Actinospica robiniae gen. nov., sp. nov. and *Actinospica acidiphila* sp. nov.: proposal for *Actinospicaceae* fam. nov. and *Catenulisporinae* subord. nov. in the order *Actinomycetales*

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Two novel Gram-positive, acidophilic bacterial strains were isolated from forest soil. According to their 16S rRNA gene sequences, these strains are related closely to each other and form a distinct cluster within the order *Actinomycetales*. They show the typical features of filamentous actinomycetes, with branched vegetative hyphae and production of aerial hyphae. The distinct phylogenetic positions and the combination of chemotaxonomic characteristics of these strains justify the proposal of *Actinospica* gen. nov. Both strains display 3-hydroxydiaminopimelic acid plus traces of meso-diaminopimelic acid, the phospholipids diphosphatidylglycerol, phosphatidylethanolamine, methylphosphatidylethanolamine and phosphatidylinositol, the predominant cellular fatty acids i-C_{15:0}, i-C_{16:0} and ai-C_{15:0} and the whole-cell sugars mannose and rhamnose. They differ in the fatty acid profiles, in the quantitative ratios of the major menaquinones MK-9(H₄), MK-9(H₆) and MK-9(H₈) and in the occurrence of additional whole-cell sugars (arabinose and xylose in strain GE134766^T and galactose in strain GE134769^T). Differences in the phenotypic characteristics and in the 16S rRNA gene sequences suggest the description of two species, *Actinospica robiniae* gen. nov., sp. nov. (the type species) and *Actinospica acidiphila* sp. nov., with the type strains GE134769^T (=DSM 44927^T=NRRL B-24432^T) and GE134766^T (=DSM 44926^T=NRRL B-24431^T), respectively. The DNA G+C contents of strains GE134769^T and GE134766^T are 70.8 and 69.2 mol%, respectively. Due to the large phylogenetic distance from known actinomycete genera, it is proposed to accommodate *Actinospica* gen. nov. in *Actinospicaceae* fam. nov. In addition, *Catenulisporinae* subord. nov. is proposed to harbour *Actinospicaceae* fam. nov. and the newly proposed family *Catenulisporaceae*, described in the accompanying paper.

INTRODUCTION

There is now substantial evidence indicating that currently known bacteria represent a small fraction of the existing diversity (Hugenholtz, 2002). Earlier data based on phylogenetic evidence from cloned DNA have contributed

to a renewed interest in strain isolation, leading to the recent cultivation of strains belonging to several novel lineages within the domain *Bacteria* (e.g. Janssen *et al.*, 2002; Hahn *et al.*, 2004; Stevenson *et al.*, 2004). It is worth emphasizing that novel taxa are often recovered from common soil sources by small but effective modifications of known isolation methods. Strains belonging to presumably novel lineages have also been isolated within the order *Actinomycetales* (Sait *et al.*, 2002; Joseph *et al.*, 2003), an order that has been subjected to intensive screening because of its biotechnological potential.

In the accompanying paper, we describe *Catenulispora acidiphila* and propose the novel family *Catenulisporaceae*

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Abbreviation: FESEM, field emission scanning electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GE134766^T and GE134769^T are AJ865861 and AJ865863, respectively.

Supplementary figures showing FESEM images and a phylogenetic tree of strains GE134766^T and GE134769^T are available in IJSEM Online.

(Busti *et al.*, 2006). Here, we report the characterization and classification of the strains GE134769^T and GE134766^T, proposing their affiliation to *Actinospica* gen. nov. as two distinct species, *Actinospica robiniae* gen. nov., sp. nov. and *Actinospica acidiphila* sp. nov., respectively. We also propose the description of *Actinospicaceae* fam. nov., in which to accommodate the novel genus *Actinospica*, and of *Catenulisporineae* subord. nov., in which to accommodate the novel families *Actinospicaceae* and *Catenulisporaceae*.

