Systematic Research on Actinomycetes Selected according to Biological Activities

Dissertation

Submitted in fulfillment of the requirements for the award

of the Doctor (Ph.D.) degree of the

Math.-Nat. Fakultät

of the Christian-Albrechts-Universität in Kiel

By MSci. - Biol. Yi Jiang

Leibniz-Institut für Meereswissenschaften,
IFM-GEOMAR, Marine Mikrobiologie,
Düsternbrooker Weg 20, D-24105 Kiel, Germany

Supervised by

Prof. Dr. Johannes F. Imhoff

Kiel 2009

Referent: Prof. Dr. Johannes F. Imhoff

Korreferent: _____

Tag der mündlichen Prüfung: Kiel, _____

Zum Druck genehmigt: Kiel, _____

Summary

Content

Summary

Actinomycetes (Actinobacteria) are the group of bacteria producing most of the bioactive metabolites. Approx. 100 out of 150 antibiotics used in human therapy and agriculture are produced by actinomycetes. Finding novel leader compounds from actinomycetes is still one of the promising approaches to develop new pharmaceuticals. The aim of this study was to find new species and genera of actinomycetes as the basis for the discovery of new leader compounds for pharmaceuticals. Unique and extreme habitats as a source for Actinomycetes combined with variation in sample treatment and isolation media have been shown to yield a great diversity of Actinomycetes including a number of new species and genera.

990 soil and sediment samples were collected from primeval tropical rain forest, secondary forests, mangroves, saline and alkaline soil and lakes in five Provinces, P. R. China and Baltic Sea. 4127 strains of actinomycetes were isolated by using various pretreatment procedures and different media. In total, 230 strains were selected for taxonomic research, among these 26 strains with anti-cancer activities, 22 with PKS genes, 32 with enzymes inhibition, 14 from salt and alkaline environments, 136 rare actinomycetes.

On the basis of partial sequences of the 16S rRNA gene, these 230 strains were identified at the genus level and found to belong to 40 genera. A selection of 62 strains was further characterized by almost complete sequences and by polyphasic taxonomic procedures. Eight new species were published previously. Two new genera, *Actinomycetospora chaingmaiensis* sp.nov. and gen. nov. and *Planosporangium flavogriseum* sp. nov. and gen. nov. and two new species, *Streptomyces hainanensis* sp. nov. and *Promicromonospora flava* sp. nov., were described in this thesis.

In order to find un-known actinomycetes for providing the new sources for discovery of new leader compounds. First, test samples should be collected from primeval and special or extreme habitats; second, Actinomycetes should be isolated by using new, specific and variable procedures; third, Isolates are characterized by using fast identifying methods, especially a part of sequencing of 16S rRNA genes.

Zusammenfassung

Actinomyceten (Aktinobakterien) sind die Gruppe der Bakterien, die die meisten bioaktiven Metabolite produzieren. Circa 100 der 150 Antibiotika, gebräuchlich in der Humanmedizin und der Agrarwirtschaft, werden von Aktinomyceten produziert. Das Finden neuer Leitstrukturen von Aktinomyceten zu ist immer noch ein viel versprechender Ansatz, um neue Medikamente zu entwickeln. Es war Ziel dieser Arbeit, neue Aktinomycetenarten und -gattungen als Grundlage zur Entdeckung neuer Leitstrukturen für Medikamente zu finden. Die Beprobung einzigartiger und extremer Lebensräume als Quelle für neue Aktinomyceten in Kombination mit unterschiedlichen Probenaufarbeitung und Isolationsmedien haben zur Entdeckung einer großen Vielfalt von Aktinomyceten, darunter auch neue Arten und Gattungen, geführt.

990 Boden- und Sedimentproben wurden aus tropischen Primärregenwäldern, Sekundärwäldern, Mangroven, salzigen und alkalischen Böden, sowie Seen in fünf Provinzen der Volksrepublik China gesammelt und Batic sea. 4127 Aktinomycetenstämme wurden durch Verwendung verschiedener Vorbehandlungsprozeduren und unterschiedlicher Kulturmedien isoliert. Insgesamt 230 Stämme wurden für taxonomische Untersuchungen ausgewählt. Unter diesen befanden sich 26 Stämme mit Anti-Krebswirkung, 22 mit PKS Genen und 32 mit Enzyminhibiton. 14 wurden aus Salz- und Alkaliumgebungen isoliert und 136 waren seltene Stämme.

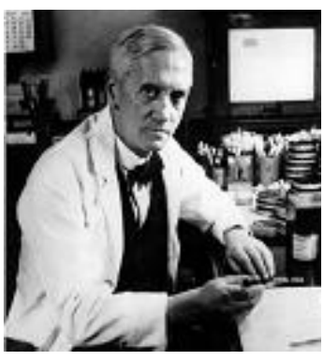
Auf der Grundlage von Teilsequenzen des 16S-rRNA Gens wurden diese 230 Stämme auf dem Gattungslevel identifiziert. Es wurde festgestellt, dass diese zu 40 verschiedenen Gattungen gehören. Eine Auswahl von 62 Stämmen wurde durch fast komplette 16S-rRNA Sequenzen und polyphasischen Taxonomieverfahren weiter charakterisiert. Fünf Beschreibungen neuer Arten wurden bereits veröffentlich. Zwei neue Gattungen, *Actinomycetospora chaingmaiensis* sp. nov. und gen. nov. *Planosporangium flavogriseum* sp. nov. und gen. nov. sowie zwei neue Arten, *Streptomyces hainanensis* sp. nov. und *Promicromonospora flava* sp. nov. wurden in dieser Arbeit beschrieben.

Um unbekannte Aktinomyceten als Quelle neuer Leitsubstanzen zu finden, sollen: Erstens; Proben von unberührten und besonderen, oder extremen Standorten genommen werden. Zweitens; Aktinomyceten durch neue, spezifische und variable Methoden isoliert werden. Drittens; Isolate mit schnellen Identifikationsmethoden, wie zum Beispiel 16S-rRNA Gen Stammbäumen, charakterisiert werden.

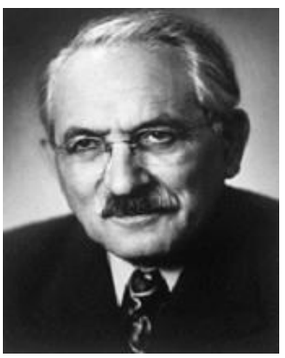
Chapter 1 Introduction Actinomycete resources and systematic

1.1 *Actinomycete* resources

The *Actinomycetes* were defined as Gram-positive bacteria with over 50 mol % of G+C (Xu *et al.* 2007). *Actinomycetes* (or *Actinobacteria*) provide important natural resources. It has been paid a great attention owing to their production of various natural drugs and other bioactive metabolites, including enzymes inhibitors and enzymes. S. A. Waksman discovered streptomycin from a *Streptomyces* in 1945, so as to promote the development, utilization and taxonomy of actinomycete resources in the world.



Alexander Fleming



Selman A. Waksman

1.1.1 Bioactive metabolites from *Actinomycetes*

The total number of discovered compounds was only 10-20 in 1940, 300-400 in 1950, approximately 800-1,000 in 1960 and 2,500 antibiotics were already known in 1970. From that time the total number of known bioactive microbial metabolites has almost doubled in every ten years. About 5,000 in 1980, 10,000 in 1990 and almost 20,000 antibiotic compounds were known in 2000. Over 22,000 bioactive secondary metabolites (including antibiotics) were published in the scientific and patent literature by the end of 2002.

Among microorganisms which produce bioactive metabolites, other bacteria produced about 3,800, *Actinomycetes* produced about 10,100; fungi produced 8,600 (Table 1-1).

Table 1-1 Approximate number of bioactive microbial metabolites according to their producers and bioactivities (Bérdy, 2005)

Source	Bioactive secondary microbial metabolites				
	Antibiotics		Bioactive metabolites		Total bioactive metabolites
	Total	(with other activity)	no antibiotic activity	(antibiotics plus "other bioactives")	
Bacteria	**2900**	**(780)**	**900**	**(1680)**	**3800**
Eubacteriales	2170	(570)	580	(1150)	2750
Bacillus sp.	*795*	(*235*)	*65*	(*300*)	*860*
Pseudomonas sp.	*610*	(*185*)	*185*	(*370*)	*795*
Myxobacter	400	(130)	10	(140)	410
Cyanobacter	300	(80)	340	(420)	640
Actinomycetales	**8700**	**(2400)**	**1400**	**(3800)**	**10100**
Streptomyces sp.	6550	(1920)	1080	(3000)	7630
Rare actinos	2250	(580)	220	(800)	2470
Fungi	**4900**	**(2300)**	**3700**	**(6000)**	**8600**
Microscopic fungi	3770	(2070)	2680	(4750)	6450
Penicillium/*Aspergillus*	1000	(450)	950	(1400)	1950
Basidiomycetes	1050	(200)	950	(1150)	2000
Yeasts	105	(35)	35	(70)	140
Slime moulds	30	(5)	20	(25)	60
Total Microbial	**16500**	**(5500)**	**6000**	**(11500)**	**22500**
Protozoa	35	(10)	5	(45)	50

Notwithstanding the recent descent, the predominant part, 45% of the presently known bioactive microbial metabolites, over 10,000 compounds have been isolated from various *Actinomycetales* species. Among the isolates which produced bioactive metabolites the dominant were, the *Streptomyces* species producing 7,600 compounds (74% of all *Actinomycetales*), while the rare *Actinomycetes* represents 26%, altogether 2,500 compounds. In this group *Micromonospora*, *Actinomadura*, *Streptoverticillium*, *Actinoplanes*, *Nocardia*, *Saccharopolyspora* and *Streptosporangium* species are the most common producers. Several hundred antibiotics are known from each of these genera. In Table 1-2 the numbers of *Actinomycetales* species, including the rare *Actinomycetes* known to produce bioactive metabolites, are summarized.

Over 2/3 of antibiotics which were applied as therapeutic agents, such as aminoglycosides, anthracycline, β-lactams, chloramphenicols, polyketides, macrolides and tetracyclines were produced by *Actinomycetes*. Microbial products share 45% of

world market of pharmaceuticals. Marketing of polyketides medicines only, including the macrolides erythromycin, rifampin and tetracyclines hit 150 million dollars in 2001, based on a statistic of National Cancer Center of America.

Table 1-2 Data of bioactive metabolites produced by *Actinomycetales* (**Bérdy,** 2005)

Streptomycetaceae		***Nocardiopsaceae***		*Proactinomyces*	14
Streptomyces	8,366	*Nocardiopsis*	41	*Micropolyspora*	13
Actinosynnemataceae		***Nocardiaceae***		*Amycolata*	12
Sacharothrix	68	*Nocardia*	357	*Frankia*	7
Actinosynnema	51	*Rhodococcus*	13	*Westerdykella*	6
Micromonosporaceae		***Streptosporangiaceae***		*Kitasatoa*	5
Micromonospora	740	*Streptosporangium*	79	*Synnenomyces*	4
Actinoplanes	257	*Microbispora*	54	*Sebekia*	3
Dactylosporangium	58	*Microtetraspora*	26	*Elaktomyces*	3
Catenuloplanes	3	*Nonomuraea*	21	*Excellospora*	3
Catellatospora	1	*Planobispora*	10	*Waksmania*	3
Pseudonocardiaceae		*Planomonospora*	2	*Faenia*	3
Saccharopolyspora	131	***Thermomonosporaceae***		*Alkalomyces*	1
Amycolatopsis	120	*Actinomadura*	345	*Thermoactinomyces*	1
Streptoalloteichus	48	*Thermomonospora*	19	*Thermoactinopolyspora*	1
Kibdellosporangium	34	*Spirillospora*	11	*Streptoplanospora*	1
Pseudonocardia	27	**Others**		*Salinospora*	1
Kutzneria	4	*Mycobacterium*	57		
Saccharomonospora	2	*Arthrobacter*	25		
Actinopolyspora	1	*Brevibacterium*	17		

Up to now, *Streptomycetes* are one of the most important producers of novel bioactive compounds. According to *Dr. Strohl in Merck*, one strain often produces different compounds. For example, *Streptomyces antibioticus*, *Streptomyces griseus*, and *Streptomyces hygroscopicus* produce 13, 32 and 46 different compounds respectively; some strains of *Streptomyces hygroscopicus* produce bialophos, hygromycin, spectinomycin, rapamycin, and others.

Today, after the characterization and description of a large number of bioactive

metabolites, the discovery of novel bioactive compounds is becoming more and more difficult. We consider that new species (genera or families) should contain new genes to produce new metabolites. The new metabolites will present a new purpose. Therefore the key point to find new compounds is to obtain new species.

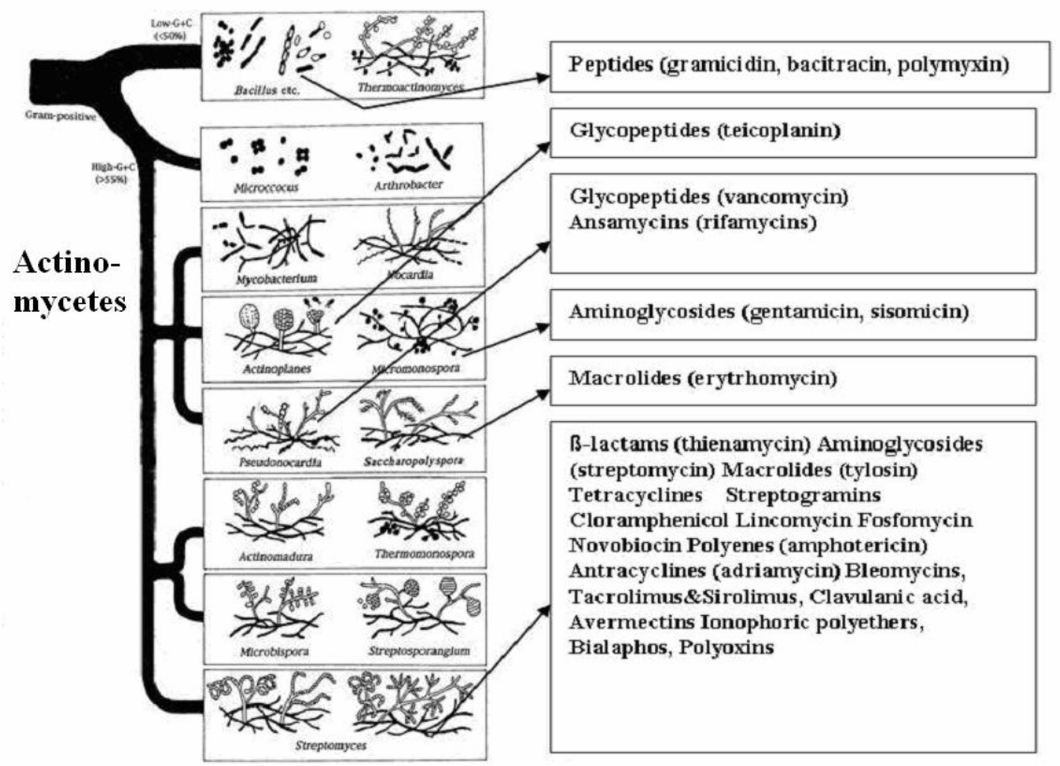


Fig. 1-1 Relationship between *Actinomycetes* and their antibiotic products

1.1.2 *Actinomycetes* in soil

Up to now, *Actinomycetes* contain 40 families and over 170 genera, and about 2,000 species were validly described and published. The genus *Streptomyces* contains about 500 species and named as "**family *Streptomycetaceae***". The rest is named "**rare *Actinomycetes***". Most cultivated Actinomycetes were isolated from soil. As one typical example, the Lechevaliers analysed 16 soil samples. *Streptomyces* is 95.34%, *Nocardia* 1.98%, *Actinomadura* 0.10%, *Thermoactinomyces* 0.14 %, *Microbispora* 0.18 %, *Thermomonospora* 0.22 %, *Microellobospora* (merged into *Streptomyces*), 0.04 %, *Actinoplanes* 0.20 %, *Streptosporangium* 0.10 %, *Nocardiopsis* 0.06 %, *Micropolyspora* 0.10 %, and *Mycobacterium* 0.14 % (Lechevalier *et al.* 1967). Nonomura *et al.* (1957-1988) isolated many *actinomycete* strains from Japanese soil,

and reported the distribution and some new species of *Microbispora*, *Microtetraspora*, *Streptosporangium*, *Thermomonospora,* and *Ampullariella* in Japanese soil in a series articles "Distribution of *Actinomycetes* in soil" (for reference see chaper Discussion). In 1970's, Williams *et al.* studied the ecology of *Actinomycetes* in soil. In the 1980's and 1990's, Jiang *et al.* studied ecological distribution of *actinomycete* isolated from soil samples collected in Yunnan, P. R. China. They related the ecological distribution of *Actinomycetes* to the tropical type, the sub-tropical plateau type, the low temperature mountain type and the snowy mountain type.

1.1.3 Aquatic *Actinomycetes*

Aquatic as an adjective, when applied to organisms and especially to *Actinomycetes*, should be regarded as a vague term. It means *Actinomycetes* living in river, lake and marine environments, despite some of them were eroded from terrestrial habitats.

T. Cross (1981) made a review on aquatic *Actinomycetes*. *Micromonospora* was an indigenous inhabitant of the water and mud from freshwater lakes. High numbers of *Micromonospora* could be isolated from lake-sediments. 10-50 % of the total microbial population in lake water belonged to *Micromonospora*. Nebish Lake had 3,300 bacteria·ml^{-1} of which 15 % was *Micromonospora* and Crystal Lake 3,600 bacteria·ml^{-1} with 16 % *Micromonospora*. *Actinoplanes* with sporangium and zoospores will grow at moist conditions and survive as spore in the dry environment. It colonizes vegetable and animal remains, ranging from pollen and hair to leaves and twigs. Rehydration stimulates the release of zoospores, which swim in the water film of soil or in stream and lake waters until able to recolonize a suitable substrate. Also representatives of *Thermoactinomycetes*, *Rhodococcus* and *Streptomycetes* are living in aquatic environments.

Xu and Jiang *et al.* (1996) studied *actinomycete* populations of 12 lakes in middle plateau of Yunnan. A total of 749 sediment and water samples were collected from 12 lakes of the Middle plateau of Yunnan. The diversity and biological characteristics of the aquatic *Actinomycetes* in these lakes were studied. Seventeen genera of *Actinomycetes* were isolated from these samples. *Micromonospora* assumed a notable

dominance (39 to 89 %) in the *actinomycete* population of these lake sediments. *Streptomyces* was the second most abundant genus. Members of *Actinoplanes, Actinomadura, Microbispora, Micropolyspora, Microtetraspora, Mycobacterium, Nocardiopsis, Nocardia, Promicromonospora, Rhodococcus, Saccharomonospora, Saccharopolyspora, Streptosporangium, Thermoactinomyces, Thermomonospora, and Thermopolyspora* were also isolated. The diversity and counts of *Actinomycetes* varied with the season. Although thermophilic *Actinomycetes* had a wide distribution in these lakes, their counts were small.

1.1.4 Marine *Actinomycetes*

Antibiotics are playing an important role in promoting the civilization and progress of the world. *Actinomycetes* (*Actinobacteria*) are the microoganisms yielding most of the bioactive metabolites. 140 to 160 of antibiotics have been used in human therapy and agriculture. 100 to 120 of them were produced by *Actinomycetes* (Berdy 2005). Research on *Actinomycete* biology has been procured much progress along with blossom of antibiotic industry. However, research efforts on marine *Actinobacteria*, especially in the deep-sea, present considerable technical and logistical difficulties. Accordingly, there is much less information and knowledge on marine *Actinobacteria* than on terrestrial Ones, although the ocean occupies about 71% of surface of the earth and is the larges ecosystem on earth.

Before the 1980's, the progress of research on marine *Actinomycetes* was slow-paced. Early work of Waksman and his co-workers was related to marine *Actinomycetes* (Waksman 1950). In the 1980's, some *Actinomycetes*, *Micromonospora, Rhodococcus* and *Streptomyces* species were isolated and characterized from shallow, inshore and deep-sea habitats. About 500 to 800 propagules·ml^{-1} wet sediment were present in the shallow North Sea on the shelf of the European continent. Up to 400,000 cfu (colony forming units) ml^{-1} were found in sediments collected from Mexican mangrove forest.

Deming and Yager (1992) compiled data on the abundance of bacteria in surface sediments (0-1 cm) over most of the range of ocean depths. Bacterial densities varied from a maximum of ca. 10^{12} ·g^{-1} dry weight (DW) in inter- and sub-tidal sediments to

ca. $10^7 \cdot g^{-1}$ DW in continental rise and abyssal plain sediments. This analysis also revealed that the intensively studied parts of the sea floor at that time were the continental shelf and slope (200-2,000 m) and not the abyssal region; pertinent studies of hadal trench systems (>6,000 m) were rare. Few comprehensive surveys of *actinobacterial* abundance in marine sediments have been made and the current consensus concluded that *Actinobacteria* constituted a minor proportion of cultured bacteria in these sediments (0.06-17%). Occasionally, however, *Actinobacteria* were reported to form a major or dominant component of the bacterial populations both at bathyal and abyssal depths (Goodfellow and Haynes, 1984; Colquhoun *et al.*, 1998).

It has been a disputed question whether *Actinobacteria* were indigenous marine microbiota or their presence in the ocean simple is the consequence of terrestrial run-off. According to Attwell and Colwell (1984) and Goodfellow and Haynes (1984), members of the genus *Thermoactinomyces* are useful markers of terrestrial wash-in and give information about the distribution and survival of terrestrial microorganisms in marine environments. If *Thermoactinomyces* were not detected in a marine sample, isolated *Actinobacteria* in the same sample were considered as indigenous marine organism. According to Matsuda *et al.* (Colquhoun *et al.*, 1998), members of the genera *Nocardia, Rhodococcus, Tsukamurella, Corynebacterium, Dietzia, Gordona and Mycobacterium* were isolated and cultured from deep-sea mud samples collected in the north-western Pacific Ocean. Ward *et al.* (2006) collected sediment samples from Mariana Trench at a depth of 10,898 m with the remotely operated submarine *Kaiko*, using sterilized mud samplers. *Actinobacteria* were isolated with 20 selective isolation media from the sample, and identified by specific primers of *Actinobacteria* with a specific 640 bp amplification product. Thirty-eight *Actinobacteria* were obtained on four of the 20 media used. Most strains (58%) were isolated from raffinose-histidine agar plates, a medium designed for the isolation of rare *Streptomycetes* (Vickers *et al.* 1984). The 38 isolates were assigned to the genera *Dermacoccus, Kocuria, Micromonospora, Streptomyces, Tsukamurella and Williamsia*. The taxonomic relationships of branches composed of the individual isolates were supported by high-bootstrap values (99-100%) that were based on neighbour-joining analyses. Members of the genera *Salinospora* (Mincer *et al.* 2002) and the proposed

genus *'Marimomyces'* (Jensen *et al.* 2004) both of which require seawater for growth and have marine chemotype signatures and *Aeromicrobium marinum* (Bruns *et al.* 2003) which has an obligate requirement for salt. Another recently characterised genus, *Salinibacterium,* tolerates up to 10% NaCl but does not have a salt requirement for growth (Han *et al.* 2003). *Verrucosispora* strain AB-18-032 (Riedlinger *et al.* 2004) also might qualify as an indigenous marine actinobacterium on these latter grounds but further physiological characterization will be necessary to confirm this designation. *Actinobacteria* also have been isolated from the deepest ocean site on Earth, the Challenger Deep of the Mariana Trench, by the JAMSTEC group (Takami *et al.* 1997) who tentatively identified organisms related to *Aureobacterium testaceum*. Up to now, cultured indigenous marine *Actinobacteria* represent at least 18 genera.

1.1.5 *Actinomycetes* derived from extreme environments

Extreme environments especially mean the places are thriving under unusual growth conditions with high and low temperature, salt, alkaline and acidic, radioactivity, high-pressure and space. Microorganisms from extreme environments have received great attention owing to special mechanism of adapting to the extreme conditions in their environments and also due to the production of various natural compounds**.** But in the long term, only few investigations have been performed with *Actinomycetes* under extreme environments. *Actinopolyspora halophila* is an accidentally discovered pioneer. In recent years, researchers of the Actinomycete Laboratority at Yunnan Institute of Microbiology of Yunnan University discovered many novel *Actinomycetes* from salt and alkaline soils in Xinjiang and Qinghai, P. R. China. They described *Yaniaceae* fam. nov. *Streptomonospora* gen. nov., *Jiangella* gen. nov., *Myceligenerans gen. nov., Naxibacter gen. nov.* and several other novel genera, and a great number of new species of the genera *Actinopolyspora, Amycolatopsis, Citricoccus, Halomonas, Isoptericola, Jonesia, Kocuria, Kribbella, Liuella, Marinococcu, Massilia, Microbacterium, Nesterenkonia, Nocardia, Nocardiopsis, Prauserella, Rhodococcus, Saccharomonospora, Saccharopolyspora, Sphingomonas, Streptomyces, Thermobifida,* and *Virgibacillus.* (Xu et al. 2003).

1.1.6 Endophytic *Actinomycetes*

A typical endophytic *actinomycete* is *Frankia* with nitrogen-fixing activity. It plays an important role in ecological system. It is known that 8 families, 24 genera and 200 species of plants produce root nodules with *Frankia* to fix nitrogen. There are 6 families and 8 genera of plants, *Alnus, Casuarina, Coriaria, Dryas, Elaeagus, Hippophae, Myrica,* and *Rubus* produced root nodule (Xu *et al.* 2007). These non-leguminous plants are important to a forestation in dry regions and deserts. Non-leguminous plants and their effective nitrogen-fixation are shown in Table 1-3.

Table 1-3 Nitrogen-fixing by part of non-leguminous plants in China

Plant species	Nitrogen-fixation (kg/hm^2·a)
Alnus crispa	157.0
Alnus glutinosa	56.0
Alnus rubra	300.0
Casuarina deplancheana	58.3
Casuarina equisetifolia	229.0
Ceanothus america)	60.0
Coriaria arborea	150.0
Hippophae rhamnides	27.0-179.0
Myrica gale	30.0

By Huang Jia-Bing *et al.*, 1988.

Taechowisan *et al.* (2003) isolated endo-phytic *Actinomycetes* of 31 species of plants. Of 330 isolates recovered, the majority were *Streptomyces* and the remainder identified as *Microbispora, Nocardia* and *Micromonospora*. Up to now, endo-phytic species of the genera, *Actinomadura, Amycolatopsis, Arthrobacter, Curtobacterium, Dietzia, Glycomyces, Janibacter, Kineosporia, Luteococcus, Microbacterium, Microbispora, Micrococcus, Microlunatus, Micromonospora, Nocardio, Nocardiopsis, Nonomuraea, Promicromonospora, Pseudonocardia, Rhodococcus, Streptomyces*, and *Streptosporangium* have been isolated from plants. Taechowisan's study had shown that 0.6-13.2% of endophytic *Actinomycetes* were strongly inhibitory to *Colletotrichum musae* and *Fusarium oxysporum.* Among the new compounds from endophytic *Actinomycetes* are fistupyrone with anti-fugal activity, munumbicins A, B, C and D

with broad spectrum anti-bacterial activity, and chloropyrrole, chlorinated anthracyclinone with inhibitory activity against drug-resistant bacteria.

1.1.7 Properties of research on *actinomycete* resources

Many authors have used the term "uncultivated microorganisms" to define those microorganisms which could not be isolated in pure cultures for a long time. But authors think, that all microorganisms should be cultivable in principle. Therefore, we suggest to use the term "cultured microorganisms", if cultures have been isolated and purified and "uncultured microorganisms", if they were not isolated in pure culture up to now. The hardly detectable and almost uncultured *Actinomycetales* species, their genetic exploitation and metabolic expression give almost unlimited possibilities. According to Berdy (2005), so far described *Actinomycetes* are only about 4,000 species. However estimated/supposed species are 35,000 to 80,000 in nature. Approximately 90 to 95% of *Actinomycetes* at least are uncultured. In other words, more than 30,000 new *Actinomycetes* are waiting for discovery and characterization. In our view, new species may contain new functional genes for synthesis of new secondary metabolites with potentially new bioactivity. It is very exciting to attempt the discovery of novel leader compounds from new genera and new species. Therefore, the first task is to isolate these uncultured *Actinomycetes*. In the future the great challenge is how to eliminate the influence carried from these known *Actinomycetes* which were isolated and described as well as utilized through selection or contest as fast and as early as possible. In order to isolate uncultured *Actinomycetes*, first, we can use some rigorous and unusual cultural condition, second, we can select samples from primeval environments, such as deep sea and third, we can ceaselessly improve and modify the isolation procedures.

First, in order to get more and more un-known microbes, designing new selective isolation procedures is a central theme. To achieve this, the procedures have already been modified in the past years.

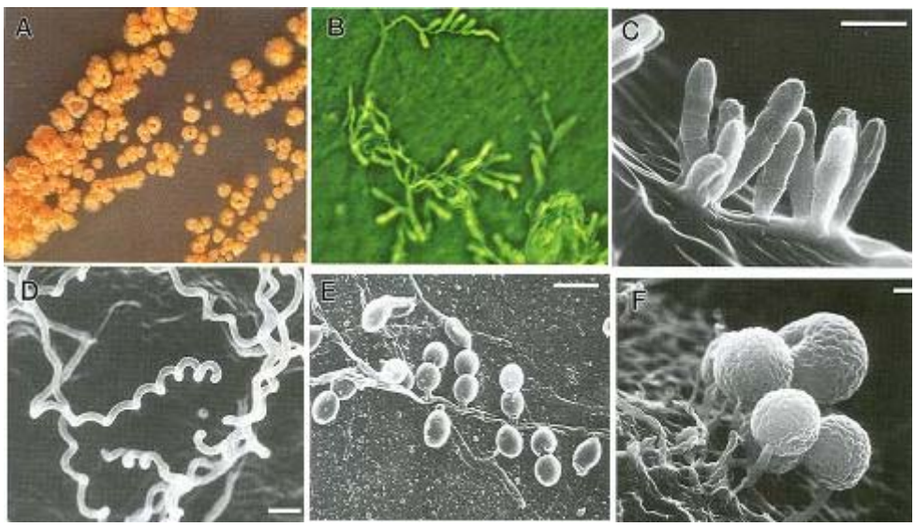
Second, in resent years, more and more new bioactive compounds were found from plant endophytic microbes. Many laboratories change their key interest of research to

these microbes. Therefore, we should study growth, reproduction and genetics of endophytic microbes in host plant, and the relationship and co-evolution between endophytic microbes and host plants at the molecular level.

Third, the oceans are tremendous biological resources. Most of the unknown microbes including *Actinomycetes* are expected to live in the marine environment. Indeed, in recent years, more and more novel bioactive compounds were discovered from marine organisms, and have revealed a huge potential. Many countries have increased the investment and research intensity in this aspect.

Fourth, for enhancing the efficiency of development and utilization of microbial resources, it is necessary to establish a fast system of analysis and identification of new compounds.

1.2 *Actinomycete* systematics



1.2.1 *Actinomycete* systematics in China

In the 1950's *Actinomycete* taxonomy was come up following the establishing of antibiotic industries in China. Founder of Chinese *Actinomycete* taxonomy is Professor Xun-Chu Yan. He and his co-workers focused on isolation and characterization of *Streptomycetes*. In the 1970's Professor Ji-Sheng Ruan and his colleagues started chemical taxonomy, isolation and characterization of rare *Actinomycetes*, especially *Actinoplanes*. In the 1980's Professor Xun-Chu Yan and his co-workers published *Actinoalloteichus*, *Microstreptospora*, *Streptomycoides*; Yun-Peng Lian published *Trichotomospora* and *Parvopolyspora* in Acta Microbiologica Sinica； Prof. Ruan and his colleagues published *Amycolata* Lechevalier and *Amycolatopsis* (Xu *et al.* 2007). Yan (1992) published his famous book on "Classification and Characterization of *Actinomycete*s". He divided the *Actinomycetes* in 14 families, *Actinomycetaceae*, *Actinoplanaceae*, *Dermatophilaceae*, *Frankiaceae*, *Microellobosporiaceae*, *Micromonosporaceae*, *Micropolysporaceae*, *Mycobacteriaceae*, *Mycoplanaceae,* *Nocardiaceae*, *Sporichthyaceae,* *Streptomycetaceae*, *Thermoactinomycetaceae* and *Thermomonosporacea*e.

At the same time, Professor Cheng-Lin Jiang and his colleagues studied the diversity of *Actinomycete*s in various climates, vegetation and soils in Yunnan, and found many new species. In 1990, Jiang and Liu Zhi-Heng start molecular taxonomy. In 2005, Liu

and Jiang published the book, "Modern Biology and Biotechnology of *Actinomycete*s". Yan, Ruan, Jiang and Liu made important contributions to establish and develop the taxonomy of *Actinomycetes*.

1.2.2 Classical Taxonomy

Actinomycete systematics and taxonomy, like all of biological systematics, is to arrange organisms in different taxa based on their similarity, and search after their natural evolutionary relationship at different taxonomic hierarchic levels. It includs classification, identification, phylogenetic analysis and nomenclature based on the International Code of Nomenclature of Bacteria (Sneath, 1992).

Waksman (1945) who discovered the streptomycin, promoted the establishment and developmet of *Actinomycete* taxonomy. At the beginning classification and description of *Actinomycetes* was based on a few characters, morphological (aerial and substrate mycelium, spore, spore chains, sporangium), cultural (colour on different media), and physiological and biochemical characters (various enzyme reaction). In the long term, *Actinomycete* taxonomy focused on *Streptomyces, Nocardia, Actinomycetes, Micromonospora, Actinoplanes* and *Thermoactinomyces*.

1.2.3 Chemotaxonomy

Since 1964, the Lechevaliers established chemotaxonomic criteria based on the achievements of her and other laboratories. They divided the composition of amino acids of cell wall into type I (containing glycine and LL-diamino pimelic acid, i.e DAP), type II (meso-DAP and glycine), type III (meso-DAP), and type IV (meso-DAP, arabinose and galactose). Sugar composition of hydrolysates of whole cells were divided into A, B, C and D (see Table 1-4).

Table 1-4 Sugar patterns of hydrolysates of whole cell of *Actinomycete*s

Sugar pattern	Arabinose	Galactose	Xylose	madurose
A	+	+	-	-
B	-	-	-	+
C	-	-	-	-
D	+	-	+	-

Phospholipids, as components of cell membranes, are considered to be relevant for classification. The Lechevaliers considered five different types of phospholipids compositions (Table 1-5).

In addition, the content of fatty acids, mycolic acids and menaquinones, polyacrylaminde gel electrophoresis of whole cell proteins and bi-directional polyacrylaminde gel electrophoresis of ribosomal protein have been applied in classification and identification of *Actinomycete*s.

Table 1-5 Type of phospholipids of *Actinomycete*s

Phospholipid type	Diagnostic phospholipids
I	None
II	Phosphatidyl ethanolamine (PE)
III	Phosphatidyl choline (PC)
IV	Unknown glucosamine-containing phospholipids (GluNU)
V	Unknown glucosamine-containing phospholipids and phosphatidyl glycerol (PG)

1.2.4 The Class *Actinobacteria*

Actinomycete systematics is dependend on molecular systematic approaches, especially on phylogenetic analysis of 16S rDNA gene sequences. Stackebrandt and colleagues (1997) collected all 16S rRNA/DNA sequences available for members of the *Actinomycete* lineage from the Ribosomal Database Project and the ARB package as well as their own entries. Based on phylogenetic analysis, they proposed to establish the Class *Actinobacteria* and five subclasses, *Acidimicrobidae*, *Actinobacteridae, Coriobacteridae, Rubrobacteridae*, and *Sphaerobacteridae*. The Subclass *Actinobacteridae* has two orders, the *Actinomycetales* and the *Bifidobacteriales*. Membership of a new strain to the class *Actinobacteria* is indicated by 16S rDNA sequence similarity values above 80% as determined by comparison of almost-complete 16S rDNA sequences of the new strain and the most deeply branching members of the class. (Figures 1-2, 1-3)

It was proposed that signature nucleotides of 16S rDNA for the higher taxa were

identified, if they are present in more than 95% of the members of the considered taxon. The signature pattern for mono-specific families or genera must be considered tentative. For example, the pattern of 16S rDNA signature nucleotides of the family *Streptosporangiaceae* is at positions 440-494 (C-G), 445-489 (G-C), 501-544 (C-G), 502-543 (G-C), no extra base between positions 453 and 479, 586-755 (U-G), 613-627 (Pyr-Pur), 681-709 (U-A), 1116-1184 (U-G), 1137 (U), 1355-1367 (A-U), 1436-1465 (GC), and 1422-1478 (G-U). In recent years, the description of higher taxa (genera and families) should include the pattern of signatures nucleotides.

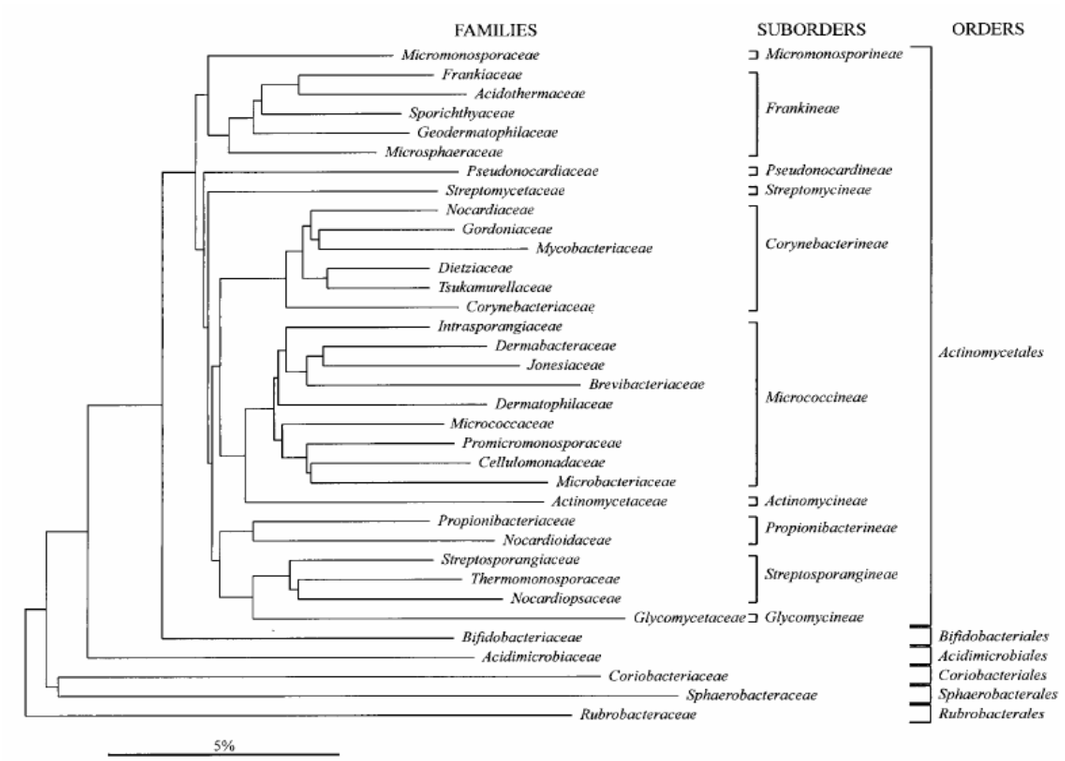


Fig. 1-2 Intraclass relatedness of *Actinobacteria* showing the presence of six orders as well as the 10 suborders of the order *Actinomycetales* based upon 16S rDNA sequence comparison. The phylogenetic relatedness of the families of the class *Actinobacteria* is outlined. The scale bar represents 5 nucleotide substitutions per 100 nucleotides.

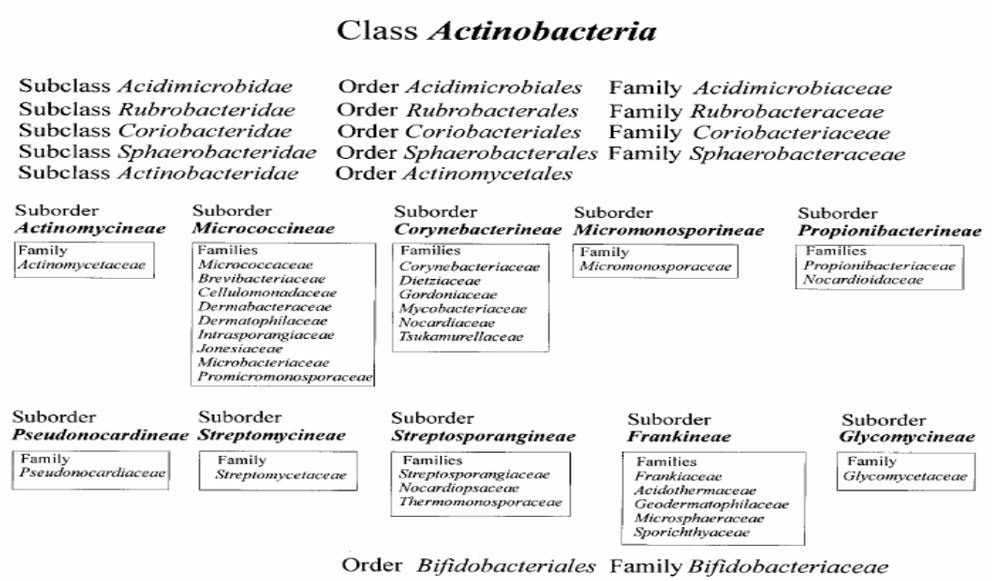


Fig. 1-3 Proposed hierarchic classification system of the class *Actinobacteria* based on the phylogenetic analyses of the 16S rDNA/rRNA sequence data.

1.2.5 The Phylum *Actinobacteria*

The *Actinobacteria* can be recognized on the basis of several molecular signatures.

1. Three new signature molecules

The first molecular signature is found in the cytochrome-c oxidase (Cox1 protein), which is an intrinsic membrane protein, and serves as the terminal enzyme of the respiratory electron transport chain. It is composed of three subunits (Michel *et al.*, 1998). In the Cox1 subunit, a 2 amino acid gap is present in a conserved region. This gap is unique to various actinobacterial species and is not seen in other bacteria (boxed in Fig. 1-4).

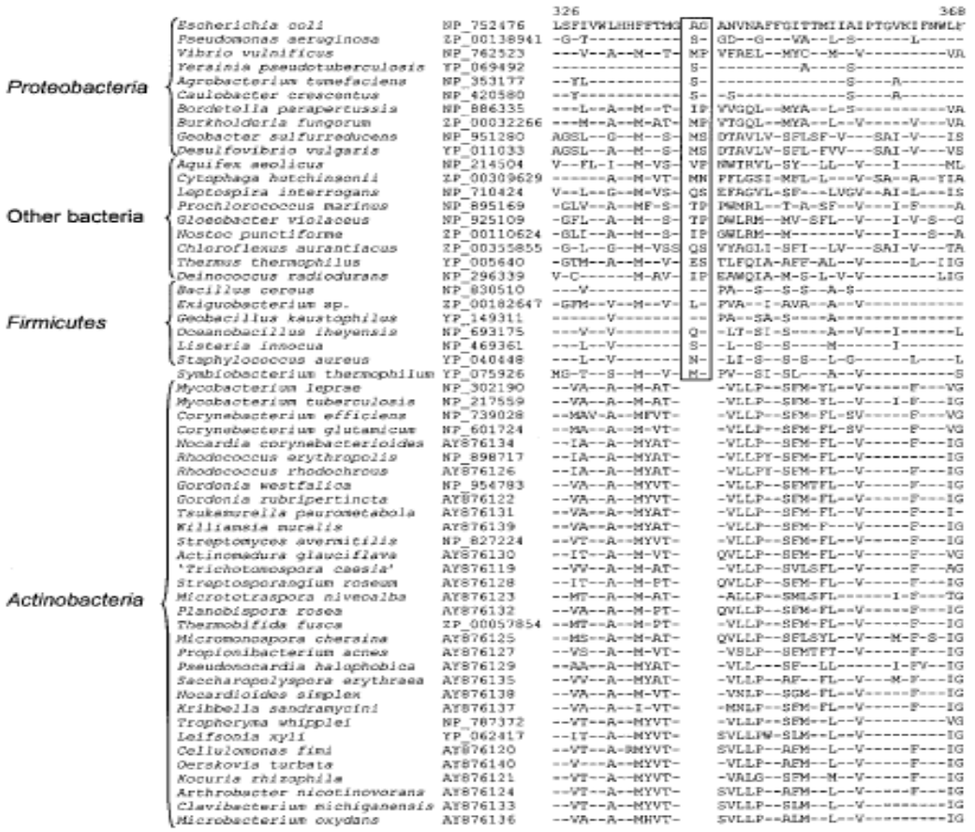


Fig. 1-4 Partial alignment of Cox1 sequences depicting a signature consisting of 2 aa deletion (underneath the boxed region) that is specific for *actinobacteria*. Dashes indicate identity with the amino acid on the top line. Accession numbers are shown in the second column. Only representative sequences from different groups are shown.

The second signature for *actinobacteria* is present in the enzyme CTP synthetase, which catalyses the conversion of UTP into CTP by transferring an amino group to the 4-oxo group of the uracil ring. Except for mycoplasma species, the gene encoding CTP synthetase is present in all other bacterial genomes and has only one copy in the genome. A 10-amino-acid-insert is distinctive of various proteobacterial species. Interestingly, in the same position where this proteobacterial insert is present, a smaller 4 amino acid insert is found in all actinobacterial species except *Tropheryma whipplei*, which contains a 3 aa insert in this position. This insert is again highly specific for *Actinobacteria* and is not present in the CTP synthetase homologues from other bacteria.

The third signature is present in glutamyl-tRNA synthetase (GluRS), which plays an essential role in protein synthesis by charging the glutamyl-tRNA. A 5 amino acid

insert is present in almost all actinobacterial species, with the exception of *Thermobifida fusca*, *Propionibacterium acnes* and *Rubrobacter xylanophilus*, but not found in any other bacterial groups.

2. 23S rRNA signatures.

Roller *et al.* (1992) previously have described a large insert of about 100 nt in the 23S rRNA that was present in a large number of *Actinobacteria*, but not found in other groups of bacteria. The original paper describing this signature included 64 high-G+C-content Gram-positive strains of 22 genera from the currently recognized phylum *Actinobacteria*. Gao and co-colleagues (2005) have successfully amplified and sequenced the 23S rRNA insert region from 13 additional actinobacterial species and genera, covering all of the major groups within *actinobacteria*. An insert of 90 to 100 bp is present in all of the actinobacterial species that we sequenced but it is not found in any other bacterial groups (Fig. 1-5).

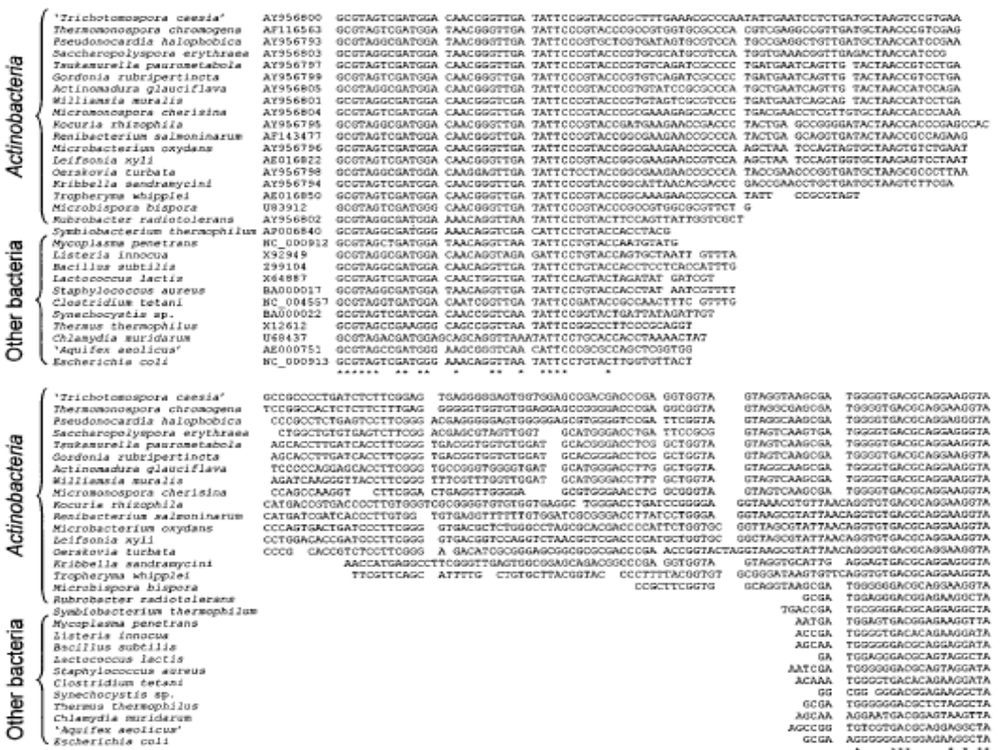


Fig. 1-5 Partial alignment of 23S rRNA gene sequences showing a large insert (99-110 nt) that is specific for *Aactinobacteria*. Positions which are completely conserved are identified by asterisks (*).

3. The composition vector (CV) method.

This method has been proposed to infer phylogenetic relationship from complete genomes of prokaryotes. The method circumvents the ambiguity of sequences of essentially different length and gene content. The topology of the CV tree converges with K increasing and stabilizes at K=5 and 6. CV tree is a K=5 genus tree based on complete genomes available at NCBI by the end of December 2004. Hao Bo (a private letter, 2005) has used a total of 222 organisms, including 21 archaea, 193 bacteria and 8 eukarya, in the calculation. The CV tree supported the SSU rRNA tree of life in overall structure and in detail. It agreed well with the Taxonomic Outline of Prokaryotes (*Bergey´s Manual of Systematic Bacteriology*, Second Edition; http://www.springer.com).

4. 16S rDNA sequences approach.

Phylogenetic trees based on 16S rRNA gene sequences indicate that a long branch is present in *actinobacteria* and other bacteria Fig. 1-6.

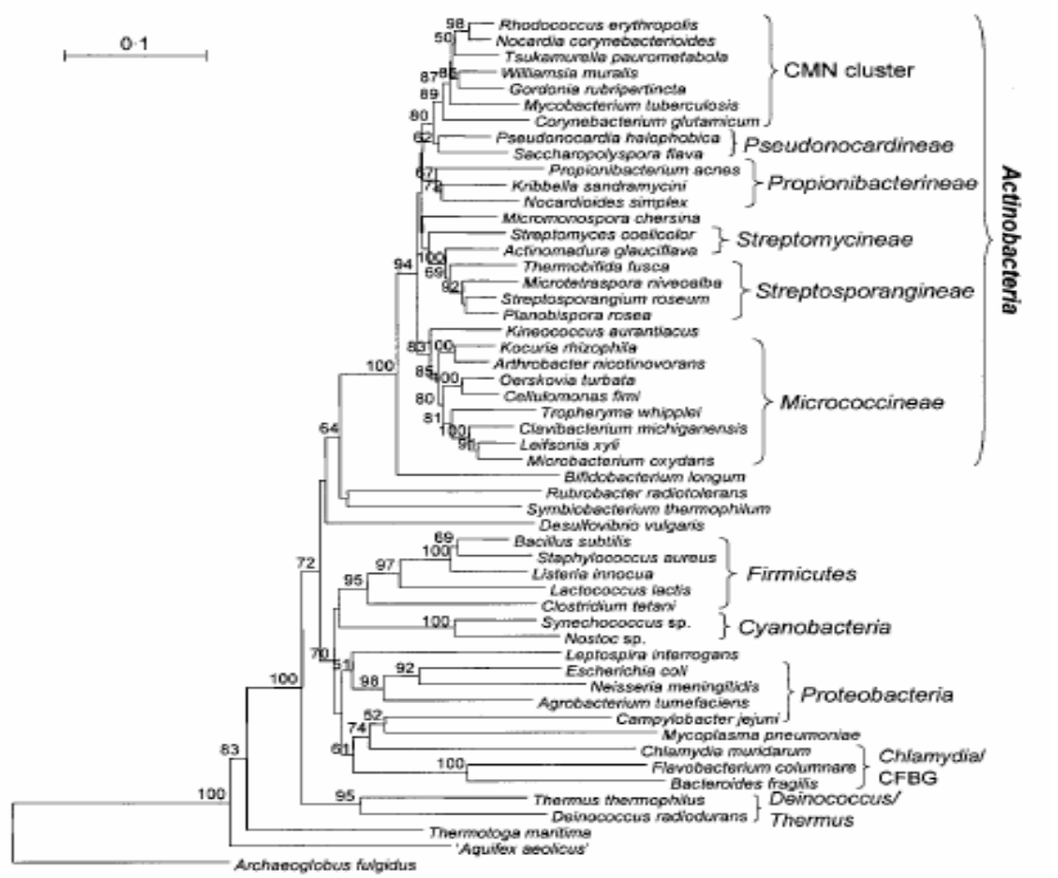


Fig. 1-6 Neighbour-joining distance tree based on 16S rRNA gene sequences. The tree is based on 1400 nucleotides excluding all indels Bootstrap scores greater than 50 are indicated.

Integrating all available evidence, the *Actinobacteria* were defined as Gram-positive bacteria with over 50 mol % of G+C. We agree with *Bergey's Manual of Systematic Bacteriology* (Second Edition) that *Actinomycete*s should be treated in the phylum *Actinobacteria*, in concert with 17 other phyla. The phylum *Actinobacteria* includes the Class *Actinobacteria* only. The class *Actinobacteria* includes five Subclasses, *Acidimicrobidae*, *Rubrobacteridae*, *Coriobactoridae*, *Sphaerobacteridae* and *Acinobacteridae*. Up to now, the *Actinobacteridae* include the orders *Actinomycetales* and *Bifidobacteriales* with 10 suborders, 40 families and 174 genera.

Table 1-6. The higher taxa of *Actinobacteria*

Class I *Actinobacteria*

Subclass I *Acidimicobidae*

Order I *Acidimicrobiales*

Suborder IV *Acidimicrobineae*

Family I ***Acidimicrobiaceae***

Acidimicrobium

Subclass II *Rubrobacteridae*

Order I *Rubrobacterales*

Suborder V *Rubrobacterineae*

Family I ***Rubrobacteraceae***

Rubrobacter, Conexibacter, Solirubrobacter, Thermoleophilum

Subclass III *Coriobacteridae*

Order I *Coriobacterales*

Suborder VI *Coriobacterineae*

Family I ***Coriobacteriaceae***

Coriobacterium, Atopium, Collinsella, Cryptobacterium, Denitrobacterium, Eggerthella, Olensella, Slackia

Subclass IV *Sphaerobacteridae*

Order I *Sphaerobacterales*

Suborder VII *Sphaerobacerineae*

Family I ***Sphaerobacteraceae***

Sphaerobacter

Subclass V *Actinobacteridae*

Order I *Actinomycetales*

Suborder VIII *Actinomycineae*

Family I ***Actinomycetaceae***

Actinomyces, Actinobaculum, Arcanobacterium, Mobiluncus,Varibaculum

Suborder IX *Micrococcineae*

Family I ***Micrococcaceae***

Micrococcus, Arthrobacter, Citricoccus, Kocuria, Nesterenkovia, Renibacterium, Rotaia, Stomatococcus, Yania

Family II ***Bogoriellaceae***

Bogoriella

Family III ***Rarobacteraceae***

Rarobacter

Family IV ***Sanguibacteraceae***

Sanguibacter

Family V ***Brevibacteraceae***

Brevibacterium

Family VI ***Cellulomonadaceae***

Cellulomans, Oerskovia, Tropheryma

Family VII ***Dermabacteraceae***

Dermabacter, Brachybacterium

Family VIII ***Dermatophilaceae***

Dermatophilus, Kineosphaera

Family IX ***Dermacoccaceae***

Dermacoccus, Demetria, Kytococcus

Family X ***Intrasporangiaceae***

Intrasporangium, Arsenicicoccus, Janibacter, Knoellia, Ornithinicoccus, Ornithinimicrobium, Nostocoidia, Terrabacter, Terracoccus, Tetrasphaera

Family XI ***Jonesiaceae***

Jonesia

Family XII ***Microbacteriaceae***

Microbacterium, Agreia, Agrococcus, Aureobacterium, Clavibacter, Cryobacterium, Curtobacterium, Frigoribacterium, Leifsonia, Leucobacter, Mycetocola, Okibacterium, Plantibacter, Rathayibacter, Rhodoglobus, Salinibacterium, Subtercola

Family XIII ***Beutenbergiaceae***

Beutenbergia, Georgenia, Salana

Family XIV ***Promicromonosporaceae***

Promicromonospora, Cellulosimicrobium, Xylanibacterium, Xylanimonas

Suborder X *Corynebacterineae*

Family I ***Corynebacteriaceae***

Corynebacterium

Family II ***Dietziaceae***

Ditzia

Family III ***Gordoniaceae***

Gorgonia, Skermania

Family IV ***Mycobacteriaceae***

Mycobacterium

Family V ***Nocardiaceae***

Nocardia, Rhodococcus

Family VI ***Tsukumurellaceae***

Tsukumurella

Family VII ***Williamsicaceae***

Williamsia

Suborder XI *Micromonosporineae*

Family I ***Micromonosporaceae***

Micromonospora, Actinoplanes, Asanoa, Catellatospora, Catenuloplanes, Couchioplanes, Dactylosporangium, Pilimelia, Spirilliplanes, Verrucosispora, Virgisporangium

Suborder XII *Propionibacterineae*

Family I ***Propionibacteriaceae***

Propionibacterium, Luteococcus, Microlunatus, Propioniferax, Propionimicrobium, Tessaracoccus

Family II ***Nocardioidaceae***

Nocardioides, Aeromicrobium, Actinopolymorpha, Friedmanniella, Hongia, Kribella, Micropruina, Marmoricola, Propionicimonas

Suborder XIII *Pseudonocardineae*

Family I ***Pseudonocardiaceae***

Pseudonocardia, Actinoalloteichus, Actinopolyspora, Amycolatopsis, Crossiella, Kibdellosporangium, Kutzneria, Prauserella, Saccharomonospora, Saccharopolyspora, Streptoalloteichus, Thermabispora, Thermocrispum

Family II ***Actinosynnemataceae***

Actinosynnema, Actinokineospora, Lechevalieria, Lentzea, Saccharothrix

Suborder XIV *Streptomycineae*

Family I ***Streptomycetaceae***

Streptomyces, Kitasatospora, Streptoverticillium

Suborder XV *Streptosporangineae*

Family I ***Streptosporangiaceae***

Streptosporangium, Acrocarpospora, Herbidospora, Microbispora, Microtetraspora, Nonomuraea, Planobispora, Planomonospora, Planopolyspora, Planotetraspora

Family II ***Nocardiopsaceae***

Nocardiopsis, Streptomonospora, Thermobifida

Family III ***Thermomonosporaceae***

Thermomonospora, Actinomadura

References

See Discussion

Chapter 2 Habitats, isolation and identification

1 Habitats

Yunnan is situated in the southwest of China, between latitudes 20°09’ and 29°15’ N and longtitudes 97°39’ and 106°12’ E. This is an area of 436,200 km^2. Yunnan has an extremely varied topography, high in the northwest and low in the southeast. Meili, Taizi, Baima, Haba, and Yulong Snow Mountain tower aloft in the northwest of Yunnan. Kalipo, the main peak, is 6,740 m above sea level. These mountains constitute the Hengduan Mountain Chain in western China. Hekou, a small town on the southeast border, is only 67 m above sea level. Several rivers, the Jinsha, Lancang, and Nu, flow through these mountains from north to south, constituting a vast gorge area. The main part of Yunnan, the Middle Plateau of Yunnan, averages about 2,000 m above sea level and is the most developed area. There, the climate is extremely varied, including all types of climates from the north Helong River to the south Hainan Island of China. There are wide ranges, from tropical rain forests and subtropical and to cool temperate primeval forests, and a half of higher plant species (about 15,000) of China are present in this province. Approx. 65% of animal species of the whole country are found in Yunnan. Therefore, Yunnan has been an ideal place to study plant and animal diversity and lately also has become into focus for studying the biodiversity of microorganisms.

Hainan Island is located on the south border area of China. The land area is 35,400 km^2. The climate there belongs to tropical type. Annual average temperature is 22.3 °C, annual average rains is 1639 mm, and have been named “the natural large green house”. There are wide tropical every green broad-leaf forests and some tropical rain forest with rich diversity of tropical plant.

Xinjiang, Qinghai and Gansu Provinces are located on the northwest border, occupy 1/4 area, far from marine, and one of dry areas in China. There are wide saline and alkaline

soils, saline lakes，like Chaka, Aiding and Talimu, and snow-mountains, like Himalayas, Kunlun and Tian Mountains in the three provinces. The salt content of soil surface in some areas is up to 58 %.

950 soil and sediment samples were collected from primeval tropical rain forest, evergreen broadleaf forest evergreen broadleaf forest，secondary forests, mangroves, saline and alkaline soil and lakes in Yunnan, Hainan, Xinjiang, Qinghai and Gansu Provinces, China. 40 sediment samples were collected from Baltic Sea.

2 Isolation of actinomycetes

2.1. Isolation media and inhibitors

2.1.1 Isolation of rare actinomycetes: Medium 3 (in Appendix) supplemented with filter sterilized solutions of nystatin 100mg/L, nalidixic acid 25mg/L; Medium 11 supplemented with filter sterilized solutions of nystatin 100mg/L, nalidixic acid 25mg/L; Medium 2 supplemented with filter sterilized solutions of cycloheximide 50mg/L; Medium 4 adding vitamins mixture of HV, nystatin 100mg/L, nalidixic acid 25mg/L; Medium 12 with filter sterilized solutions of cycloheximide 50 mg/L, nalidixic acid 10mg/L, novobiocin 10 mg/L and nystatin 100 mg/L.

2.1.2 Isolation of actinomycetes in saline soil samples: Medium12 supplemented with $MgCl_2 \cdot 6H_2O$ 200g/L and trace salt 1ml/L; Medium 6 supplemented with a mixture of salts ($MgCl_2 \cdot 6H_2O$, NaCl, KCl) 150g/L.

2.2.3 Isolation of marine actinomycetes: Medium 8, Medium 9, Medium 10 and Medium 1 in natural sea water 1,000 ml.

All samples were dried at room temperature for 1 week and 120 °C for 1 hour. These treatments can restrain the growth of other bacteria on isolation plates. The samples were added in 0.1% $Na_4P_2O_5$, and treated by ultrasonication (80 W) to release cells of actinomycetes from the soil granules. The isolation protocol is following as.

2.2 Isolation Protocol

Soil (or sediment) samples → dry in room temperature for 1 week → 120°C for 1 hr

↓

0.1% $Na_4P_2O_5$ 18ml, soil 2g，shake for 60 min

↓

80W ultrasonication for 40 sec

↓

Dilute 10, 100, 1000, 0.2ml/dish based on sample source and medium

↓

Incubation at 28°C for 15 up to 35 days

↓

Transfer to YIM 38 agar

3 Identification of actinomycete strains

3.1 Strains

230 selected strains were identified by using polyphasic procedures (protocol). Some typical strains were deposited in the Collection Center of Typical Cultures (CCTCC), Wuhan, P. R. China, DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen (GmbH), Leibniz-Institut für Meereswissenschaften an der Universität Kiel, Germany, and Yunnan Institute of Microbiology, Yunnan University, P. R. China.

3.2 Identification Protocol

Selection of strains

↓

Cultivation

↓

DNA-extraction and analysis

↓

16S rRNA sequences (500 to 750 bp)

↓

Phylogenetic analysis

↓

Possible novel species (if similarity lower than 97 %)

↓

Polyphasic taxonomy

(morphology, physiology, biochemistry, chemistry, phylogenetic analysis)

4. Results

4,127 strains were isolated from the 990 soil and sediment samples by Prof. Xu Lihua and Ms Duan Ruolin using the media described above in Yunnan Institute of Microbiology, Yunnan University. I took part in this isolation work. 124 strains including 14 of strains from saline and alkaline habitats and 110 special strains were selected from the 3,847 strain for characterization in this research.

Activities of extracts from 4,608 strains of actinomycetes were determined by using 6 types of cancer cells in vitro by Oncotest, Germany. 1080 strains were analyzed using PKS gene screening procedures by Dr Ping Xu. Anti-oxidation, AchE (acetylcholine enzyme) inhibition, AGE (Advanced Glycosylation End Products) inhibition and α-glucosidease activity of extracts ferment broths of 2,821 of actinomycete strains were determined by using corresponding models in vitro at Pharmaceutical Institute, Beijing, during the course of my master thesis. 80 strains including 26 strains with anti-cancer activities, 22 with PKS genes and 32 showing enzymes inhibition were selected from these strains for characterization in this research.

280 strains were isolated from the samples collected in Batic Sea. 26 strains were selected for identification.

Partial 16S rRNA gene sequences (600-1100bp) of total 230 selected strains were determined and primarily grouped at a level of genus.

The characterization of the 204 selected strains based on partial 16S rRNA gene sequences are shown in the appedix of this thesis and indicate the number of strains (in bracketes) identified as belonging to the listed 33 genera: *Actinobispora* (2 strains), *Actinomadura* (1), *Actinomycetospora* (1), *Aerococcus* (1), *Arthrobacter* (2), *Bacillus* (6), *Catellatospora* (1), *Chelatococcus* (2), *Chryseobacterium* (1), *Cryobacterium* (1), *Dactylosporangium* (1), *Dietzia* (1), *Duganella* (1), *Glycomyces* (2), *Gordonia* (2), *Isoptericola* (1), *Kitasatospora*

(5), *Kocuria* (1), *Leifsonia* (1), *Methylobacterium* (5), *Micrococcus* (1), *Micromonospora* (12), *Mycobacterium* (2), *Nocardia* (38), *Nocardiopsis* (9), *Nonomuraea* (8), *Paenibacillus* (1), *Planosporangium* (1), *Promicromonospora* (5), *Pseudonocardia* (3), *Rhodococcus* (11), *Streptomyces* (64)，*Streptosporangium* (11)**.** 26 identified strains from marine samples belonged to 13 actinomycete genera, *Promocromonospora*, *Myceligenerans*, *Rhodococcus*, *Agrococcus, Exiguobacterium, Microbacterium, Kocuria, Nocardiopsis, Agrobacterium, Isoptericola, Mycobacterium*, *Actinomadura* and *Amycolatopsis,* and other bacteria, *Phyllobacterium, Zobellia=F.uliginosum, Rhizobium, Chromohalobacter,* , *Pseudomonas, Cellulomonas* and *Bacillus.*

Complete sequences of 16S rRNA of 54 of the 230 strains were determined, and their taxonomy was carried out by using polyphasic taxonomic procedures. Out of these isolates, five have been identified as new species (*Nocardia polyresistens* sp. nov. YIM 33361, *Nocardia lijiangensis* sp. nov. YIM 33378, *Nocardia alba* sp. nov. YIM 30243, *Streptomyces nanningensis* sp.nov. YIM 33098, *Streptomyces roseoalbus* sp.nov. YIM 31634) and have been published (see list of publications nos. 7, 8, 9, 10 and 14). Seven possible new species or new subspecies (*Micromonospora purpureochromogenes* subsp. *hongheensis* subsp. nov. YIM 45197, *Nocardia flava* sp. nov. YIM 45193, *Pseudonocardia flava* sp. nov. YIM 47705, *Streptomyces violaceogriseus* sp. nov. YIM 47663, *Streptomyces xianggelilaensis* sp. nov. YIM 32234，*Streptomyces flavoablus* sp. nov. YIM 32342 and *Streptomyces sclerotialus* sp. nov. YIM 90018) and four known species (*Duganella violaceinigra* YIM 37272, *Nocardia jejuensis* (99.5%) YIM 36723，*Nocardiopsis alba* YIM 90039, and *Streptomyces avermectinius* YIM 31339 and 32196) were characterized (not prepared the manuscripts). CC 0387^{T} from Batic Sea was identified as a new species, *Promicromonospora flava* sp. nov., and published on Int. J. Syst. Evol. Microbiol.

Two new genera (*Actinmycetospora chiangmaiensis* and *Planosporangium flavogriseum*) and two new species (*Streptomyces hainanensis* sp. nov and *Promicromonospora flava* sp. nov.) will be described in chapters 3 to 6 of this thesis.

References

Al-Tai, A. M. & Ruan, J. S. (1994). *Nocardiopsis halophila* sp. nov., a new halophilic actinomycete isolated from soil. *Int J Syst Bacteriol* **44,** 474- 478.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSIBLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25,** 33389-3402.

Al-Zarban, S.S., Abbas, I., Al-Musallam, A.A., Steiner, U., Stackebrandt, E., & Kroppenstedt, R.M. (2002). *Nocardiopsis halotolerans* sp. nov., isolated from salt march soil in Kuwait. *Int J Syst Evol Microbiol* **52**, 525- 529.

Becker, B., Lechevalier, M. P., Gordon, R. E., and Lechevalier, H. A. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl Microbiol* **12,** 421-423.

Berdy, J. (1974). Recent development of antibiotic research and classification of antibiotics according to chemical structure. *Adv Appl Microbiol* **18,** 309-405.

Brock, T.D. (1994). Life at high temperatures. Yellowstone Association for National Science, History & Education, Inc, Wyoming.

Betina, V. (1983). The chemisty and biology of antibiotics. Edited by Mauta, W. Th., and R.F. Rekker, Elsevier Scientific Publishing Company, New York.

Madigan, M.T., and Marrs, B.L. (1997). Extremophiles. Scientific American April, pp.66-70.

Brosiuus, J., M. L. Palmer, J. P. Kennedy, and H. P. Noller. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75,** 4801-4805.

Chun, J., Bae, K. S., Moon, E. Y., Jung, S. O., Lee, H. K. & Kim, S. J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50,** 1909- 1913.

Collins, M.D. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267- 287. Edited by M. Goodfellow & D.E. Minnikin. London, Academic Press.

Colquhoun, J.A., Heald, S.C., Li, L., Tamaoka, J., Kato. C., Horikoshi, K., and Bull, A.T. (1998). Taxonomy and biotransformation activities of some deep-sea actinomycetes. *Extremophiles* **2,** 269-277.

Crueger , W., and A. Crueger. (1990). Biotechnology: A textbook of industrial microbiology, pp. 229-273, Science Tech. Publishers, London.

Cui, X. L., Mao, P. H., Zeng, M., Li, W. J., Zhang, L. P., Xu, L. H. & Jiang, C. L. (2001). *Streptomonospora salina* gen. nov., sp. nov., a new member of the family

Nocardiopsaceae. *Int J Syst Evol Microbiol* **51**, 357- 363.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133- 142.

Demain, A.L. (1975). Why mode of action studies? *Chem. Technol* **5,** 287-289.

Deming, J.W. (1998). Deep ocean environmental biotechnology. *Curr Opin Biotechnol* **9,** 283-287.

Duckworth, A. W., Grant, S., Grant, W. D., Jones, B. E. & Meijer, D. (1998). *Dietzia natronolimnaios* sp. nov., a new member of the genus *Dietzia* isolated from an East African soda lake. *Extremophiles* **2**, 359- 366.

Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei subsp. albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **50,** 73- 81.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368- 376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783- 791.

Groth, I., Schumann, P., Rainey, F.A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Borgoriella caseilytica* gen. nov., sp. nov., a new alkalophilic actinomycete from a soda lake in Africa. *Int J Syst Bacteriol* **47**, 788- 794.

Grund, E., & Kroppenstedt, R.M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5- 11.

Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319- 322.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. & Ward, J. M. (1985). Genetic manipulation of *Streptomyces*- a laboratory manual. In preparation of chromosomal, plasmid and phage DNA, pp. 79- 80. Norwich, F. Crowe & Sons.

Horikoshi, K. (1999). Alkaliphiles: Some applications of their products for biotechnology. *Microbiol Mol Biol Rev* **63**, 735- 750.

Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenus aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141- 145.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21- 132. Edited by H.N. Munro. New York: Academic Press.

Kämpfer, P., Busse, H. & Rainey, F. A. (2002). *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* **52**, 621- 627.

Kelly, K. L. (1964). Inter-society color council-national bureau of standards color name charts illustrated with centroid colors published in US.

Kroppenstedt, R. M. (1992). The genus *Nocardiopsi*s. In *The Prokaryotes*, 2nd edn, pp. 1139- 1156. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York, Springer.

Küster, E. & Williams, S.T. (1964). Production of hydrogen sulphide by streptomycetes and methods for its detection. *Appl Microbiol* **12**, 46- 52.

Ladeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1984). *Saccharothrix*: a new genus of the *Actinomycetales* related to *Nocardiopsis*. *Int J Syst Bacteriol* **34**, 426- 431.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435- 443.

Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes, p. 227-291. In A. Dietz and D.W. Thayer (ed.), *Actinomycete taxonomy*. Special publications no. 6. Society For Industrial Microbiology, Arlington, Va.

Lechevalier, M. P., DeBievre C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249- 260.

Li, M. G., Li, W. J., Xu, P., Cui, X. L., Xu, L. H., & Jiang, C. L. (2003). *Nocardiopsis xinjiangensis* sp. nov., a halophilic actinomycete isolated from a saline soil samples in China. *Int J Syst Evol Microbiol* **53,** 317-321.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195- 206.

Marmur, J., & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol*. **5**, 109-118.

Mandel, M., & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B,** 195-206.

Meyer, J. (1976). *Nocardiopsis,* a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 487- 493.

Mikami, Y., Miyashita, K. & Arai, T. (1982). Diaminopimelic acid profiles of alkalophilic and alkaline-resistant actinomycetes. *J Gen Microbiol* **128**, 1709- 1712.

Mikami, Y., Miyashita, K. & Arai, T. (1986). Alkalophilic actinomycetes. *Actinomycetes* **19**, 176- 191.

Minnikin, D.E., Patel, P.V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104- 117.

Miyashita, K., Mikami, Y. & Arai, T. (1984). Alkalophilic actinomycete isolate, *Nocardiopsis dassonvillei* subsp. *prasina* subsp. nov., isolated from soil. *Int J Syst Bacteriol* **34**, 405- 409.

Orsini, M. & Romano-Spica, V. (2001). A micrwave-based method for nucleic acid isolation from environmental samples. *Lett Appl Microbiol* **33**, 17- 20.

Peltola, J. S. P., Andersson, M. A., Kämpfer, P., Auling, G., Kroppenstedt, R. M., Busse, H. J., Salkinoja-Salonen, M. S. & Rainey, F. A. (2001). Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. *Appl Environ Microbiol* **67**, 4293- 4304.

Rothschild, L.J., and Mancinelli, R. L. (2001). Life in extreme environments. Nature 409: 1092-1102.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406- 425.

Sato, M., Beppu, T. & Arima, K. (1983). Studies on antibiotics produced at high alkaline

pH. *Agric Biol Chem* **47 (9)**, 2019- 2027.

Schippers, A., Bosecker, K., Willscher, S., Spröer, C., Schumann, P. & Kroppenstedt, R.M. (2002). *Nocardiopsis metallicus* sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump. *Int J Syst Evol Microbiol* **52**, 2291- 2295.

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

Stackebrandt, E. & Goebel, B.M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rDNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846- 849.

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

Sun, M. E., Chen, Y. H. & Du, G. H. (2002). High throughput screening method of nitric oxide synthase inhibitors and enhancers. *Acta Pharmaceutica Sinica* (Chinese). **37**, 161-164.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeammnougin, F., & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24,** 4876-4882.

Waksman, S.A. (1961). The actinomycetes, vol. 2. Classification, identification and descriptions of genera and species. The Williams & Wilkins Co., Baltimore.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463- 464.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M.J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Micrbiol* **129**, 1743- 1813.

Yassin, A. F., Galinski, E. A., Wohlfarth, A., Jahnke, K.D., Schaal K. P. & Trüper, H. G. (1993). A new actinomycete species, *Nocardiopsis lucentensis* sp. nov. *Int J Syst Bacteriol* **43**, 266- 271.

Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, & Schaal, K. P. (1997). Description of *Nocardiopsis synnemataformans* sp. nov., elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*. *Int J Syst Bacteriol* **47**, 983- 988.

Chapter 3 *Streptomyces hainanensis* sp. nov., a new member of the genus *Streptomyces*

Yi Jiang,[1, 2] Shu-Kun Tang,[2] Jutta Wiese,[1] Li-Hua Xu,[2] Johannes F. Imhoff [1] and Cheng-Lin Jiang[2]

(**Int. J. Syst. Evol. Microbiol. 57:2694-2698, 2007)**

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

[2] Yunnan Institute of Microbiology, Yunnan University, Kunming, 650091 China.

Authors for correspondence:

Johannes F. Imhoff

Tel: +49-431 600 4450/51; **Fax:** +49-431 600 4452

E-mail: jimhoff@ifm-geomar.de

Li-Hua Xu

Tel: +86-871-5035263; **Fax:** +86-871-5173878

E-mail: lihxu@ynu.edu.cn

Subject category: Gram-positive bacteria

Running title: *Streptomyces hainanensis* gen. nov., sp. nov

The 16S rRNA gene sequences of strain YIM 47672 [T] has been deposited in EMBL under the accession number AM398645.

A new isolate belonging to the genus *Streptomyces,* strain YIM 47672^T, was obtained from a soil sample in Hainan, an island of P. R. China. The characterization of this isolate was performed by a polyphasic approach. The strain forms long spore chains in aerial mycelia, the cell wall contains L-DAP, traces of *meso*-DAP and glycine. Whole-cell hydrolysates contain galactose and xylose, the main phospholipids are PE and DPG. The 16S rRNA gene sequence of the strain was compared with closed species of *Streptomyces*. 16S rRNA sequence similarity values with closest relatives of the genus were less than 95.5 %. *Streptomyces hainanensis* sp. nov. is proposed . Type strain of the new species is YIM 47672^T (= CCTCC AA 205017^T = DSM 41900^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943). Classification of this genus has been attached a great attention owing to their production of various natural products with considerable commercial value. The development of numerical taxonomic systems, which utilized phenotypic traits helped to resolve the intergeneric relationships within the family *Streptomycetaceae* and resulted in the reclassification of six additional genera (*Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa* and *Microellobosporia*) to the *Streptomyces* genus (William et al., 1983; Goodfellow et al., 1986). Despite the genera *Streptomyces* and *Streptoverticillium* having cell-wall type 1 (Lechevalier et al., 1970) are lysed by the same phages (Wellington et al., 1981), and are phylogenetically closely related (Stachebrandt et al., 1981), the two genera were classified to two distinct genera (Witt et al., 1990) concluded based on 16S and 23S rRNA comparisons that the genus *Streptoverticillium* should be regarded as a synonym of *Streptomyces*. In our research on new actinomycete resources, strain YIM 47672^T was identified as a new species of the genus *Streptomyces.* Results of morphological, physiological and chemical studies as well as phylogenetic analysis are reported here and a novel species, *Streptomyces hainanensis* will be proposed.

Strain YIM 47672^T was isolated, using the dilution plating method, from a soil sample collected in evergreen broadleaf forest in Wuzhi Mountain, Hainan province, P. R.

China. Selective isolation was achieved using mycose-proline agar (Jiang *et al*. 2006) and incubation at 28°C for 35 days. Strain YIM 47672^{T} was maintained on YIM 38 medium [malt extracts 10 g, yeast extracts 4 g, glucose 4 g, vitamin mixture (thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, calcium pantothenate, p-aminobenzoic acid, each 0.5 mg, and biotin 0.25 mg)，agar 20 g, pH 7.2] (Hayakawa & Nonomura, 1987) at 4°C and preserved in 20% (v/v) glycerol at -20°C. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (at 180 rpm) using YIM 38 broth at 28°C for 4 to 7 days. Cultural characteristics were determined after 2 weeks at 28°C by methods used in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). Czapek´s agar and nutrient agar were prepared according to Dong & Cai (2001). Morphological properties were examined by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (Philips XL30 ESEM-TMP). Color determination was done with color chips from the ISCC-NBS COLOR CHARTS standard samples No. 2106 (Kelly 1964).

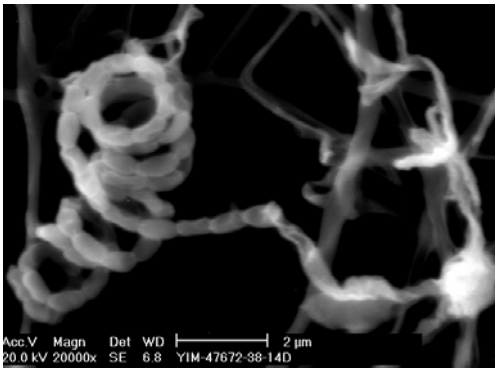


Fig. 1. Scanning electron micrograph of spiral spore chains of strain YIM 47672^{T} after growth on YIM 38 medium for 14 days. Bar, 2 µm.

Strain YIM 47672^{T} was growing well on most of the tested media (Table 1). Aerial mycelia developed well. They were long branched, white and pink white to pink gray. Vegetative mycelia developed well, and were pale to deep orange yellow. Light brown to orange yellowish soluble pigments were produced. Spore chains were spirales or *Retinaculiaperti* (looped in the top) (Fig. 1). Spores were elliptical or short rod-shaped in 0.4-0.6×0.7-1.0 µm. Spore surface was smooth.

Table 1. Cultural characteristics of strain YIM 47672^{T}

Legend: +, poor; ++ moderate; +++, well; -, not present

Medium	Growth	Aerial mycelium		Substrate mycelium		Soluble pigment
		Presence	Color	Presence	Color	
Czapek's agar	Moderate	++	Pink gray	++	Orange yellow	Light brown
Glycerol-asparagines agar(ISP 5)	Poor	-	None	+	Pale yellow	None
Inorganic salts-starch agar (ISP 4)	Moderate	++	Pink white	++	Light reddish brown	Pale orange-yellow
Yeast ext.-malt ext. agar (ISP 2)	Moderate	++	Pink white	++	Brill orange yellow	Orange yellow
Oatmeal agar (ISP 3)	Well	+++	Pale pink	+++	Deep orange yellow	Orange yellow
Nutrient agar	Poor	+	White	+	Soft orange yellow	None

A range of phenotypic properties was examined using standard procedures (Goodfellow, 1971; Williams *et al*. 1983). In addition, acid production from carbohydrates was carried out using media and methods described by Gordon *et al*. (1974). The utilization of carbon and nitrogen sources was determined by using the methods of Gordon & Mihm (1962) and Tsukamura (1966). Gelatine liquefaction, milk coagulation and peptonisation, arginase, phenylalanine deaminase, DNAase and melanin production of strain YIM 47672^{T} were negative. Starch hydrolysation, arginine decarboxylase, nitrate

reduction, gas production from nitrate, growth on cellulose and H_2S production were positive. Growth occurs at pH 6.0-9.0 and 0-10% of NaCl, with optimum at pH 7.0 and without NaCl. Glucose, cellobiose, starch, esculin, mycose, galactoside, and urea were utilized. Acids were not produced from these carbon sources. Galactose, mannose, fructose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, turanose, melicitose, sorbin, dextrin, salicine, adonitol, inositol, mannitol, sorbitol, xylitol, galactitol, erythritol, amygdaloside, sodium citrate, sodium acetate, gluconate, malonate, tartrate, lysine, ornithine and acetamide were not utilized.

Strain YIM 47672^T was resistant to penicillin G (10 IU), amoxicllin/clavulanic acid (20/10 μg), novobiocin (30 μg), rifampin (5 μg) and ampicillin (10 μg), and sensitive to erythromycin (15 IU), gentamicin (10 μg), kanamycin (30 μg), tetracycline (30 μg), vancomycin (30 μg), midecamycin (15 μg), clindamycin (2 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), chloramphenicol (30 μg), polymyxin B (300 IU) and norfloxacin (10 μg).

The amino acid and sugar analysis of whole cell hydrolysates followed procedures described by Stanek & Roberts (1974). Phospholipid analysis was carried out as described by Lechevalier *et al*. (1981). Menaquinones were determined using the procedures of Collins *et al*. (1997). Biomass for the quantitative fatty acid analysis was prepared by scraping cell mass from TSB agar plates [Trypticase soy broth (BBL), 3% (w/v); Bacto agar (Difco), 1.5% (w/v)] that had been incubated for 5 days at 28^{o}C. The fatty acids were extracted, methylated and analysed by using the standard MIDI (Microbial Identification) system (Kämpfer & Kroppenstedt, 1996; Sasser, 1990). Chromosomal DNA of strain YIM 47672^T was extracted as described by Marmur (1961). The DNA G+C base content was determined by HPLC (Tamaoka & Komagata, 1984) with an Agilent 1100 LC system (IRIS Technologies, U.S.A).

The cell wall of strain YIM 47672^T contained LL-diaminopimelic acid (A_2pm), traces of *meso*-diaminopimelic acid and glycine. Whole-cell hydrolysates contained galactose and xylose. Main phospholipids were phosphatidyl ethanolamine and diphosphatidyl glycerol (phospholipid type II). The predominant menaquinones were MK-9 (H_4)

(45.4%), MK-9 (H_6) (14.0%), MK-9（H_8) (13.6%）and MK-10 (H_0) (27.0%). The predominant components were iso $C_{15:0}$ (1.1%), iso $C_{16:0}$ (30.9%), $_{16:1}$ w7c/$_{16:1}$ w6c (2.1%), $C_{16:0}$ (13.7%), iso w9c $C_{17:1}$ (1.4%), iso $C_{17:0}$ (2.8%), anteiso $C_{17:0}$ (10.8%), w8c $C_{17:1}$ (7.1%), cyclo $C_{17:0}$ (1.4%), $C_{17:0}$ (5.6%), anteiso $C_{18:0}$ /$_{18:2}$ w6,9c (9.7%), w9c $C_{18:1}$ (4.9%), $C_{18:0}$ (1.2%). In addition, the sum of $_{16:1}$ w7c/$_{16:1}$ w6c was 2.1%, sum of anteiso $_{18:0}$ /$_{18:2}$ w6,9c was 9.7% and sum of iso w9c C17:1 or $_{16:0}$ 10-methyl was 1.4%.

The G+C content of genomic DNA of the strain was 73.4 mol %.

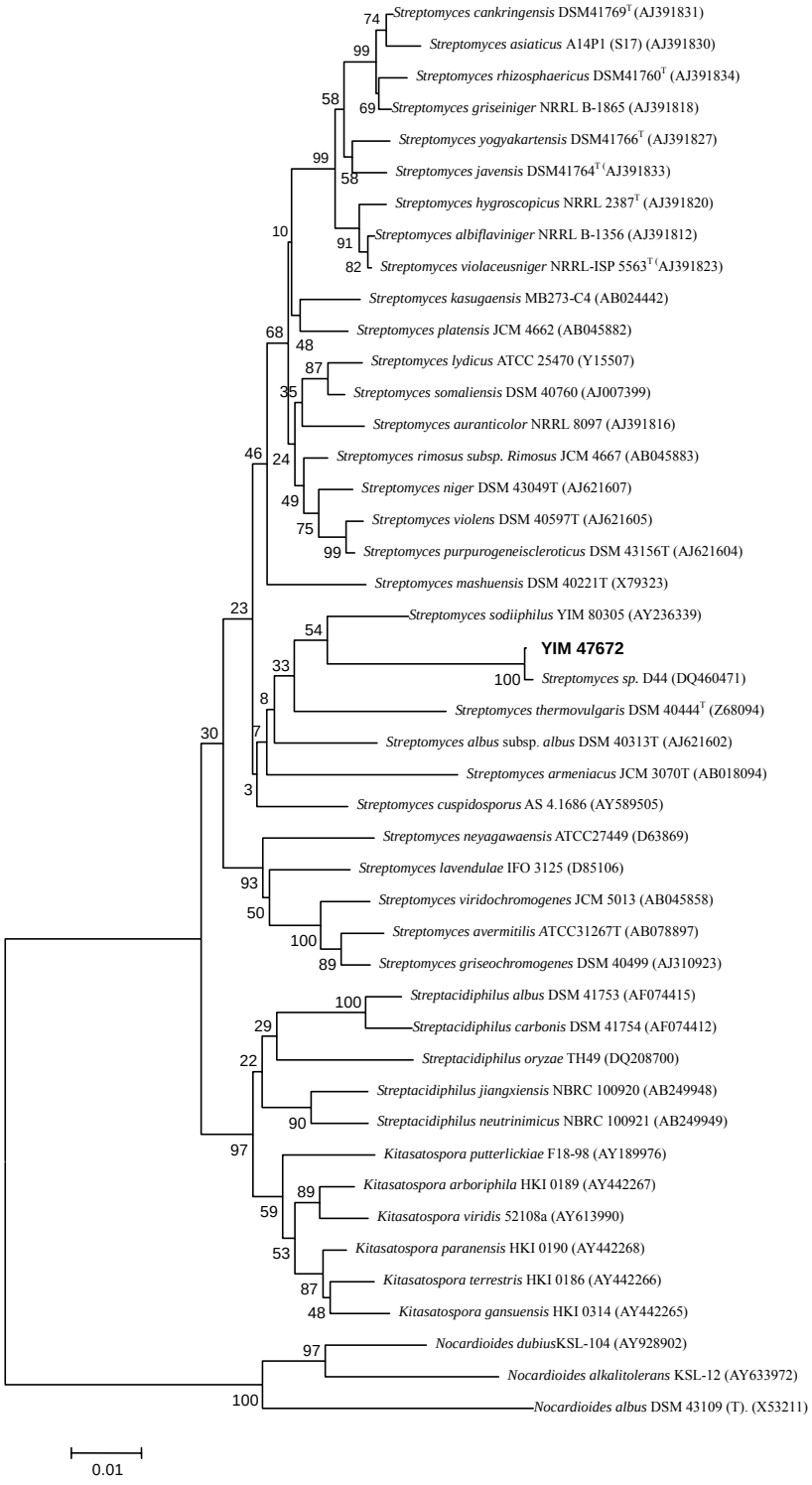


Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of YIM 47672^{T} to type species of 3 genera of the family *Streptomycetaceae* with species of *Nocardioides* as outgroup. Numbers at branching points indicate percentages of occurrence using least squares analysis applying bootstrap values based on 1000 replications.

The almost complete sequence 16S rDNA of strain YIM 47672^T was aligned with representative sequences of related type strains of three genera of the family *Streptomycetaceae* from the GenBank/EMBL/DDBJ database. The evolutionary tree, rooted with *Nocardioides albus* DSM 43109^T (X53211), *Nocardioides alkalitolerans* KSL-12 (AY633972) and *Nocardioides dubius* KSL-104 (AY928902) as the out group, was inferred by using the neighbour-joining method (Saitou & Nei, 1987) formed the evolutionary distance data corrected by Kimura's 2 parameter model (Kimura, 1980). The topology of the tree was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1,000 resamplings. The ClustalX program (Thompson *et al*. 1997) was used for multiple alignment and phylogenetic analysis. TreeView program (Page, 1996) was used to display, edit and print phylogenetic trees.

The phylogenetic analysis demonstrated that strain YIM 47672^T belongs to the genus *Streptomyces.* The rooted phylogenetic tree of strain YIM 47672^T and related type species of the genera *Streptomyces*，*Kitasatospora* and *Streptacidiphilus* indicated that YIM 47672^T formed a separate cluster with *Streptomyces* sp. and *Streptomyces sodiiphilus* (Fig. 2). The 16S rRNA gene sequence similarity of YIM 47672^T with closed species were *Streptomyces cuspidosporus* (Skerman *et al*. 1980) (95.84%), *Streptomyces rimosus subsp. Rimosus* (Skerman *et al.* 1980) (95.48%), *Streptomyces lavendulae* (95.44%) (Skerman *et al.* 1980), *Streptomyces somaliensis* (Skerman et al. 1980) (94.26%), *Streptomyces hygroscopicus* (Skerman *et al.* 1980) (95.10%) and *Streptomyces sodiiphilus* (Li *et al*. 2005) (96.20%) respectively, and is below so much the border among species of *Streptomyces*. *Streptomyces cuspidosporus* produces spherical spores with wart surface, green to black soluble pigment, Gelatine liquefaction is positive reaction, and utilizes 22 carbon sources. *Streptomyces rimosus subsp. Rimosus* produces elliptical spores, light brown soluble pigment, milk coagulation and peptonisation are positive, and utilizes 11 carbon sources. *Streptomyces lavendulae* produces elliptical spores, pale brown soluble pigment, Gelatine liquefaction and milk peptonisation are positive, produces melanin, and utilizes glucose, arabinose, xylose and maltose. *Streptomyces hygroscopicus* produces spores with wart and sting surface, yellowish soluble pigment, milk peptonisation is positive, and utilizes 8 carbon sources.

Above-mentioned characteristics of strain YIM 47672^T were different with these species. *Streptomyces sodiiphilus* and strain YIM 47672^T were clustered always in one same cluster in five trees with different amount and members. But their similarity is only 96.2%. Whole cell hydrolysates of former mainly contain galactose and glucose, the predominant menaquinones are MK-9(H_4) (13%), MK-9(H_6) (68%) and MK-9(H_8) (19%), and the main phospholipid is phosphatidylethanolamine. Whole cell hydrolysates of the latter contained galactose and xylose, main phospholipids were phosphatidyl ethanolamine and diphosphatidyl glycerol , and the predominant menaquinones were MK-9 (H_4) (45.4%), MK-9 (H_6) (14.0%), MK-9 (H_8) (13.6%) and MK-10 (H_0) (27.0%). Strain YIM 47672^T was 99.9% similar to an isolate from traditional Chinese medicinal plant, from which sequence data are available but no description of its characteristics have been published *Streptomyces sp.* D44 (DQ460471). Therefore, strain YIM 47672^T should be considered as novel members of the genus *Streptomyces* for which the name *Streptomyces hainanensis* sp. nov. is proposed.

Description of *Streptomyces hainanensis* sp. nov.

Streptomyces hainanensis (hai.nan.en'sis. N.L. adj. *hainanensis* pertaining to Hainan, a province of south China from where the type strain was isolated).

Aerial mycelia are white and pink white to pink gray. Spore chains are spiral or looped. Spores are elliptical or short rod-shaped. Spore surface is smooth. Vegetative mycelia are pale to deep orange yellow. Produces light brown to orange yellowish soluble pigments. Gelatine liquefaction, milk coagulation and peptonisation, arginase, phenylalanine deaminase, DNAase and melanin production are negative. Starch hydrolysis, arginine decarboxylase, nitrate reduction, gas production from nitrate, growth on cellulose and H_2S production are positive. Optimal growth occurs at pH 7.0 (range pH 6.0 – 9.0) and without NaCl (range 0 – 10% NaCl). Glucose，cellobiose，starch, esculin, mycose, galactoside, and urea are utilized. Acids are not produced from the 5 carbon sources. Galactose, mannose, fructose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, turanose, melicitose, sorbin, dextrin, salicine, adonitol, inositol, mannitol, sorbitol, xylitol, galactitol, erythritol,

amygdaloside, sodium citrate, sodium acetate, gluconate, malonate, tartrate, lysine, ornithine and acetamide are not utilized. Resistant to penicillin G (10 IU), amoxicllin/clavulanic acid (20/10 μg), novobiocin (30 μg), rifampin (5 μg) and ampicillin (10 μg), and sensitive to erythromycin (15 μg IU), gentamicin (10 μg), kanamycin (30 μg), tatracycline (30 μg), vancomycin (30 μg), midecamycin (15 μg), clindamycin (2 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), chloramphenicol (30 μg), polymyxin B (300 IU) and norfloxacin (10 μg). Cell wall contains L-DAP, trace *meso*-DAP and glycine. Whole-cell hydrolysates contain galactose and xylose. Main phospholipids are phosphatidyl ethanolamine and diphosphatidyl glycerol (phospholipid type II). The predominant menaquinones are MK-9(H_4) (45.4%), MK-9 (H_6) (14.0%), MK-9（H_8）（13.6%）and MK-10 (H_0) (27.0%). Fatty acid compositions are iso $C_{15:0}$ (1.1%), iso $C_{16:0}$ (30.9%), $_{16:1}$ w7c/$_{16:1}$ w6c (2.1%), $C_{16:0}$ (13.7%), iso w9c $C_{17:1}$ (1.4%), iso $C_{17:0}$ (2.8%), anteiso $C_{17:0}$ (10.8%), w8c $C_{17:1}$ (7.1%), cyclo $C_{17:0}$ (1.4%), $C_{17:0}$ (5.6%), anteiso $_{18:0}$ /$_{18:2}$ w6,9c (9.7%), w9c $C_{18:1}$ (4.9%), $C_{18:0}$ (1.2%). In addition, the sum of $_{16:1}$ w7c/$_{16:1}$ w6c was 2.1%, sum of anteiso $_{18:0}$ /$_{18:2}$ w6,9c was 9.7% and sum of iso w9c C17:1 or $_{16:0}$ 10-methyl was 1.4%. G+C content of genomic DNA is 73.4 mol %. The type strain YIM 47672^T (= CCTCC AA 205017^T = DSM 41900 ^{T}) was isolated from a soil sample collected from evergreen broadleaf forest in Wuzhi Mountain, Hainan province, P. R. China.

Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (No. 30560001), the Yunnan Provincial International Cooperative Program (No. 2005GH21), the Yunnan Provincial Natural Science Foundation (No. 2004 C0002Q), the Program for New Century Excellent Talents in University and the *Zentrum für Marine Wirkstoffe* by the Ministerium für Wissenschaft, Wirtschaft und Verkehr des Landes Schleswig Holstein. We thank Miss Cai Xiang-Feng and Mr. Chen Yun for their technical assistance.

References

Berdy，J. (2005). Bioactive microbial metabolites, a personal view. *J Antibiotics* **58**, 1-26.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1997). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100,** 221-230.

Dong, X. Z. & Cai, M. Y. (2001). *Manual of systematics and identification of general bacteria.* Science Press, Beijing.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783-791.

Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* **69**, 33-80.

Goodfellow, M., Willimas, S. T. and Alderson, G. (1986). Transfer of *Kitasatoa purpurea* Matsumae and Hata to the genus *Streptomyces* as *Streptomyces purpureus* comb.nov. *Syst Appl Microbiol* **8**, 65-66.

Gordon, R. E. & Mihm J. M. (1962). The type species of the genus *Nocardia*. *J Gen Microbiol* **27**, 1-10.

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

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65,** 501-509.

Jiang, Y., Duan, S. R., Tang, S. K., Cheng, H. H., Li, W. J. & Xu, L. H. (2006).

Isolation methods of rare actinomycetes. *Microbiology* **33,** 181-183.

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,* US Government Printing Office, Washington, DC.

Kim, S. B., Lonsdale, J., Seong, C. N. & Goodfellow, M. (2003). *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici 1943^{AL}) emend. Rainey *et al*. 1997. *Antonie van Leeuwenhoek* **83,** 107-116.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J *Mol Evol* **16,** 111-120.

Lechevalier, M. P. and Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20,** 435-443.

Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). *Phospholipids in the taxonomy of actinomycetes,* In *Actinomycetes*, pp. 11-116. Edited by K. P. Schaal & G. Pulverer. New York: Gustav Fischer.

Li, W. J., Zhang, Y. Q., Tang, S. K., Xu, P., Xu, L. H. & Jiang, C. L. (2005). *Streptomyces sodiiphilus* sp. nov., a novel alkaliphilic actinomycete. *Int J Syst Evol Microbiol* **55**, 1329-1333.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208-218.

Omura, S., Takshsdhi，Y., Iwai, Y. & Tanaka, H. (1982). *Kitasatosporia*, a new genus of the order *Actinomycetales. J Antibiot* **35,** 1013-1019.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comp Appl Biosci* **12,** 357-358.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids, *USFCC Newslett* **20**, 1-6.

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

Skerman,V. B. D., McGowan, V. and Sneath, P. H. A. (1980), Approved Lists of Bacterial Names. *Int J Syst Bacteriol* **30**, 225-420.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Evol Microbiol* **47,** 479-491.

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

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25,** 125-128.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin. F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence

alignment aided by quality analysis tools. *Nucleic Acids Res* **24,** 4876-488.

Tsukamura, M. (1966). Adansonian classification of mycobacteria, *J Gen Microbiol* **45**, 253-273.

Waksman, S. A. & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *J Bacteriol* **46**, 337-341.

Wellington, E. M. H. and Williams S. T. (1981). Host ranges of phages isolated to *Streptomyces* and other genera. *Zentralbl Bakteriol Microbiol Hyg* I Abt. Suppl. **11,** 93-98.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera, *J Gen Microbiol* **129,** 1743-1813.

Witt, D. and Stackebrandt, E. (1990). Unification of the genea *Stretoverticillum* and *Sreptomyces*, and amendation of *Streptomyces* Waksman and Henrici 1943.339^{AL}. *Syst Appl Microbiol* **13,** 361-371.

Zhang, Z., Wang, Y. & Ruan, J. (1997). A proposal to revive the genus *Kitastospora* (Omura, Takahashi, Iwai, and Tanaka 1982). *Int J Syst Bacteriol* **47**, 1048-1054.

Chapter 4 *Actinomycetospora chiangmaiensis* gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*

Yi Jiang, Jutta Wiese, Shu-Kun Tang, Li-Hua Xu, Johannes F. Imhoff and Cheng-Lin Jiang

(Int. J. Syst. Evol. Microbiol. 58:408–413, 2008)

A novel actinomycete strain, YIM 0006^T, was isolated from soil of a tropical rainforest in northern Thailand. The isolate displayed the following characteristics: aerial mycelium is absent, short spore chains are formed directly on the substrate mycelium, contains meso-diaminopimelic acid, arabinose and galactose (cell-wall chemotype IV), the diagnostic phospholipid is phosphatidylcholine, MK-9(H_4) is the predominant menaquinone and the G+C content of the genomic DNA is 69.0 mol%. Phylogenetic analysis and phenotypic characteristics showed that strain YIM 0006^T belongs to the family Pseudonocardiaceae but can be distinguished from representatives of all genera classified in the family. The novel genus and species *Actinomycetospora chiangmaiensis* gen. nov., sp. nov. are proposed, with strain YIM 0006^T (=CCTCC AA 205017^T =DSM 45062^T) as the type strain of *Actinomycetospora chiangmaiensis.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 0006^T is AM398646.

The first description of the family *Pseudonocardiaceae* was given by Embley et al. (1988), and the description was emended by Stackebrandt et al. (1997) on the basis of 16S rRNA gene sequence analysis. The family currently consists of 14 genera with validly published names: *Actinoalloteichus* (Tamura *et al*., 2000), *Actinopolyspora* (Gochnauer et al., 1975), *Amycolatopsis* (Lechevalier et al., 1986), *Crossiella* (Labeda, 2001), *Goodfellowia* (Labeda & Kroppenstedt, 2006), *Kibdelosporangium* (Shearer *et al*., 1986), *Kutzneria* (Stackebrandt *et al*., 1994), *Prauserella* (Kim & Goodfellow, 1999), *Pseudonocardia* (Henssen, 1957), *Saccharomonospora* (Nonomura & Ohara, 1971), *Saccharopolyspora* (Lacey & Goodfellow, 1975), *Streptoalloteichus* (Tomita *et al*., 1987), *Thermobispora* (Wang *et al*., 1996) and *Thermocrispum* (Korn-Wendisch *et al*., 1995). Strain YIM 0006^T was isolated during an investigation of actinomycete diversity in soil from a tropical rainforest in Chiang Mai, in northern Thailand. Here, we report on the classification and characterization of strain YIM 0006^T and propose a novel genus and species of the family *Pseudonocardiaceae* to accommodate the strain.

Strain YIM 0006^T was isolated from a soil sample after 2 weeks incubation at 28 °C on starch-glycerol medium as described by Jiang *et al*. (2006). Cultural characteristics of the strain were determined after growth at 28 °C for 2 weeks by methods used in the International Streptomyces Project (ISP; Shirling & Gottlieb, 1966) as well as by using Czapek's medium and nutrient agar (Dong & Cai, 2001). Colour determination was performed with colour chips from the ISCC-NBS Color Charts Standard Samples no. 2106 (Kelly, 1964). Morphological observations of spore chains and mycelia were made by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (Philips XL30 ESEM-TMP) after 20–50 days incubation. Gram staining (Hucker's modification; Society for American Bacteriologists, 1957) and Ziehl–Neelsen preparations (Gordon, 1967) were evaluated by light microscopy.

Growth of strain YIM 0006^T was poor on most media tested, although the strain grew well but slowly on yeast extract malt extract agar (ISP 2). Soluble pigments were not produced under any conditions tested in this study. Aerial mycelium was not observed on any of the tested media. The vegetative mycelium fragmented into rod-shaped elements

(Fig. 1a) and was pale to brilliant orange–yellow in colour. Short spore chains were formed directly on the vegetative mycelium (Fig. 1a, b). The strain displayed bud-like structures of the spore chains, as has also been described for members of the genus *Pseudonocardia* (Huang *et al*., 2002). Spores were short and rod-shaped, 0.3–0.6×0.8–1.2 mm. The spore surface was smooth.

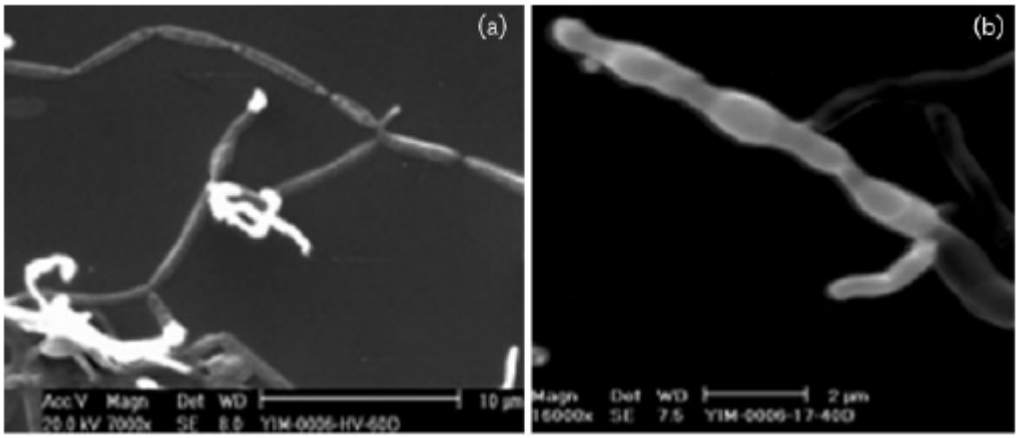


Fig. 1. (a) Scanning electron micrograph of fragments of vegetative mycelium and short spore chains of strain YIM 0006^T on HV medium (Hayakawa & Nonomura, 1987) after incubation for 60 days. Bar, 10 mm. (b) Scanning electron micrograph of short spore chains of strain YIM 0006^T on glycerolasparagine medium (ISP 5) after incubation for 40 days. Bar, 2 mm.

All tests of physiological and biochemical characteristics of strain YIM 0006^T were performed at 28 °C and recorded after 7, 14, 20 and 30 days, except for the nitrate reduction test, which was recorded after 1, 3 and 5 days. Carbon- and nitrogen-source utilization as well as acid production from sugars under aerobic conditions were examined according to the method of Kämpfer et al. (1991). The isolate used a range of carbon sources (see species description). Galactose, arabinose, mannose, raffinose, inositol, mannitol and sodium citrate were not utilized. Tests of gelatin liquefaction, milk coagulation, milk peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, H_2S and melanin production were negative.

Cell material for the extraction of chromosomal DNA and chemotaxonomic studies was obtained after cultivation at 28°C for 7–10 days in ISP 2 broth (Shirling & Gottlieb, 1966) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987)

as a shaking culture. Procedures for analysis of diagnostic cell-wall amino acids and sugars followed those described by Staneck & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al*. (1984). Menaquinones were extracted according to Minnikin et al. (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). Chromosomal DNA for genomic DNA G+C content analysis was extracted as described by Marmur (1961).

The DNA G+C content was determined by the HPLC method (Tamaoka & Komagata, 1984) with an Agilent 1100 LC system (IRIS Technologies).

The cell wall of strain YIM 0006^T contained *meso*-diaminopimelic acid as the diagnostic peptidoglycan diamino acid. Whole-cell hydrolysates contained arabinose and galactose as diagnostic sugars (cell-wall chemotype IV; Lechevalier & Lechevalier, 1970). Analysis of phospholipids revealed phosphatidylcholine, phosphatidylinositol and phosphatidylglycerol, indicating phospholipid type PIII (Lechevalier et al., 1977). The predominant menaquinone was MK-9(H_4). The fatty acid profile consisted mainly of iso-branched saturated hexadecanoic acid. The predominant components, as proportions of the total fatty acid composition, were iso-$C_{14:0}$ (1.1 %), iso-$C_{15:0}$ (3.2 %), iso-$C_{16:1}$ H (2.6 %), iso-$C_{16:0}$ (29.8 %), $C_{16:1}\omega7c$/iso-C_{15} 2-OH (17.6%), $C_{16:0}$ (10.8 %), $C_{16:0}$ 10-methyl (7.2 %), iso-$C_{17:0}$ (2.5 %), anteiso-$C_{17:0}$ (5.5 %), $C_{17:1}\omega8c$ (4.4%), $C_{17:0}$ (1.6%), $C_{17:0}$ 10 methyl (1.3%), $C_{18:1}\omega9c$ (2.3%) and $C_{18:0}$ (3.7 %). The G+C content of genomic DNA of the strain was 69.0 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain YIM 0006^T were carried out using procedures described by Xu *et al*. (2003). The 16S rRNA gene sequence (1456 nucleotides) was compared with corresponding sequences of the family Pseudonocardiaceae from the GenBank/EMBL/DDBJ database by using BLAST (Altschul et al., 1997), BLAST 2 sequences (Tatusova & Madden, 1999) and FASTA (Pearson, 1990). The alignment was performed using CLUSTAL_X (Thompson et al.,

1997) and corrected manually. Phylogenetic analysis was conducted using MEGA version 3.1 (Kumar et al., 2004) and the PhyML online web server (Guindon et al., 2005). A distance matrix was generated according to Kimura's two parameter model (Kimura, 1980, 1983) and a phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. A maximum-likelihood tree was calculated using the GTR (general time-reversible) substitution model and bootstrap values from 500 resamplings.

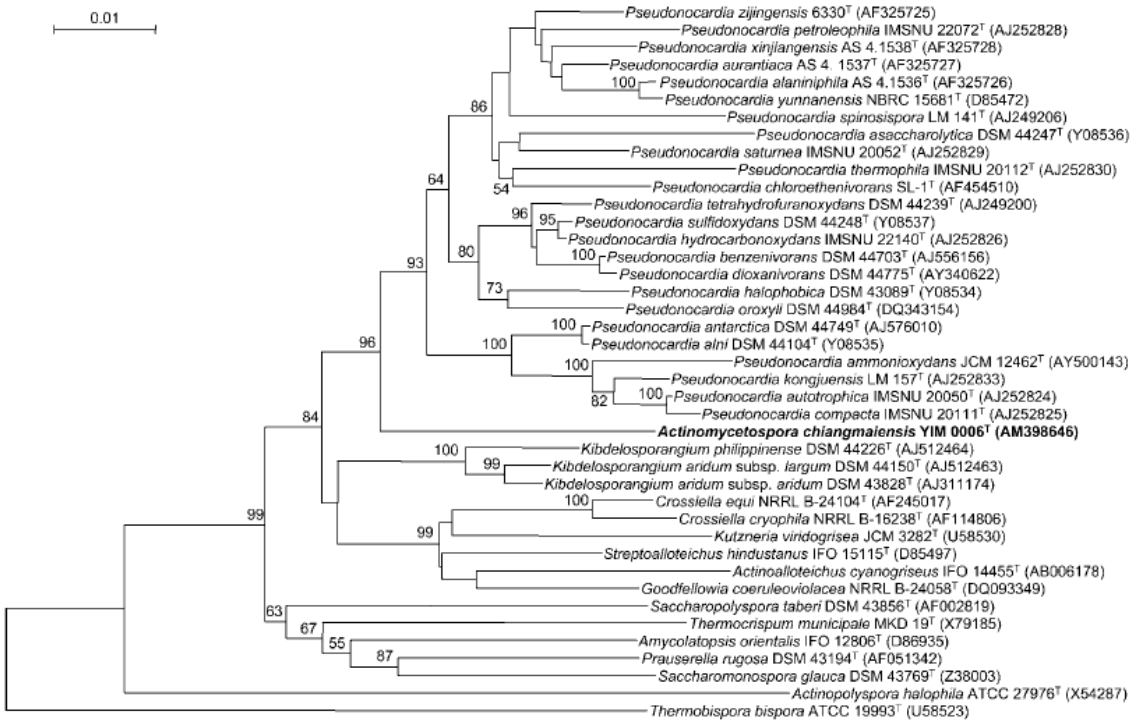


Fig. 2. Neighbour-joining tree derived from 16S rRNA gene sequences showing the relationship of YIM 0006^T and representative species of the 14 genera of the family *Pseudonocardiaceae*. Numbers at branch nodes are bootstrap percentages (1000 resamplings; only values over 50% are given). Bar, 1% sequence divergence.

Database search demonstrated that strain YIM 0006^T belongs to the family *Pseudonocardiaceae* (Stackebrandt *et al*., 1997). Phylogenetic study was performed with 16S rRNA gene sequences of type strains of *Pseudonocardia* species with validly published names, as far as available, including [*Actinobispora*] *xinjiangensis*,

[*Actinobispora*] *aurantiaca*, [*Actinobispora*] *alaniniphila* and [*Actinobispora*] *yunnanensis*, which were combined into the genus *Pseudonocardia* by Huang *et al*. (2002) as well as with sequences of representative type strains of the other 13 genera of the *Pseudonocardiaceae*. The closest relatives of strain YIM 0006^T were *Pseudonocardia halophobica* DSM 43089^T, with 95.24% sequence identity, *Pseudonocardia antarctic*a DSM 44749^T (95.17 %), *Pseudonocardia benzenivorans* DSM 44703^T and *Pseudonocardia alni* IMSNU 20049^T (both 95.10 %). The sequence identity of YIM 0006^T to *Kibdelosporangium aridum* DSM 43828^T was 94.28 %, and the identity to type strains belonging to other genera of the family *Pseudonocardiaceae* was below 94.20 %.

Table 1. Morphological and chemotaxonomic characteristics of strain YIM 0006^T and related genera of the family *Pseudonocardiaceae*

Data for reference genera were derived from Shearer *et al.* (1986), Mertz & Yao (1988), Tomita *et al.* (1993), Henssen (1957), Huang *et al.* (2002), Reichert *et al.* (1998) and Kämpfer *et al.* (2006). Abbreviations: Ara, arabinose; Gal, galactose; Glc, glucose; Mad, madurose; Man, mannose; Rha, rhamnose; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine; GlcNu, *N*-acetylglucosamine-containing phospholipids; v, variable.

Genus	Some key morphological characteristics	Sugar pattern	Phospholipids*	Major menaquinone(s)	Fatty acid type†	G+C content (mol%)
Kibdelosporangium	Long chains of spores, sporangium-like structure of the aerial mycelium, fragmentation of the substrate mycelium	Ara, Gal, Mad (v), Glc (v), Rha (v)	*PE*, PI, PME, PG, DPG, PIM	MK-9(H_2, H_4, H_6)	3c	66
Pseudonocardia	Long or short chains of spores, fragmentation of aerial and substrate mycelia (v), production of spores by budding or septation (v), swollen hyphal segments (v)	Ara, Gal	*PE* or *PC*, PME, PI, PG, DPG, GlcNu	MK-8(H_4)	2b	68–79
Actinomycetospora gen. nov. (YIM 0006^T)	Short chains of spores, bud-like structures, fragmentation of substrate mycelium, absence of aerial mycelium	Ara, Gal	*PC*, PI, PG	MK-9(H_4)	2b	69

*Data given in italics represent the diagnostic phospholipid according to Lechevalier *et al.* (1977).
†According to the classification of Kroppenstedt (1985).

According to the phylogenetic tree (Fig. 2), strain YIM 0006^T formed a distinct subclade between the genera *Pseudonocardia* and *Kibdelosporangium*. Although the 16S rRNA gene sequence similarity between strain YIM 0006^T and members of the genus *Pseudonocardia* fell into the range between the *Pseudonocardia* species (99.6–93.6 %) given by Huang et al. (2002), the separate branching of the isolate is clearly supported by high bootstrap values of 96% (percentage of 1000 resamplings) and 97% (percentage of 500 resamplings) after calculation of the neighbour-joining tree (Fig. 2) and the maximum-likelihood tree (not shown), respectively.

Strain YIM 0006^T and representatives of the next most closely related genus *Pseudonocardia* have the same cellwall chemotype (chemotype IV; meso-diaminopimelic acid, arabinose and galactose), fatty acid type and DNA G+C content, while the menaquinone pattern clearly distinguishes the new isolate from members of the genus *Pseudonocardia* (Table 1). