# *Actinopolymorpha alba* sp. nov., isolated from a rhizosphere soil

Yan-Ru Cao,[1] Yi Jiang,[1,2] Jin-Yuan Wu,[1] Li-Hua Xu[1] and Cheng-Lin Jiang[1]

Correspondence
Cheng-Lin Jiang
lihxu@ynu.edu.cn
Li-Hua Xu
lihxu@ynu.edu.cn

[1]Yunnan Institute of Microbiology, The National Engineering Center for Research of Microbial Pharmaceuticals, Yunnan University, Kunming, Yunnan 650091, PR China

[2]Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Düsternbrooker Weg 20, D-24105 Kiel, Germany

A Gram-positive, milk–white coloured, aerobic strain, YIM 48868$^T$, was isolated from the rhizosphere soil of *Maytenus hookeri* Loes in Xishuangbanna, China. 16S rRNA gene sequence similarity studies showed that strain YIM 48868$^T$ was a member of the genus *Actinopolymorpha*, showing 96.8 % sequence similarity to *Actinopolymorpha singaporensis* IM 7744$^T$ and 97.0 % similarity to *Actinopolymorpha rutila* YIM 45725$^T$. Chemotaxonomic data (peptidoglycan type I, LL-diaminopimelic acid; sugar pattern C, glucose, rhamnose and ribose; polar lipids PI, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides and phosphatidylinositol) were characteristic of the genus *Actinopolymorpha*. A phylogenetic tree based on 16S rRNA gene sequences showed that strain YIM 48868$^T$ formed a distinct phylogenetic lineage within the genus *Actinopolymorpha*. Strain YIM 48868$^T$ could be differentiated from recognized species by means of phenotypic properties and the predominant menaquinones [MK-9($H_6$), MK-9($H_8$), MK-10($H_6$), MK-10($H_8$)]. The DNA G+C content was 66.6 mol%. The DNA–DNA relatedness values between strain YIM 48868$^T$ and the type strains of *A. singaporensis* and *A. rutila* were 48.7 % and 53.1 %, respectively. These data, in combination with phenotypic and chemotaxonomic data, demonstrate that strain YIM 48868$^T$ represents a novel species in the genus *Actinopolymorpha*, for which the name *Actinopolymorpha alba* sp. nov. is proposed. The type strain is YIM 48868$^T$ (=CCTCC AA 208030$^T$=DSM 45243$^T$).

The genus *Actinopolymorpha* encompasses two recognized species: *Actinopolymorpha singaporensis* (Wang *et al.*, 2001) and *Actinopolymorpha rutila* (Wang *et al.*, 2008). Species of the genus *Actinopolymorpha* show sparse or no aerial mycelium, irregular cell shapes and are able to tolerate at least 5 % NaCl.

During an investigation of the relationship between plant rhizosphere actinomycetes and plant endophytic actinomycetes, strain YIM 48868$^T$ was isolated from rhizosphere soil collected from Xishuangbanna, China, using Gauze 1 agar (Gauze *et al.*, 1983). The strain was maintained on medium YIM 38 (Jiang *et al.*, 2007) at 28 °C, on which it developed a milk–white substrate mycelium, but no aerial hyphae.

The cultural characteristics of strain YIM 48868$^T$ were determined by using 14 day-old cultures growing on ISP (International *Streptomyces* project) 2, ISP 3, ISP 4 and ISP 5 media (Shirling & Gottlieb, 1966), Czapek's agar (Pridham & Lyons, 1980), potato agar and nutrient agar at 28 °C. The colony colour was determined with ISCC-NBS colour charts (Kelly, 1964). Cell morphology was observed under a light microscope (BH-2; Olympus) and a scanning electron microscope (XL30; Philips) after incubation at 28 °C for 12 and 28 days on ISP 2 medium. Strain YIM 48868$^T$ grew on all media tested except for nutrient agar and ISP 4, and showed grey–white to milk–white substrate mycelium. No diffusible pigments were produced. Sparse aerial hyphae were observed on ISP 2 medium. No aerial hyphae were observed on any of the other media. The cell morphology (Fig. 1) was characteristic of the genus *Actinopolymorpha*, exhibiting variable shape, lateral budding and swelling on the top of the branched hyphae.

The physiological characteristics were determined as described by Gordon *et al.* (1974). Tolerance of different NaCl concentrations (1, 3, 5, 7, 8, 10, 15, 20 %, w/v) was tested on ISP 2 as the basal medium. Growth was tested

Abbreviation: ISP, International *Streptomyces* project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 48868$^T$ is EU706350.

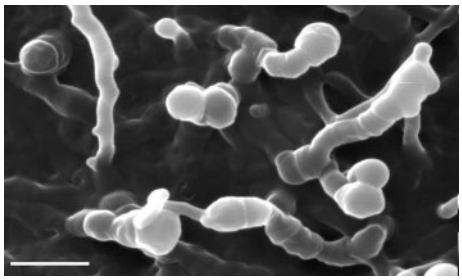

**Fig. 1.** Scanning electron micrograph of cells of strain YIM 48868$^T$ grown on ISP 2 agar for 12 days at 28 °C. Bar, 2 μm.

over a range of temperatures (4, 10, 15, 20, 28, 37, 45, 55, 65 °C) and at different pH values (pH 4–10) as described by Xu *et al.* (2005). Enzyme activities were determined by using the API ZYM test system (bioMérieux) according to the manufacturer's recommendations. Catalase and oxidase activities were detected according to the method of Wang *et al.* (2008). Growth occurred at pH 6.0–7.0. Strain YIM 48868$^T$ was positive for catalase, oxidase and gelatin hydrolysis, but negative for milk coagulation and peptonization, reduction of nitrate, starch degradation and cellulose hydrolysis. The physiological characteristics that differentiate strain YIM 48868$^T$ from recognized species of the genus *Actinopolymorpha* are shown in Table 1. Other physiological characteristics are given in the species description.

Analysis of the characteristic diamino acid of the cell-wall peptidoglycan and determination of the whole-cell sugar pattern were carried out as described by Staneck & Roberts (1974). Polar lipids were extracted by the method of Minnikin *et al.* (1979) and identified by two-dimensional TLC (Collins & Jones, 1980). Menaquinones were extracted according to Collins *et al.* (1977) and were analysed by HPLC as described by Tamaoka *et al.* (1983). Cells used for fatty acid analysis were harvested from TSA plates [3 % (w/v) trypticase soy broth (BBL); 1.5 % (w/v) Bacto agar (Difco)] incubated for 5 days at 28 °C and the analysis was performed by using the standard MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of the genomic DNA was determined by HPLC according to Mesbah *et al.* (1989). The diamino acid of the cell wall was LL-diaminopimelic acid. The whole-cell sugar pattern (type C) included glucose, rhamnose and ribose. The phospholipids were of type PI, including diphosphatidylglycerol,

**Table 1.** Differential characteristics between strain YIM 48868$^T$ and the recognized species of the genus *Actinopolymorpha*

Strains: 1, YIM 48868$^T$; 2, *A. singaporensis* KCTC 19907$^T$; 3, *A. rutila* YIM 45725$^T$. Data for reference strains are taken from Wang *et al.* (2001, 2008). +, Positive; −, negative.

| Characteristic | 1 | 2 | 3 |
|---|---|---|---|
| Substrate mycelium colour | | | |
| ISP 2 agar medium | Milk white | Brilliant orange | Deep orange–yellow |
| ISP 3 agar medium | Light grey–white | Yellow | Brilliant orange–yellow |
| NaCl tolerance up to | 7 % | 15 % | 5 % |
| Growth temperature (°C) | 10–45 | 25–37 | 15–37 |
| Milk coagulation and peptonization | − | + | − |
| Reduction of nitrate | − | + | − |
| Utilization as carbon source: | | | |
| D-Arabinose | + | − | + |
| Cellobiose | − | − | + |
| D-Fructose | − | + | + |
| D-Galactose | − | + | − |
| *myo*-Inositol | − | + | − |
| Lactose | − | − | + |
| D-Mannitol | − | + | − |
| Raffinose | + | − | + |
| Sorbitol | − | + | − |
| Sucrose | − | + | + |
| Xylitol | − | + | + |
| Predominant menaquinone(s) | MK-9($H_6$), MK-9($H_8$), MK-10($H_6$), MK-10($H_8$) | MK-9($H_6$), MK-9($H_4$), MK-9($H_8$), MK-10($H_4$) | MK-9($H_4$), MK-9($H_6$), MK-9($H_8$), MK-10($H_4$) |
| Major fatty acids | iso-$C_{15:0}$ (28.81 %), anteiso-$C_{15:0}$ (14.46 %), 3-OH-iso-$C_{14:0}$ (11.75 %), iso-$C_{16:0}$ (7.1 %), iso-H-$C_{16:1}$ (6.58 %) | iso-$C_{15:0}$ (33.32 %), iso-$C_{16:0}$ (19.39 %), iso-H-$C_{16:1}$ (16.04 %) | iso-$C_{15:0}$ (22.09 %), iso-$C_{16:0}$ (22.36 %), iso-H-$C_{16:1}$ (21.57 %) |
| DNA G+C content (mol%) | 66.6 | 69.5 | 67.7 |

phosphatidylglycerol, phosphatidylinositol mannosides and phosphatidylinositol; all of which are characteristic for the genus *Actinopolymorpha*. The other chemotaxonomic features (shown in Table 1) were different from the type strains of the genus *Actinopolymorpha*, which confirmed that strain YIM 48868$^T$ represents a novel species. The G+C content of the genomic DNA was 66.6 mol%.