METHODS

Isolation and cultivation of strains GE134766^T and GE134769^T. Strains GE134766^T and GE134769^T were isolated from a soil sample collected in a wooded area in Gerezano, Italy, as described in the accompanying paper (Busti *et al.*, 2006). Strains GE134766^T and GE134769^T were maintained on ISP2 agar (Shirling & Gottlieb, 1966) acidified to pH 5.0–5.5 with HCl. Inocula for cultural, physiological and chemotaxonomic analyses were obtained by growing strains GE134766^T and GE134769^T for 4–8 days (until the end of the vegetative growth phase) at 28 °C on a rotary shaker at 200 r.p.m., using ATSB medium (Busti *et al.*, 2006).

Morphological and physiological characterization. For morphological, cultural and physiological characterization, we used the media (acidified to pH 4.8–5.5) and procedures described previously (Busti *et al.*, 2006). For carbon-source utilization, ISP4 (without starch and acidified to pH 5.0–5.5) and ISP9 were used as basal media, both supplemented with the CMM vitamin solution (Busti *et al.*, 2006). Field emission scanning electron microscopy (FESEM) was performed as described previously (Busti *et al.*, 2006).

Chemotaxonomic characterization. Menaquinones and polar lipids were analysed as described by Groth *et al.* (1997). Analysis of the amino acid composition of the peptidoglycan followed the method described by Schleifer & Kandler (1972), as modified by Willems *et al.* (1997). Whole-cell sugars were determined according to Stanek & Roberts (1974). Cellular fatty acid methyl esters were obtained by the method of Miller (1982). Identification and quantification of the fatty acid methyl esters were performed by using standard MIS Library Generation software (Microbial ID).

DNA base composition. The DNA G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

DNA sequencing and phylogenetic analysis. Genomic DNA was purified with a GenElute Bacterial Genomic DNA kit (Sigma). Amplification of the nearly complete 16S rRNA gene, DNA sequencing, similarity searches and phylogenetic analyses were performed as described previously (Monciardini *et al.*, 2003).

RESULTS AND DISCUSSION

Strain morphology

Strains GE134766^T and GE134769^T appeared on isolation plates only after prolonged incubations at 28 °C. As observed by light microscopy on HSA5 plates after a 3 week incubation at 28 °C, strain GE134766^T forms branched vegetative and aerial mycelia. Aerial mycelium appears as single filaments or as tufts of relatively long, straight to flexuous hyphae. FESEM images show aerial hyphae proceeding towards different maturation stages, as

revealed by the different level of hyphal septation (Fig. 1a), to produce chains of arthrospores. Spores are cylindrical (Fig. 1b), except those borne on the apical part of the sporogenous hyphae, which have a rounded tip (arrows) and are 0.6–0.8 µm long with a mean diameter of 0.5 µm. Spore chains contain up to 30 spores (or occasionally more). Spore surface is slightly rugose; spores do not show motility.

The aerial mycelium of strain GE134769^T appears as tufts of short, straight to slightly flexuous hyphae starting to septate into chains of squat to cylindrical (0.6–0.7 to 1.0–1.2 µm long by 0.6–0.7 µm wide) arthrospores (Fig. 2a). Up to 30 spores were counted in a single chain. Spores showed a slightly rugose surface (Fig. 2b). No motile elements were observed. Further images of the strains are available as Supplementary Figs S1 and S2 in IJSEM Online.

Cultural and physiological characteristics

Table 1 reports the appearance of strains GE134766^T and GE134769^T on various agar media. Strain GE134766^T usually grows better than GE134769^T. On ISP5 and ISP7 plates, GE134766^T also shows a higher degree of differentiation than GE134769^T, which does not produce any aerial mass or pigmentation under these conditions.

Both strains grow in the temperature range 17–33 °C, are incapable of growing at 14 or 37 °C, are positive for H₂S

Fig. 1. Scanning electron microscopy of strain GE134766^T grown on HSA5 agar for 3 weeks at 28 °C. (a) A young, unseptated hypha runs together with a partially septated one and with a matured one, divided into a spore chain; (b) cylindrical spores with rugose surface. See text for description. Bars, 2 µm.

