Five novel *Kitasatospora* species from soil: *Kitasatospora arboriphila* sp. nov., *K. gansuensis* sp. nov., *K. nipponensis* sp. nov., *K. paranensis* sp. nov. and *K. terrestris* sp. nov.

Ingrid Groth,¹ Carlos Rodríguez,² Barbara Schütze,¹ Petra Schmitz,³ Eckhard Leistner³ and Michael Goodfellow²

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹Hans-Knöll-Institut für Naturstoff-Forschung eV, D-07745 Jena, Germany

²School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

³Rheinische Friedrich-Wilhelms-Universität, Institut für Pharmazeutische Biologie, D-53115 Bonn, Germany

A polyphasic study was carried out to establish the taxonomic positions of six strains isolated from diverse soil samples and provisionally assigned to the genus *Kitasatospora*. The isolates were found to have chemical and morphological properties consistent with their classification as *Kitasatospora* strains. Direct 16S rRNA gene sequence data confirmed the taxonomic position of the strains following the generation of phylogenetic trees by using three tree-making algorithms. Five of the isolates were considered to merit species status using complementary genotypic and phenotypic data. These organisms were designated *Kitasatospora arboriphila* sp. nov. (HKI 0189^T=2291-120^T=DSM 44785^T=NCIMB 13973^T), *Kitasatospora gansuensis* sp. nov. (HKI 0314^T=2050-015^T=DSM 44786^T=NCIMB 13974^T), *Kitasatospora nipponensis* sp. nov. (HKI 0315^T=2148-013^T=DSM 44787^T=NCIMB 13975^T), *Kitasatospora paranensis* sp. nov. (HKI 0190^T=2292-041^T=DSM 44788^T=NCIMB 13976^T) and *Kitasatospora terrestris* sp. nov. (HKI 0186^T=2293-012^T=DSM 44789^T=NCIMB 13977^T). The remaining organism, isolate HKI 0316 (=2122-022=DSM 44790=NCIMB 13978), was considered to be a strain of *Kitasatospora kifunensis* on the basis of 16S rRNA gene sequence, DNA–DNA relatedness and phenotypic data.

The genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* form the family *Streptomycetaceae* Waksman and Henrici 1943 emend. Kim *et al.* 2003. The genus *Kitasatospora* (formerly *Kitasatosporia*) was proposed by Ōmura *et al.* (1982) and currently includes aerobic, Gram-positive, non-motile, chemo-organotrophic actinomycetes that form an extensively branched substrate mycelium, aerial hyphae that differentiate into long chains of spores, contain *N*-acetylated muramic acid, major proportions of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type 2 *sensu* Lechevalier *et al.*, 1977), predominant amounts of hexahydrogenated menaquinones with nine isoprene units, fatty acids with major

amounts of iso and anteiso components and whole-organism hydrolysates that are typically rich in galactose, and in LL- and meso-diaminopimelic acid (*meso*-A₂pm). Aerial spores on solid culture and submerged spores in liquid media contain LL-A₂pm whereas mycelia grown under both cultural conditions have mainly *meso*-A₂pm (Ōmura *et al.*, 1981, 1982; Takahashi *et al.*, 1984; Groth *et al.*, 2003).

The 13 species of *Kitasatospora* that have validly published names at the time of writing are *Kitasatospora azatica* (Nakagaito *et al.* 1993) Zhang *et al.* 1997, *Kitasatospora cheerisanensis* Chung *et al.* 1999, *Kitasatospora cineracea* Tajima *et al.* 2001, *Kitasatospora cochleata* (Nakagaito *et al.* 1993) Zhang *et al.* 1997, *Kitasatospora cystarginea* Kusakabe and Isono 1992, *Kitasatospora griseola* Takahashi *et al.* 1985, *Kitasatospora kifunensis* (Nakagaito *et al.* 1993) Groth *et al.* 2003, *Kitasatospora mediocidica* Labeda 1988, *Kitasatospora niigatensis* Tajima *et al.* 2001, *Kitasatospora paracochleata* (Nakagaito *et al.* 1993) Zhang *et al.* 1997, *Kitasatospora phosalacinea* Takahashi *et al.* 1985,

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains HKI 0315^T, HKI 0316, HKI 0189^T, HKI 0314^T, HKI 0186^T and HKI 0190^T are AY442263–AY442268, respectively.

Kitasatospora putterlickiae Groth *et al.* 2003 and *Kitasatospora setae* Ômura *et al.* 1983 [species name corrected by Ômura *et al.* (1985)]; *K. setae* is the type species of the genus. Members of these species form a distinct phyletic line in the 16S rRNA gene tree and can be delineated using a combination of genotypic and phenotypic data (Nakagaito *et al.*, 1992a, b; Groth *et al.*, 2003).

Six out of 18 *Kitasatospora* strains isolated from soil samples collected from several geographical locations during a study designed to improve the isolation and rapid differentiation of kitasatosporae from phylogenetically similar streptomycetes were the subject of a polyphasic taxonomic study. This showed that five of the six strains should be recognized as novel species. Strains HKI 0315^T (=2148-013^T), HKI 0316 (=2122-022) and HKI 0314^T (=2050-015^T) were isolated from soil samples collected in Kumagura and Kyoto, Japan, and in Gansu Province, China, respectively, using PY-BHI agar (Yokota *et al.*, 1993) and a standard dilution plate procedure; these isolates were originally misclassified as streptomycetes. Strains HKI 0190^T (=2292-041^T), HKI 0186^T (=2293-012^T) and HKI 0189^T (=2291-120^T) were isolated from rhizosphere soil of *Maytenus* plants in Brazil using either PY-BHI agar (strains HKI 0186^T and HKI 0190^T) or yeast extract/starch agar (Kudo *et al.*, 1993) (strain HKI 0189^T) and a phage-assisted isolation method.

The phage-assisted isolation method involved heating air-dried soil (1 g) at 80 °C in a dry oven for 1 h, making a soil suspension in 10 ml aqueous sodium pyrophosphate (0.2 % w/v), mixing on a rotary shaker for 30 min, and allowing the soil particles to settle prior to decanting the supernatant, which was kept at 4 °C. The soil fraction was treated with the same amount of solvent and the resultant preparation was ultrasonicated for 5 min at 50 W. The second supernatant was added to the first, the combined preparation was diluted and 0.1 ml aliquots of the various dilutions were spread over casein mineral (Altenburger *et al.*, 1996), humic acid (Hayakawa & Nonomura, 1987), PY-BHI agar (Yokota *et al.*, 1993) and yeast extract starch agar isolation plates, which were carefully overlaid with 100 µl per plate of a high titre suspension (about 10⁸–10⁹ plaque-forming units) of polyvalent *Streptomyces* phage S7 (DSM 49153) and incubated at 28 °C for up to 4 weeks. Colonies that exhibited a *Streptomyces*-like morphology were transferred to oatmeal agar plates (ISP medium 3; Shirling & Gottlieb, 1966) and incubated for further growth. These isolates were checked again for their susceptibility to *Streptomyces* phage S7 as described below. All S7-resistant strains were grown on organic medium 79 (Prauser *et al.*, 1987) and oatmeal agar, which favour the growth of vegetative mycelium (contains meso-A₂pm) and spores (contain LL-A₂pm), respectively. Strains found to contain both of these isomers were studied further.

Biomass for the chemotaxonomic analyses of the six isolates and marker strains of *K. azatica* (DSM 41650^T), *K. kifunensis* (DSM 41654^T), *K. mediocidica* (DSM 43929^T),

K. phosalacinea (DSM 43860^T) and *K. putterlickiae* (DSM 44665^T) was prepared by cultivating them for 24–48 h at 28 °C in liquid organic medium 79 and bacto-tryptic soy broth (Sigma-Aldrich). The strains were grown on ISP media 2, 3, 4 and 5 (Difco; Shirling & Gottlieb, 1966) at 28 °C for up to 21 days, after which cultural and morphological properties were recorded; morphological features were observed by light and electron microscopy. The dimensions of the spores were measured by using an Axioskop 2 microscope equipped with image-analysing AXIO VISION 2.05 software (both from Zeiss). Samples for electron microscopy were prepared from heavily sporulating cultures grown on ISP medium 3 following Shirling & Gottlieb (1966) and observed by using a Zeiss EM 902A electron microscope at an acceleration voltage of 80 kV.

Physiological tests were carried out as described by Groth *et al.* (2003). Susceptibility to most of the tested antibiotics was examined by placing antibiotic discs (Difco) on organic medium 79 agar plates that were seeded with suspensions of the test strains grown in a soft agar layer for up to 2 days at 28 °C. Nalidixic acid and novobiocin were added to ISP 2 agar plates, which were then seeded with spores of the strains. Susceptibility to polyvalent *Streptomyces* phages was tested by dropping high-titre suspensions of phage S7 (DSM 49153) onto agar plates seeded with spores of the test strains held in a soft agar layer; the phage was propagated as described by Groth *et al.* (2003). Standard procedures were used to determine the isomers of A₂pm in whole-organism hydrolysates (Rhuland *et al.*, 1955; Hasegawa *et al.*, 1983), type of muramic acid (Uchida & Aida, 1984) and presence of mycolic acids (Minnikin *et al.*, 1975), together with whole-organism fatty acid (MIDI system; Agilent), menaquinone (Collins *et al.*, 1977), polar lipid (Minnikin *et al.*, 1979; Collins & Jones, 1980) and sugar profiles (Becker *et al.*, 1965; Saddler *et al.*, 1991).

16S rRNA gene sequence amplification and analysis of strains HKI 0186^T, HKI 0189^T and HKI 0190^T were carried out as described by Edwards *et al.* (1989), and of strains HKI 0314^T, HKI 0315^T and HKI 0316 as described by Kim *et al.* (1998). The resultant sequences were aligned manually with corresponding almost complete sequences of representatives of most actinomycete genera; in each case reference sequences were retrieved from DDBJ/EMBL/GenBank. Phylogenetic analysis was carried out using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP 3.5c package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were generated according to the model of Jukes & Cantor (1969). The stability of the resultant unrooted tree topologies were evaluated by carrying out bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings, using the SEQBOOT and CONSENSE options from the PHYLIP 3.5c software.

DNA was extracted from the strains included in the

DNA–DNA relatedness studies using a French pressure cell (Thermo Spectronic) and then purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridizations were carried out in $2 \times$ SSC and DMSO (10%, v/v) at 70 °C, using modifications (Escara & Hutton, 1980; Huß *et al.*, 1983) of the procedure described by De Ley *et al.* (1970) and a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates were computed using the TRANSFER.BAS program of Jahnke (1992).

Preliminary phylogenetic analysis of the almost complete 16S rRNA gene sequences of the six isolates (1368–1444 nt) placed them within the evolutionary radiation occupied by the genus *Kitasatospora* (data not shown). The isolates were aerobic, Gram-positive, non-motile, formed non-fragmenting, extensively branched substrate mycelia, aerial hyphae that differentiated into long chains of smooth-surfaced spores (Fig. 1) and grew well on ISP media (Table 1) and within the pH range 5.0–9.5. They were also characterized by the presence of *N*-acetylated muramic acid, galactose, predominant proportions of hexahydrogenated menaquinones with nine isoprene units, major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides, LL- and *meso*-A₂pm in whole-organism hydrolysates and contained varying

proportions of iso- and anteiso-branched, straight-chain and unsaturated fatty acids (Table 2) but lacked mycolic acids. The isolates were resistant to polyvalent *Streptomyces* phage S7. All of these properties are consistent with their classification in the genus *Kitasatospora* (Ōmura *et al.*, 1982; Zhang *et al.*, 1997). This phenotypic profile also serves to distinguish the isolates from members of the genera *Streptacidiphilus* and *Streptomyces* (Kim *et al.*, 2003a). The isolates were sensitive to low concentrations of novobiocin ($5 \mu\text{g ml}^{-1}$) and hence are unlikely to grow on the novobiocin-containing agar medium used by Tajima *et al.* (2001) to isolate *K. cineracea* and *K. niigatensis* strains.

It is evident from Fig. 2 that the six isolates are scattered throughout the *Kitasatospora* 16S rRNA gene sequence tree. The closest relationship between any of the isolates is between strain HKI 0316 and *K. kifunensis* NBRC 15206^T. This relationship is supported by all three tree-making algorithms and by a bootstrap value of 77% in the neighbour-joining analysis. The two strains share a 16S rRNA gene sequence similarity of 99.6%, which corresponds to 6 nt differences over 1365 locations. However, they have a DNA–DNA relatedness of 78%, a value well above the 70% cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). The two strains also share key phenotypic properties, notably the ability to form short cylindrical, smooth-surfaced spores

Fig. 1. Electron micrographs of spores of *Kitasatospora* isolates grown on ISP3 agar for 21 days at 28 °C. (a) *K. arboriphila* 2291-120^T (=HKI 0189^T); (b) *K. gansuensis* 2050-015^T (=HKI 0314^T); (c) *K. nipponensis* 2148-013^T (=HKI 0315^T); (d) *K. paranensis* 2292-041^T (=HKI 0190^T); (e) *K. terrestris* 2293-012^T (=HKI 0186^T). Bars, 0.54 μm .