Extraction of genomic DNA and amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Phylogenetic analysis was performed using the MEGA software package, version 3.1 (Kumar *et al.*, 2004) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using bootstrap values based on 1000 replications. It is evident from Fig. 2 that strain YIM 48868$^T$ consistently formed a single cluster within the genus *Actinopolymorpha*, supported by a high bootstrap value (100 %). 16S rRNA gene sequence similarity calculations revealed that strain YIM 48868$^T$ shared 96.8 % and 97.0 % similarity with the type strains of *A. singaporensis* and *A. rutila*, respectively. DNA–DNA hybridization was performed according to the methods described by He *et al.* (2005). The low DNA–DNA hybridization values with *A. singaporensis* KCTC 19907$^T$ (48.7 %) and *A. rutila* DSM 18448$^T$ (53.1 %), indicated that strain YIM 48868$^T$ was distinct from the recognized species of the genus *Actinopolymorpha*.

The phylogenetic analysis, peptidoglycan type, sugar patterns and polar lipid data revealed that strain YIM 48868$^T$ belongs to the genus *Actinopolymorpha*. The phenotypic characteristics of the novel strain, including colony colour (milk–white) and growth temperature (10–45 °C), utilization of some carbon sources, major fatty acids [iso-$C_{15:0}$, (28.81 %), anteiso-$C_{15:0}$, (14.46 %), 3-OH-iso-$C_{14:0}$ (11.75 %), iso-$C_{16:0}$, (7.1 %) and iso-H-$C_{16:1}$ (6.58 %)] and the predominant menaquinones [MK-9($H_6$), MK-9($H_8$), MK-10($H_6$) and MK-10($H_8$)] (shown in Table 1) were very different from the recognized species of the genus *Actinopolymorpha*. On the basis of this study, it is suggested that strain YIM 48868$^T$ represents a novel species of the genus *Actinopolymorpha* for which the name *Actinopolymorpha alba* sp. nov. is proposed.

## Description of *Actinopolymorpha alba* sp. nov.

*Actinopolymorpha alba* (al′ba. L. fem. adj. *alba* white, referring to the white substrate mycelium).

Forms a milk–white substrate mycelium. Sparse white aerial hyphae occur on ISP 2 medium. No pigment is produced on any of the agars tested. Good growth occurs on all agars tested at 28 °C except nutrient agar and ISP 4. Cells show variable shapes. Gram-positive, strictly aerobic. Catalase- and oxidase-positive. Grows at 10–45 °C, pH 6.0–7.0 and in the presence of up to 7 % NaCl (w/v) on ISP 2 medium. Positive for gelatin hydrolysis, but negative for nitrate reduction, milk coagulation and peptonization, starch hydrolysis and cellulose hydrolysis.