Fig. 2. Scanning electron microscopy of strain GE134769^T grown on HSA5 agar for 3 weeks at 28 °C. (a) Tufts of sporogenous aerial hyphae undergoing division into cylindrical arthrospores; (b) cylindrical spores with slightly rugose surface. Flat faces of the spores deriving from the sporogenous hypha are evident. A terminal spore is distinguishable by the rounded tip. Bars, 5 µm (a); 1 µm (b).

production and negative for gelatin liquefaction, tyrosine reaction and nitrate reduction. In addition, they are sensitive to 100 µg lysozyme ml⁻¹. Whilst both strains are obligate acidophiles, they exhibit different pH ranges (4.2–6.0 and 4.8–6.2 for GE134766^T and GE134769^T, respectively) and optima (5.0 and 5.5, respectively) for growth. In addition, 1% NaCl disturbs the growth of GE134769^T significantly, whereas it is well tolerated by GE134766^T. We could observe starch hydrolysis only with GE134766^T. Traces of casein hydrolysis could be observed for strains GE134769^T and GE134766^T only after 5 and 6 weeks at 28 °C, respectively, even if good growth was observed after 3 weeks. No significantly enhanced growth was observed when the basal media were supplemented with any carbon source. Thus, we cannot say whether GE134766^T or GE134769^T differ in carbon-source utilization.

Chemotaxonomic characteristics

Strains GE134766^T and GE134769^T contain 3-hydroxydiaminopimelic acid as diagnostic diamino acid of the peptidoglycan in addition to glycine, glutamic acid and alanine. Only small traces of *meso*-diaminopimelic acid could be detected. The occurrence of 3-hydroxydiaminopimelic acid, alone or in combination with *meso*-diaminopimelic acid, has already been reported for members of the family *Micromonosporaceae* (Koch *et al.*, 1996; Lee & Hah, 2002) within the order *Actinomycetales*.

The major menaquinones of strains GE134766^T and GE134769^T are MK-9(H₄), MK-9(H₆) and MK-9(H₈), with different ratios in the two strains (see Table 2). Among members of the suborder *Frankineae*, the closest phylogenetic cluster (see below), the major menaquinone patterns differentiate the isolates from *Nakamurella multipartita* and *Quadrisphaera granulorum*, showing MK-8(H₄)

and MK-8(H₂), respectively, whilst other members of that suborder also contain partially hydrogenated menaquinones with nine isoprenoid units (Tamura *et al.*, 1998; Tao *et al.*, 2004; Maszenan *et al.*, 2005). The isolates show cellular fatty acid profiles with the predominant components i-C_{15:0}, i-C_{16:0} and ai-C_{15:0}. Detailed profiles are reported in Table 3. The cellular fatty acid profiles of the two isolates differ in amounts and in the content of the C_{17:1} isomers and do not match any entry of the TSBA40 identification database.

Both strains display the phospholipids diphosphatidylglycerol, phosphatidylethanolamine, methylphosphatidylethanolamine and phosphatidylinositol and contain the whole-cell sugars mannose and rhamnose. Whereas strain GE134769^T shows the additional whole-cell-wall sugar galactose, strain GE134766^T additionally contains arabinose and xylose.

Phylogenetic analysis

The almost-complete 16S rRNA gene sequences for strains GE134769^T and GE134766^T [1421 and 1446 nt, corresponding to 92.0 and 93.6 %, respectively, of the *Escherichia coli* sequence (Brosius *et al.*, 1978)] were determined. The two sequences share 97.5 % identity with each other. The highest pairwise identity levels with cultured bacteria (ranging from 97.0 to 99.3 %) were found with the recently released sequences of several undescribed strains, identified as 'acidophilic actinobacteria' (e.g. GenBank accession numbers AB180758, AB180762 and AB180777). Apart from these, the closest relative (93.1 % sequence identity) was *Catenulispora acidiphila* ID139908^T (Busti *et al.*, 2006). Among species with validly published names, the highest pairwise identity (92.3 %) was found between the sequences of GE134766^T and of *Cryptosporangium aurantiacum* IMSNU 22120 (GenBank accession no. AJ293746), but

Table 1. Culture characteristics of strains GE134766^T and GE134769^T after 3 weeks growth at 28 °C

All media were acidified to pH 4.8–5.5.

