriate to reclassify *Humihabitans oryzae* in the genus *Intrasporangium*.

Based on analysis of its distinct phylogenetic position, DNA–DNA relatedness level, chemotaxonomic traits and physiological properties, it is concluded that strain Q5-1^T

represents a novel species of the genus *Intrasporangium*, for which the name *Intrasporangium chromatireducens* sp. nov. is proposed.

Emended description of the genus *Intrasporangium* Kalakoutskii *et al.* 1967

This emended description is based on that given by Schumann *et al.* (1997) and Stackebrandt & Schumann (2006), with the following changes. Positive for catalase activity, but negative for oxidase activity and H₂S production. Esterase (C4), esterase lipase (C8), α -glucosidase, valine arylamidase, cystine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and acid phosphatase are detected. The major menaquinone is MK-8(H₄). Iso- and anteiso-branched fatty acids predominate, including iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0}. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The DNA G + C content is in the range 68.2–71.5 mol%. The type species is *Intrasporangium calvum*.

Description of *Intrasporangium* *chromatireducens* sp. nov.

Intrasporangium chromatireducens (chro.ma.ti.re.du'cens. N.L. n. *chromas* -atis chromate; L. part. adj. *reducens* leading back, bringing back, and, in chemistry, converting to a different oxidation state; N.L. part. adj. *chromati-reducens* reducing chromate).

Gram-positive, non-motile micro-organism that forms branching hyphae with diameters of 0.3–0.5 µm. Colonies are pale yellow to cream-whitish, circular, smooth and convex with entire margins on LB, TSA, NA and GPM media. Grows aerobically at 10–45 °C (optimum at 37 °C), but not at 4 °C or 50 °C. Grows at pH 5–10, with optimum growth at pH 8. Grows in 0–4 % (w/v) NaCl (optimum 1 %, w/v). Cannot grow under anaerobic conditions. Catalase-positive. H₂S is not produced. Voges–Proskauer and methyl red tests are negative. Positive for nitrate reduction, aesculin hydrolysis, arginine dihydrolase and urease, but negative for gelatin hydrolysis, oxidase, indole production and β-galactosidase (API 20NE test strips). Acid is produced from D-glucose, D-mannose, D-mannitol, D-galactose, D-xylose, sucrose and maltose, but not from lactose, inositol or cellobiose. The following substrates are assimilated as sole carbon sources: D-glucose, D-mannose, D-mannitol, D-ribose, maltose, DL-malate, valerate, N-acetylglucosamine, aesculin, adipate, 2-ketogluconate, acetate, DL-lactate, L-alanine, L-histidine, gluconate, pentanoate and 3-hydroxybutyrate. The following substrates are not utilized: gelatin, L-arabinose, caprate, citrate, phenylacetate, lactose, sucrose, suberate, malonate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconic acid, N-acetylglucosamine, starch, 5-ketogluconate, L-proline, L-fucose, melibiose, L-serine, propionate, D-sorbitol, melezitose, raffinose, L-rhamnose, D-salicin and inositol (API 20NE and ID 32GN test strips). Esterase (C4), esterase lipase (C8), α-glucosidase, valine arylamidase, cystine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase and β-glucuronidase are detected; negative for trypsin, lipase (C14), β-fucosidase, alkaline phosphatase, N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-mannosidase and β-glucosidase. A weak reaction for chymotrypsin is detected (API ZYM). The major menaquinone is MK-8(H₄). The cell-wall peptidoglycan is of type A3γ and contains LL-A₂pm, glycine, alanine and glutamic acid. Major fatty acids (>10 %) are iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0}. The polar lipids contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and an unidentified phospholipid. The type strain is highly chromate-resistant and is able to grow at 17 mM K₂CrO₄ and aerobically reduce 0.5 mM K₂CrO₄ in LB medium within 16 h.

The type strain is Q5-1^T (=KCTC 19811^T=CCTCC AA 2010019^T=CGMCC 1.10750^T=NRRL B-59521^T), isolated from subsurface soil of a manganese mine in Hunan Province, central-south China. The DNA G+C content of the type strain is 71.5 mol%.

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References

- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.
- 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., Faulkner, M. & Keddle, R. M. (1984). Menaquinone composition of some sporeforming actinomycetes. *Syst Appl Microbiol* **5**, 20–29.
- Eccles, H. (1995). Removal of heavy metals from effluent streams: why select a biological process? *Int Biodeterior Biodegradation* **35**, 5–16.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* **73**, 15–27.
- Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kageyama, A., Takahashi, Y. & Ōmura, S. (2007). *Humihabitans oryzae* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **57**, 2163–2166.
- Kalakoutskii, L. V. (1989). Genus *Intrasporangium* Kalakoutskii, Kirillova and Krasil'nikov 1967, 7^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2395–2397. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams and Wilkins.
- Kalakoutskii, L. V., Kirillova, I. P. & Krassilnikov, 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.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.
- 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.
- Ryu, E. (1938). On the Gram-differentiation of bacteria by the simplest method. *J Jpn Soc Vet Sci* **17**, 58–63.
- 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-diamino-pimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Schumann, P., Kämpfer, P., Busse, H. J., Evtushenko, L. I. & Subcommittee on the Taxonomy of the Suborder Micrococcineae of the International Committee on Systematics of Prokaryotes (2009).** Proposed minimal standards for describing new genera and species of the suborder *Micrococcineae*. *Int J Syst Evol Microbiol* **59**, 1823–1849.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Schumann, P. (2000).** Description of *Bogoriellaceae* fam. nov., *Dermacoccaceae* fam. nov., *Rarobacteraceae* fam. nov. and *Sanguibacteraceae* fam. nov. and emendation of some families of the suborder *Micrococcineae*. *Int J Syst Evol Microbiol* **50**, 1279–1285.
- Stackebrandt, E. & Schumann, P. (2006).** Introduction to the taxonomy of actinobacteria. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, pp. 297–321. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997).** Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983).** Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010).** Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & 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.
- Wilson, K. H., Blitchington, R. B. & Greene, R. C. (1990).** Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol* **28**, 1942–1946.
- Yang, J., He, M. & Wang, G. (2009).** Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of *Intrasporangium* sp. Q5-1. *World J Microbiol Biotechnol* **25**, 1579–1587.
- Zhi, X.-Y., Li, W.-J. & Stackebrandt, E. (2009).** An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589–608.