Table 1. Growth of *Kitasatospora* isolates on ISP media

None of the strains produced soluble pigment on ISP2, ISP4 or ISP5 media.

Characteristic	HKI 0315 ^T	HKI 0189 ^T	HKI 0316 ^T	HKI 0314 ^T	HKI 0190 ^T	HKI 0186 ^T
ISP2 medium						
Growth	Good	Good	Abundant	Good	Moderate	Abundant
Aerial mycelium	Absent or sparse; white	White to grey	White, pale grey	White	Sparse; white	Grey
Reverse colour	Yellowish brown	Yellowish brown	Orange, brown	Orange, brown	Yellowish brown	Yellowish brown to dark brown
ISP3 medium						
Growth	Abundant	Abundant	Abundant	Good	Abundant	Abundant
Aerial mycelium	Grey	Grey to dark grey	Grey	White	Grey	Grey
Reverse colour	Reddish brown, dark brown	Greyish brown, dark brown	Yellowish brown	Orange-brown to brown	Yellowish brown, dark brown	Yellowish brown to brown
Soluble pigment	Purple	None	None	None	None	None
ISP4 medium						
Growth	Good	Good	Good	Good	Moderate	Moderate
Aerial mycelium	Absent or sparse; white, grey	Grey to dark grey	Grey	Sparse; white, beige	Sparse; white to pale grey	Sparse; white, grey
Reverse colour	Yellowish brown, brown	Yellowish grey, olive	Beige, brown	Yellowish brown to dark brown	Yellowish brown	Yellowish brown, greyish brown
ISP5 medium						
Growth	Moderate	Good	Good	Good	Moderate	Good
Aerial mycelium	Absent or sparse; white	Grey	Grey	White	Pale grey	Sparse; grey
Reverse colour	Reddish brown	Yellowish grey, dark greyish brown	Beige, brown	Yellowish brown to dark brown	Yellowish brown to brown	Yellowish grey

Table 2. Cellular fatty acid compositions (%) of the *Kitasatospora* isolates and *K. kifunensis* DSM 41654^T

Strains: 1, HKI 0190^T; 2, HKI 0186^T; 3, HKI 0189^T; 4, HKI 0315^T; 5, HKI 0316^T; 6, *K. kifunensis* DSM 41654^T; 7, HKI 0314^T.
–, Not detected.

Fatty acid	1	2	3	4	5	6	7
Saturated:							
C _{14:0}	1.0	0.6	0.4	0.4	0.7	1.4	0.8
C _{15:0}	5.0	1.1	2.1	1.2	2.1	3.7	1.4
C _{16:0}	14.1	9.3	7.4	5.9	17.0	25.3	18.6
Unsaturated:							
C _{16:1} ω7c	6.8	8.3	4.9	9.4	12.4	6.8	9.7
C _{17:1} ω7c	1.7	1.0	0.5	0.6	1.6	0.7	0.5
C _{18:1} ω7c	0.7	2.4	–	0.2	0.7	0.5	1.0
Branched:							
iso-C _{14:0}	4.2	2.3	2.6	4.6	0.6	0.5	1.3
iso-C _{15:0}	9.4	16.0	16.7	10.2	15.8	12.9	10.3
anteiso-C _{15:0}	16.3	10.4	11.6	8.7	12.9	16.8	21.4
iso-C _{16:1} H	2.6	3.9	6.6	7.0	0.6	0.4	2.0
iso-C _{16:0}	19.3	21.4	25.4	37.7	6.4	3.9	10.1
anteiso-C _{17:1} C	1.8	2.3	2.3	1.4	2.4	1.5	2.4
iso-C _{17:0}	2.0	5.3	4.6	1.6	5.0	2.6	3.1
anteiso-C _{17:0}	5.4	5.8	5.8	3.0	7.3	5.4	8.9
9-Methyl-C _{16:0}	2.0	7.2	6.4	4.2	8.3	4.5	4.3
C _{17:0} cyclo	3.6	0.7	1.2	1.9	3.9	9.4	2.2

in straight to spiral spore chains (*rectiflexibiles* to *spirales*), to produce melanin pigments on tyrosine agar, to hydrolyse starch, to produce β -galactosidase, α -glucosidase, α -mannosidase and naphthol-AS-BI phosphohydrolase, to use (+)-D-mannitol and (+)-D-sucrose as sole carbon sources and to grow in the presence of NaCl (2.5 %, w/v), lincomycin hydrochloride (2 μ g), penicillin G (10 IU) and polymyxin B (300 IU). In contrast, neither of the organisms is able to liquefy gelatin, peptonize milk, reduce nitrate, grow on (+)-D-raffinose or (+)-L-rhamnose as sole carbon sources or to produce β -glucosidase or *N*-acetyl- β -glucosamidase. The two strains have a fatty acid profile rich in C_{16:0}, anteiso-C_{15:0} and iso-C_{15:0} components. These data are consistent with the assignment of isolate HKI 0316 (=2122-022) to *K. kifunensis* (Nakagaito *et al.* 1993) Groth *et al.* 2003.

Isolates HKI 0190^T and HKI 0186^T are closely related to *K. cystarginea* (Fig. 2); they share 16S rRNA gene sequence similarities with this organism of 98.5 and 99.0 %, respectively, values equivalent to 15 and 14 nt differences at 1430 locations. However, the two isolates are more closely related to one another, having a 16S rRNA gene sequence similarity of 99.2 %, which corresponds to 12 nt differences at 1444 sites, but clearly belong to distinct genomic species as they share a DNA–DNA relatedness of 40.1 %. It is also evident from Tables 2 and 3 that these organisms

Fig. 2. Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the position of the six isolates in the *Kitasatospora* tree. Asterisks indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Felsenstein, 1993) algorithms; 'ml' denotes branches that were formed using the maximum-likelihood tree-making method. Numbers at nodes are bootstrap values (%) based on 1000 resampled datasets; only values > 50 % are given. Bar, 0.02 nucleotide substitutions per nucleotide position.

Isolate HKI 0315^T was most closely related to the type strains of *K. azatica* and *K. kifunensis*; it showed a 16S rRNA gene sequence similarity to these organisms of 99.1% and 99.2%, values that correspond to 13 and 11 nt differences at 1387 and 1377 locations, respectively. DNA-DNA relatedness studies were not carried out between this isolate and the type strains of *K. azatica* and *K. kifunensis* as it has been established that kitasatosporae with similar 16S rRNA gene sequence similarities show relatedness values well below the cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). This is exemplified in the present study by

The relatively close phylogenetic relationship found between isolate HKI 0314^T and *K. medicicida* NBRC 14789^T is supported by the results from all of the tree-making algorithms and by a bootstrap value of 90 % in the neighbour-joining analysis (Fig. 2). The two organisms share a 16S rRNA gene sequence similarity of 98.7 %, a value equivalent to 19 nt differences at 1429 sites. The two strains can be separated readily using a combination of phenotypic properties (Table 3). It is proposed that isolate HKI 0314^T be recognized as a novel *Kitasatospora* species, for which the name *Kitasatospora gansuensis* sp. nov. is proposed.

Description of *Kitasatospora arboriphila* sp. nov.

Aerobic, Gram-positive, non-acid-fast actinomycete that produces a yellowish brown to dark brown or olive substrate mycelium and a grey to dark grey aerial spore mass on glycerol/asparagine, inorganic salts/starch, oatmeal and yeast extract/malt extract agars. Soluble pigments are not formed, but melanoid pigments are produced on peptone/yeast extract/iron and tyrosine agars. Spore chains are long, straight to spiral with hooks and loops with 20 or more cylindrical, smooth-surfaced spores ($1.1\text{--}1.7 \times 1.3\text{--}2.4\text{ }\mu\text{m}$) per chain (Fig. 1a). Submerged spores are formed sparsely in liquid culture. Temperature range for growth is $15\text{--}40\text{ }^{\circ}\text{C}$ (optimum $28\text{--}32\text{ }^{\circ}\text{C}$); growth does not occur at $10\text{ }^{\circ}\text{C}$ or above $40\text{ }^{\circ}\text{C}$. pH range for good growth is pH $5.0\text{--}8.0$; growth does not occur at either pH 4.5 or pH 9.0 . Additional phenotypic properties are given in Table 3. The cell wall contains *meso*- and LL- A_2pm ; the muramic acid moiety is *N*-acetylated. Whole-organism hydrolysates contain galactose, mannose, glucose and ribose. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The predominant fatty

Table 3. Phenotypic properties that separate the novel isolates from one another and from representatives of closely related *Kitasatospora* species