Medium	GE134766 ^T				GE134769 ^T			
	Growth	Colour of reverse*	Aerial mass	Soluble pigments	Growth	Colour of reverse*	Aerial mass	Soluble pigments
ISP2	Good	Beige (9G3)	Scant, thin, whitish	Absent	Good	Light yellowish/brown (1114)	Scant, thin, whitish	Absent
ISP3	Good	Cream to greenish (1112 to 12I5)	Scant to abundant along the streak margins, whitish	Absent	Good	Beige to yellowish/brown (11E3 to 11I4)	Discrete, thin, whitish	Absent
ISP4	Good	Whitish (11A1)	Absent	Absent	Discrete	Whitish (ND)	Absent	Absent
ISP5	Discrete	Light greenish (17E3)	Scant, whitish	Scant, greenish	Scant	Whitish (ND)	Absent	Absent
ISP6	Scant	Whitish (ND)	Absent	Absent	Absent	–	–	–
ISP7	Good	Greenish (12I4)	Discrete, white	Moderate, brownish	Discrete	Thin, whitish (ND)	Absent	Absent
Suter + tyrosine	Moderate	Whitish to greenish (ND)	Absent	Absent	Discrete	Thin, whitish (ND)	Absent	Absent

*Colour codes in parentheses are according to Maerz & Paul (1950). ND, Not determined.

representatives of several actinomycete families have sequence identities to GE134766^T and GE134769^T of between 90 and 92 %. Although strains GE134766^T and GE134769^T clearly belong to the order *Actinomycetales*, they – together with the above-mentioned acidophilic strains – do not seem to belong to any of the described families within the order.

The 16S rRNA gene sequences of strains GE134766^T and GE134769^T, together with those of some of the closest relatives found in GenBank, were aligned with those of representatives of the major actinomycete lineages. For the suborder *Frankineae*, we included several type species in order to have a better representation of the diversity within this suborder containing the closest described relatives. The resulting phylogenetic tree is shown in Fig. 3. Strains GE134766^T and GE134769^T represent, together with strains Aac-2, Aac-35, Aac-50 and Aac-85, a novel group within the *Actinomycetales* line of descent. They are associated consistently with another coherent clade, represented by the family *Catenulisporaceae* (Busti *et al.*, 2006). Bootstrap analysis suggests a monophyletic origin for the two clades. In order to better understand the degree of diversity within the novel group, we aligned the 16S rRNA gene sequences of strains GE134766^T and GE134769^T with those of similar strains isolated in our laboratory and of strains reported as GenBank entries only (see Supplementary Fig. S3, available in IJSEM Online). The lineage presents two major clades, represented respectively by strains GE134766^T and GE134769^T, but phylogenetic diversity of each of the two groups appears quite large.

The 16S rRNA gene sequences of all the 28 sequences included in Supplementary Fig. S3 (available in IJSEM Online) were scanned for the signature nucleotide patterns of the order *Actinomycetales* and its suborders (Stackebrandt *et al.*, 1997). All signature nucleotides of the order *Actinomycetales* were found, with two exceptions. The first is at position 449, where a C is found instead of an A, as also observed for other members of the *Actinomycetales*. The second exception is at positions 450:483, where C–G is found instead of G–C, as observed for the order *Bifidobacteriales* (Stackebrandt *et al.*, 1997). However, sequences of strains GE134766^T and GE134769^T do not present any of the other signatures typical of the order *Bifidobacteriales* and clearly cluster within the *Actinomycetales* in phylogenetic analyses. In addition, they do not contain the signature pattern of any of the described suborders. Phylogenetic data therefore suggest that these strains belong to a novel family within the *Actinomycetales*. Relatively high bootstrap values and the presence of a common pattern of signature nucleotides (see below) support the placement of this family in the same suborder as the newly proposed family *Catenulisporaceae* (Busti *et al.*, 2006).

The combination of morphological, chemotaxonomic and phylogenetic data reported here for strains GE134766^T and GE134769^T support the proposal for a novel genus to include them both. Due to the morphology of the aerial structures, the name *Actinospica* gen. nov. is proposed.