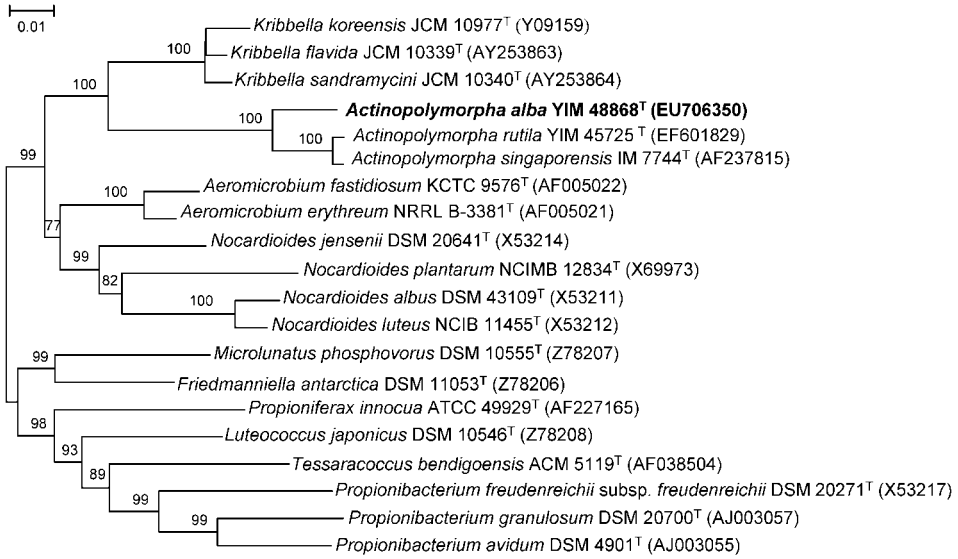


**Fig. 2.** Phylogenetic tree showing the recognized species of the genus *Actinopolymorpha* and strain YIM 48868$^T$, based on 16S rRNA gene sequences available from GenBank/EMBL/DDBJ (accession numbers are given in parentheses), constructed after multiple alignment of data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using the MEGA version 3.1 software package (Kumar *et al.*, 2004). Bootstrap percentages based on 1000 replications are given at branch points. Bar, 0.01 nucleotide substitutions per nucleotide position.

The type strain can use dextrin, fucose, maltose, mannose and ribose as sole carbon sources, but cannot utilize glycerol, L-rhamnose or D-xylose. Other carbon source utilization data are given in Table 1. Hydrolyses L-alanine, L-asparagine, L-proline, L-phenylalanine, L-tyrosine and L-valine, but not L-arginine, L-histidine, hypoxanthine, urea or xanthine. Positive for the enzymes α-chymotrypsin, esterase (C4), β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, but negative for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase lipase (C8), α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), trypsin and valine arylamidase. The cell-wall peptidoglycan contains LL-diaminopimelic acid as the diagnostic diamino acid. Glucose, rhamnose and ribose are present in the whole cell hydrolysate. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides and phosphatidylinositol. The predominant menaquinones are MK-9($H_6$), MK-9($H_8$), MK-10($H_6$) and MK-10($H_8$). Major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, 3-OH-iso-$C_{14:0}$, iso-$C_{16:0}$ and iso-H-$C_{16:1}$.

The type strain, YIM 48868$^T$ (=CCTCC AA 208030$^T$ =DSM 45243$^T$), was isolated from a soil sample collected in Yunnan Province, south-west China. The DNA G+C content of the type strain is 66.6 mol%.

## Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (No. 30560001, 30600001), the International Cooperative Program of the Ministry of Science of Technology, P. R. China (2006DFA33550) and the Yunnan Provincial International cooperative Program (No. 2005GH21).

## References

**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.

**Gauze, G. F., Preobrazhenskaya, T. P., Sveshnikova, M. A., Terekova, L. P. & Maksimova, T. S. (1983).** *Opredelitel' Aktinomycetov: Rody Streptomyces, Streptoverticillium.* Chainia, Moscow: Izd. Nauka (in Russian).

**Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H. N. (1974).** *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

**He, L., Li, W., Huang, Y., Wang, L. M., Liu, Z. H., Lanoot, B. J., Vancanneyt, M. & Swings, J. (2005).** *Streptomyces jietaisiensis* sp. nov., isolated from soil in northern China. *Int J Syst Evol Microbiol* **55**, 1939–1944.

**Jiang, Y., Tang, S. K., Wiese, J., Xu, L. H. & Imhoff, F. & Jiang, C. L. (2007).** *Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces*. *Int J Syst Evol Microbiol* **57**, 2694–2698.

**Kämpfer, P. & Kroppenstedt, R. M. (1996).** Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

**Kelly, K. L. (1964).** *Inter-Society Color Council–National Bureau of Standards Color Name Charts Illustrated with Centroid Colors.* Washington, DC: US Government Printing Office.

**Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

**Li, W.-J., Xu, P., Schumann, P., Zhang, Y.-Q., Pukall, R., Xu, L.-H., Stackebrandt, E. & Jiang, C.-L. (2007).** *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

**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. & Lyons, A. J. (1980).** Methodologies for Actinomycetales with special reference to streptomycetes and streptoverticillia. In *Actinomycete Taxonomy*, pp. 153–224. Special publication no. 6. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society for Industrial Microbiology.

**Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

**Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

**Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl Microbiol* **28**, 226–231.

**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., 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.

**Wang, Y. M., Zhang, Z. S., Xu, X. L., Ruan, J. S. & Wang, Y. (2001).** *Actinopolymorpha singaporensis* gen. nov., sp. nov., a novel actinomycete from the tropical rainforest of Singapore. *Int J Syst Evol Microbiol* **51**, 467–473.

**Wang, Y. X., Zhang, Y. Q., Xu, L. H. & Li, W. J. (2008).** *Actinopolymorpha rutila* sp. nov., isolated from a forest soil. *Int J Syst Evol Microbiol* **58**, 2443–2446.

**Xu, P., Li, W. J., Tang, S. K., Zhang, Y. Q., Chen, G. Z., Chen, H. H., Xu, L. H. & Jiang, C. L. (2005).** *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family *Oxalobacteraceae* isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.