Taxa: 1, *K. nipponensis* sp. nov. HKI 0315^T; 2, *K. azatica* DSM 41650^T; 3, *K. putterlickiae* DSM 44665^T; 4, *K. arboriphila* sp. nov. HKI 0189^T; 5, *K. phosalacinea* DSM 43860^T; 6, *K. kifunensis* HKI 0316; 7, *K. kifunensis* DSM 41654^T; 8, *K. gansuensis* sp. nov. HKI 0314^T; 9, *K. medicidica* DSM 43929^T; 10, *K. paranensis* sp. nov. HKI 0190^T; 11, *K. terrestris* sp. nov. HKI 0186^T; 12, *K. cystarginea* DSM 41680^T. +, Positive; −, negative; (+), weakly positive; ±, doubtful; ND, not determined. For the following properties, tests for which strain DSM 41680^T was not tested are indicated by 'a'. Spores of all of the tested strains are cylindrical with a smooth surface. All strains were positive for the production of H₂S^a, growth on (+)-D-glucose, and produced acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase (API ZYM tests). Good growth occurred at temperatures of 15–32 °C and pH 5.0–7.0. All strains were sensitive to chloramphenicol (30 µg)^a, ciprofloxacin (5 µg)^a, imipenem (10 µg)^a, kanamycin sulphate (30 µg)^a, nalidixic acid (50 µg ml^{−1} agar)^a, oxytetracycline (30 µg)^a, rifampicin (30 µg)^a, streptomycin sulphate (10 µg)^a and vancomycin (30 µg)^a. They did not use cellulose^a as a sole carbon source; did not produce α-chymotrypsin, cystine arylamidase, α-galactosidase, β-glucuronidase, α-fucosidase, lipase (C14), trypsin or valine arylamidase (API ZYM tests) and did not grow in the presence of NaCl (4%, w/v) or at 42 °C and pH 4.0 or pH 10.

Test	1	2	3	4	5	6	7	8	9	10	11	12
Spore chain morphology*	RF, RA, S	RF	RF	RF, RA, S	RF	RF, S	RF, S	RF	RF, RA	RF	RF, RA, S	S†
Formation of melanoid pigment	−	−	+	+	−	+	+	+	+	+	+	−†
Degradation of casein	+	+	+	+	+	−	+	+	−	+	+	ND
Liquefaction of gelatin	+	+	+	+	+	−	−	+	−	+	+	−†
Hydrolysis of potato starch	+	+	−	+	+	+	+	+	+	+	+	++†
Peptonization of milk	+	+	+	+	+	−	−	+	−	+	+	++†
Nitrate reduction	−	+	+	+	+	−	−	+	−	+	+	−†
Growth on sole carbon sources:												
(+)-L-Arabinose	−	+	−	+	++‡	−	+	+	+	+	+	±†
(+)-D-Fructose	(+)	+	(+)	+	++‡	+	−	+	+	−	+	±†
i-Inositol	−	−	−	−	−‡	−	−	−	−	−	−	++†
(+)-D-Mannitol	−	−	−	−	−‡	+	+	−	−	−	−	−†
(+)-D-Raffinose	−	−	−	+	++‡	−	−	−	−	−	+	−†
(+)-L-Rhamnose	−	−	−	−	++‡	−	−	−	−	+	(+)	−†
(−)-D-Sucrose	(+)	+	(+)	−	++‡	+	+	+	+	−	(+)	−†
(+)-D-Xylose	−	+	−	+	++‡	−	+	+	+	+	+	−†
Enzyme assay (API ZYM):												
N-Acetyl-β-glucosamidase	+	−	−	−	−	−	−	(+)	−	−	−	−
β-Galactosidase	+	+	+	(+)	+	+	+	+	−	+	+	+
α-Glucosidase	−	(+)	−	+	+	(+)	+	+	−	+	(+)	+
β-Glucosidase	−	−	+	−	−	−	−	+	−	−	−	−
α-Mannosidase	−	−	−	−	−	+	(+)	−	−	−	−	−
Naphthol-AS-BI-phosphohydrolase	+	+	−	+	+	+	+	+	+	+	+	+
Temperature for growth:												
6 °C	−	−	−	−	−	−	−	(+)	(+)	−	−	−†
10 °C	+	(+)	(+)	−	+	+	(+)	+	+	(+)	−	−†
35 °C	(+)	(+)	+	+	+	+	−	−	−	+	+	++†
37 °C	−	−	+	+	+	+	−	−	−	+	+	++†
40 °C	−	−	−	(+)	+	−	−	−	−	−	+	++†
42 °C	−	−	−	−	++‡	−	−	−	−	−	−	−†
Growth in the presence of NaCl (%):												
2.0	+	−	+	+	+	+	+	+	+	+	+	+
2.5	(+)	−	+	−	+	+	+	+	+	+	+	+
3.0	−	−	+	−	−	+	−	−	−	+	(+)	−
3.5	−	−	+	−	−	+	−	−	−	+	−	−
Growth at pH:												
8.0	+	+	+	+	+	+	+	+	−	+	+	+
9.0	−	−	+	−	+	−	+	+	−	+	+	+
9.5	−	−	+	−	+	−	−	+	−	−	−	−
Antibiotic susceptibility:												
Ampicillin (10 µg)	−	+	+	−	+	−	(+)	(+)	−	(+)	−	ND

Table 3. cont.

Test	1	2	3	4	5	6	7	8	9	10	11	12
Lincomycin hydrochloride (2 µg)	–	–	–	–	–	–	–	–	(+)	(+)	–	ND
Methicillin (5 µg)	(+)	+	(+)	–	–	–	(+)	–	–	(+)	–	ND
Norfloxacin (10 µg)	–	(+)	–	–	–	+	–	–	(+)	–	(+)	ND
Novobiocin (5 µg ml ⁻¹)	+	+	+	+	–	+	+	+	+	+	+	ND
Penicillin G (10 IU)	–	+	+	–	+	–	–	–	–	–	–	ND
Polymyxin B (300 IU)	(+)	+	(+)	+	(+)	–	–	–	(+)	+	+	ND
Sulfonamide (200 µg)	(+)	+	–	–	–	–	(+)	+	–	(+)	–	ND

*RF, *Rectiflexibiles*; RA, *retinaculiaperti*; S, *spirales*.

†Data from Kusakabe & Isono (1988).

‡Data from Takahashi *et al.* (1984).

acids are iso-C_{16:0} (25 %), iso-C_{15:0} (17 %) and anteiso-C_{15:0} (12 %); mycolic acids are absent. The major menaquinones are MK-9(H₆) (49 %) and MK-9(H₈) (28 %).

The type and only strain is HKI 0189^T (= 2291-120^T = DSM 44785^T = NCIMB 13973^T), which was isolated from a soil sample collected from the roots of the tree *Maytenus aquifolia* in Ribeirao Preto, Brazil.

Description of *Kitasatospora gansuensis* sp. nov.

Kitasatospora gansuensis (gan.su.en'sis. N.L. fem. adj. *gansuensis* pertaining to Gansu, a province in China, the origin of the soil from which the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast actinomycete that produces a yellowish- or orange-brown to dark-brown substrate mycelium and a white to beige aerial spore mass on glycerol/asparagine, inorganic salts/starch, oatmeal and yeast extract/malt extract agars; soluble pigments are not formed on these media but melanoid pigments are produced on peptone/yeast extract/iron and tyrosine agars. Spore chains are long, straight to flexuous, with 20 or more cylindrical, smooth-surfaced spores (0.8–1.3 × 1.6–3.0 µm) per chain (Fig. 1b). Submerged spores and irregular fragments are formed in liquid culture. Temperature range for growth is 6–32 °C (optimum 25–28 °C); growth does not occur below 6 °C or at 35 °C. pH range for good growth is pH 5.0–9.5; growth does not occur at pH 4.5 or above pH 9.5. Additional phenotypic properties are given in Table 3. The cell wall contains *meso*- and LL-A₂pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, ribose, mannose and rhamnose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and an unknown glycolipid. The predominant fatty acids are anteiso-C_{15:0} (21 %), C_{16:0} (19 %), iso-C_{15:0} (10 %) and iso-C_{16:0} (10 %); mycolic acids are absent. The major menaquinone is MK-9(H₆) (75 %).

The type and only strain is HKI 0314^T (= 2050-015^T = DSM 44786^T = NCIMB 13974^T), which was isolated from a sample of forest soil collected in the Lianhua Shan Reservation, Gansu Province, China.

Description of *Kitasatospora nipponensis* sp. nov.

Kitasatospora nipponensis (nip.pon.en'sis. N.L. fem. adj. *nipponensis* pertaining to Nippon, the native name for Japan, the origin of the soil from which the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast actinomycete that produces a yellowish- or reddish-brown substrate mycelium and a grey aerial spore mass on glycerol/asparagine, inorganic salts/starch, oatmeal and yeast extract/malt extract agars. A purple soluble pigment is formed on oatmeal agar, but melanoid pigments are not produced on peptone/yeast extract/iron and tyrosine agars. Spore chains are open spirals, long straight loops and hooks with 20 or more cylindrical, smooth-surfaced spores (1.1–1.6 × 1.2–2.3 µm) per chain (Fig. 1c). Submerged spores are formed in liquid culture. Temperature range for growth is 10–32 °C (optimum 25–28 °C); growth does not occur at 6 °C or at 37 °C. pH range for good growth is pH 5.0–8.0; growth does not occur at pH 4.5 or above pH 8.5. Additional phenotypic properties are given in Table 3. The cell wall contains *meso*- and LL-A₂pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose, ribose and glucose. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and an unknown phospholipid. The predominant fatty acids are iso-C_{16:0} (38 %) and iso-C_{15:0} (10 %); mycolic acids are absent. The major menaquinone is MK-9(H₆) (74 %).

The type and only strain is HKI 0315^T (= 2148-013^T = DSM 44787^T = NCIMB 13975^T), which was isolated from a soil sample collected at Kumagura, Japan.

Description of *Kitasatospora paranensis* sp. nov.

Kitasatospora paranensis (pa.ra.nen'sis. N.L. fem. adj. *paranensis* pertaining to Parana, a state of Brazil, the origin of the soil from which the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast actinomycete that produces a yellowish-brown to dark-brown substrate mycelium and a grey aerial spore mass on glycerol-asparagine, inorganic salts/starch, oatmeal and yeast extract/malt extract agars. Soluble pigments are not formed, but melanoid pigments are produced on peptone/yeast extract/iron and tyrosine agars. Spore chains are long straight to flexuous with 20 or more cylindrical, smooth-surfaced spores ($1.1\text{--}1.4 \times 1.2\text{--}2.1\text{ }\mu\text{m}$) per chain (Fig. 1d). Submerged spores are rarely formed in liquid culture. Temperature range for growth is $10\text{--}37^\circ\text{C}$ (optimum $25\text{--}28^\circ\text{C}$); growth does not occur at 6°C or at 40°C . pH range for good growth is pH $5.0\text{--}9.0$; growth does not occur at either pH 4.0 or pH 9.5 . Additional phenotypic properties are given in Table 3. The cell wall contains both *meso*- and LL-A₂pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose and glucose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylserine and an unknown phospholipid. The predominant fatty acids are iso-C_{16:0} (19%), anteiso-C_{15:0} (16%) and C_{16:0} (14%); mycolic acids are absent. The major menaquinone is MK-9(H₆) (53%) with minor components MK-9(H₄) (22%) and MK-9(H₂) (14%).

The type and only strain is HKI 0190^T (= 2292-041^T = DSM 44788^T = NCIMB 13976^T), which was isolated from rhizosphere soil of *Maytenus ilicifolia*, Contenda, Parana State, Brazil.

Description of *Kitasatospora terrestris* sp. nov.

Kitasatospora terrestris (ter.res'tris. L. fem. adj. *terrestris* of the earth, terrestrial).

Aerobic, Gram-positive, non-acid-fast actinomycete that produces a yellowish-brown to dark-brown or greyish-brown substrate mycelium and a grey aerial spore mass on glycerol/asparagine, inorganic salts/starch, oatmeal and yeast extract/malt extract agars. Soluble pigments are not formed. The formation of melanoid pigments is weak on peptone/yeast extract/iron and tyrosine agars. Spore chains are straight, hooked and spiral with 20 or more cylindrical, smooth-surfaced spores ($1.1\text{--}1.5 \times 1.3\text{--}2.8\text{ }\mu\text{m}$) per chain (Fig. 1e). Submerged spores are formed in liquid culture. Temperature range for growth is $15\text{--}40^\circ\text{C}$ (optimum $28\text{--}32^\circ\text{C}$); growth does not occur at 10°C or at 42°C . pH range for good growth is pH $5.0\text{--}9.0$; growth does not occur at pH 4.5 or at pH 9.5 . Additional phenotypic properties are shown in Table 3. The cell wall contains *meso*- and LL-A₂pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose and glucose. The polar lipids are

phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol (traces), phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylserine and an unknown glycolipid. The predominant fatty acids are iso-C_{16:0} (21%), iso-C_{15:0} (16%) and anteiso-C_{15:0} (10%); mycolic acids are absent. The major menaquinone is MK-9(H₆) (76%).

The type and only strain is HKI 0186^T (= 2293-012^T = DSM 44789^T = NCIMB 13977^T), which was isolated from a soil sample of the roots of *Maytenus aquifolia*, Ribeirao Preto, Brazil.

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