Table 2. Chemotaxonomic characteristics of strains GE134766^T and GE134769^T

Abbreviations: Dpm, diaminopimelic acid; Ara, arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; Xyl, xylose; PI, phosphatidylinositol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine.

Chemotaxonomic marker	GE134766 ^T	GE134769 ^T
Peptidoglycan diamino acids	hydroxy-Dpm, meso-Dpm (traces)	hydroxy-Dpm, meso-Dpm (traces)
Whole-cell sugar pattern	Man, Rha, Ara, Xyl	Man, Rha, Gal
Major menaquinones (%)		
MK-9(H ₄)	23	2
MK-9(H ₆)	40	25
MK-9(H ₈)	20	59
Polar lipids	PI, DPG, PE, methyl-PE	PI, DPG, PE, methyl-PE
G + C content of DNA (mol%)	69.2	70.8

Although the level of DNA relatedness between the two strains was not analysed, we consider the differences observed between the two strains in terms of morphology, physiology (pH growth range and NaCl tolerance), chemotaxonomy (menaquinones and fatty acid patterns), G + C content of their DNAs and level of 16S rRNA sequence identity to be sufficient to support the description of two different species.

Stackebrandt *et al.* (1997) proposed that affiliation to higher hierarchical taxa in the class *Actinobacteria* should be based on phylogenetic criteria. Accordingly, strains GE134766^T and GE134769^T are related phylogenetically to, but are clearly distinct from, representatives of the genus *Catenulispora* (Busti *et al.*, 2006). We thus propose *Actinospicaceae* fam. nov. to harbour *Actinospica* gen. nov. and, on the basis of the branch depth observed in phylogenetic trees, we also propose the description of *Catenulisporineae* subord. nov. to harbour the families *Actinospicaceae* and *Catenulisporaceae*.

Table 3. Cellular fatty acid composition (%) of strains GE134766^T and GE134769^T

Fatty acid	GE134766 ^T	GE134769 ^T
Branched		
i-C _{14:0}	3.88	5.26
i-C _{15:0}	30.17	14.88
i-C _{16:0}	27.40	37.65
i-C _{17:0}	4.60	1.82
ai-C _{15:0}	8.56	15.41
ai-C _{17:0}	6.19	10.52
Saturated		
C _{15:0}	1.48	2.72
C _{16:0}	2.86	1.10
Unsaturated		
C _{17:1ω8c}	—	2.94
C _{17:1ω9c}	7.18	—
i-C _{17:1ω9c}	—	2.72
ai-C _{17:1ω9c}	1.06	1.19
Other	6.62	3.78

Description of *Catenulisporineae* subord. nov.

(Ca.te.nu.li.spo.ri'ne.ae. N.L. fem. n. *Catenulispora* type genus of the suborder; *-ineae* ending to denote a suborder; N.L. fem. pl. n. *Catenulisporineae* the *Catenulispora* suborder).

The suborder contains the families *Catenulisporaceae* and *Actinospicaceae*. The pattern of 16S rRNA gene signatures consists of nt 127:234 (G–C), 138:225 (U–A), 139:224 (C–G), 140:223 (C–G), 141:222 (A–U), 157:164 (G–C), 449 (C), 589:650 (C–G), 602:636 (R–U), 603:635 (A–U), 694 (G) and 1251 (G). The phylogenetic analysis is presented in this study. The type genus of the suborder is *Catenulispora*.

Description of *Actinospicaceae* fam. nov.

(Ac.ti.no.spi.ca'ce.ae. N.L. fem. n. *Actinospica* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Actinospicaceae* the *Actinospica* family).

The family contains the type genus *Actinospica*. The pattern of 16S rRNA gene signatures consists of nt 127:234 (G–C), 129:232 (C–G), 344 (G), 449 (C), 450:483 (C–G), 560 (U), 576 (G), 590:649 (C–G), 591:648 (U–R), 859 (G), 952:1229 (C–G), 1122:1151 (G–C), 1123:1150 (U–G), 1124:1149 (A–U), and of seven to nine extra nucleotides between positions 1134 and 1140. The phylogenetic analysis is presented in this study.

Description of *Actinospica* gen. nov.

(Ac.ti.no.spi'ca. Gr. n. *actinos* a ray; L. fem. n. *spica* tuft; N.L. fem. n. *Actinospica* an actinomycete with tufts of aerial hyphae).

Aerobic, Gram-positive micro-organisms that form non-fragmenting vegetative mycelia. The aerial mycelium appears as tufts of straight to slightly flexuous hyphae, carrying at maturity chains of cylindrical spores. Tufts originate from very short sporophorous branching in few sporogenous hyphae. Motile elements are not produced. Obligately acidophilic, growing at pH values not higher than 6.2. Mesophilic; best growth occurs at 22–28 °C. Grow

Fig. 3. Neighbour-joining tree based on 1291 aligned positions within the 16S rRNA gene, omitting regions of ambiguous alignment. The tree was rooted by using the 16S rRNA gene sequence of *Bifidobacterium bifidum* NBRC 14252^T (GenBank accession no. S83624). Numbers at nodes are bootstrap values based on 100 resamplings; only values >80 are shown. Bar, 1 inferred nucleotide substitution per 100 nt.

better on acidic yeast extract–malt extract agar and acidic oatmeal agar. Cell wall contains 3-hydroxydiaminopimelic acid. The polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, methylphosphatidylethanolamine and phosphatidylinositol. The predominant cellular fatty acids are i-C_{15:0}, i-C_{16:0} and ai-C_{15:0}. Contain the major menaquinones MK-9(H₄), MK-9(H₆) and MK-9(H₈) and the cell-wall sugars mannose and rhamnose. The G + C content of the DNA ranges between 69 and 71 mol%. The type species of the genus is *Actinospica robiniae*.

Description of *Actinospica robiniae* sp. nov.

Actinospica robiniae (ro.bi'ni.æ. N.L. fem. n. *Robinia* scientific name of a genus of tree; N.L. fem. gen. n. *robiniae* of *Robinia*, isolated from a wood of *Robinia pseudoacacia*).

The chemotaxonomic and general characteristics are the same as given above for the genus. The type strain contains galactose as an additional whole-cell sugar. Aerial mycelium appears as enlarged tufts of sporogenous aerial hyphae that divide into chains of squat to cylindrical arthrospores (0.6–0.7 to 1.0–1.2 × 0.6 µm). Spore surface is slightly rugose. Growth occurs between pH 4.8 and 6.2 and is optimal at pH 5.5. H₂S is produced. Nitrates are not reduced. Starch is not hydrolysed. Gelatin is not liquefied. Catalase-positive. Growth occurs between 17 and 33 °C and is optimal in the range 22–28 °C; no growth occurs at 14 or 37 °C. NaCl [1% (w/v)] is not tolerated; nor is 100 µg lysozyme ml⁻¹. The G + C content of the DNA is 70.8 mol%.

The type strain, GE134769^T (=DSM 44927^T=NRRL B-24432^T), was isolated from temperate forest soil.

Description of *Actinospica acidiphila* sp. nov.

Actinospica acidiphila (a.ci.di'phi.la. N.L. neut. n. *acidum* acid; Gr. adj. *philos* loving; N.L. fem. n. *acidiphila* acid-loving).

The chemotaxonomic and general characteristics are the same as given above for the genus. The type strain contains arabinose and xylose as additional whole-cell sugars. Aerial mycelium produces long chains of up to 30 cylindrical spores (0.6–0.8 × 0.5 µm) and occasionally more. Spore surface is rugose. Growth occurs between pH 4.2 and 6.0, with optimal rates at pH 5.0. Greenish pigments are produced in some media. H₂S is produced. Nitrates are not reduced. Starch is hydrolysed. Gelatin is not liquefied. Catalase-positive. Growth occurs between 17 and 33 °C and is optimal at 28 °C; no growth occurs at 14 or 37 °C. Up to 1% (w/v) NaCl is tolerated, whereas 100 µg lysozyme ml⁻¹ is not. The G + C content of the DNA is 69.2 mol%.

The type strain, GE134766^T (=DSM 44926^T=NRRL B-24431^T), was isolated from temperate forest soil.

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REFERENCES

- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Busti, E., Cavaletti, L., Monciardini, P., Schumann, P., Rohde, M., Sosio, M. & Donadio, S. (2006). *Catenulispora acidiphila* gen. nov., sp. nov., a novel, mycelium-forming actinomycete, and proposal of *Catenulisporaceae* fam. nov. *Int J Syst Evol Microbiol* **56**, 1741–1746.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Bogoriella caseilytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a soda lake in Africa. *Int J Syst Bacteriol* **47**, 788–794.
- Hahn, M. W., Stadler, P., Wu, Q. L. & Pöckl, M. (2004). The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. *J Microbiol Methods* **57**, 379–390.
- Hugenholtz, P. (2002). Exploring prokaryotic diversity in the genomic era. *Genome Biol* **3**, REVIEWS0003.
- Janssen, P. H., Yates, P. S., Grinton, B. E., Taylor, P. M. & Sait, M. (2002). Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. *Appl Environ Microbiol* **68**, 2391–2396.
- Joseph, S. J., Hugenholtz, P., Sangwan, P., Osborne, C. A. & Janssen, P. H. (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol* **69**, 7210–7215.
- Koch, C., Kroppenstedt, R. M., Rainey, F. A. & Stackebrandt, E. (1996). 16S ribosomal DNA analysis of the genera *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium*, and *Pilimelia* and emendation of the family *Micromonosporaceae*. *Int J Syst Bacteriol* **46**, 765–768.
- Lee, S. D. & Hah, Y. C. (2002). Proposal to transfer *Catellatospora ferruginea* and '*Catellatospora ishihariensis*' to *Asanoa* gen. nov. as *Asanoa ferruginea* comb. nov. and *Asanoa ishihariensis* sp. nov., with emended description of the genus *Catellatospora*. *Int J Syst Evol Microbiol* **52**, 967–972.
- Maerz, A. & Paul, M. R. (1950). *Dictionary of Color*, 2nd edn. New York: McGraw-Hill.
- Maszenan, A. M., Tay, J.-H., Schumann, P., Jiang, H.-L. & Tay, S. T.-L. (2005). *Quadrisphaera granulorum* gen. nov., sp. nov., a Gram-positive polyphosphate-accumulating coccus in tetrads or aggregates isolated from aerobic granules. *Int J Syst Evol Microbiol* **55**, 1771–1777.
- 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.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Monciardini, P., Cavaletti, L., Schumann, P., Rohde, M. & Donadio, S. (2003). *Conexibacter woesei* gen. nov., sp. nov., a novel representative of a deep evolutionary line of descent within the class *Actinobacteria*. *Int J Syst Evol Microbiol* **53**, 569–576.
- Sait, M., Hugenholtz, P. & Janssen, P. H. (2002). Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environ Microbiol* **4**, 654–666.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- 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.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Stevenson, B. S., Eichorst, S. A., Wertz, J. T., Schmidt, T. M. & Breznak, J. A. (2004). New strategies for cultivation and detection of previously uncultured microbes. *Appl Environ Microbiol* **70**, 4748–4755.
- Tamura, T., Hayakawa, M. & Hatano, K. (1998). A new genus of the order *Actinomycetales*, *Cryptosporangium* gen. nov., with descriptions of *Cryptosporangium arvum* sp. nov. and *Cryptosporangium japonicum* sp. nov. *Int J Syst Bacteriol* **48**, 995–1005.
- Tao, T. S., Yue, Y. Y., Chen, W. X. & Chen, W. F. (2004). Proposal of *Nakamurella* gen. nov. as a substitute for the bacterial genus *Microsphaera* Yoshimi *et al.* 1996 and *Nakamurellaceae* fam. nov. as a substitute for the illegitimate bacterial family *Microsphaeraceae* Rainey *et al.* 1997. *Int J Syst Evol Microbiol* **54**, 999–1000.
- Willems, A., Moore, W. E. C., Weiss, N. & Collins, M. D. (1997). Phenotypic and phylogenetic characterization of some *Eubacterium*-like isolates containing a novel type B wall murein from human feces: description of *Holdemania filiformis* gen. nov., sp. nov. *Int J Syst Bacteriol* **47**, 1201–1204.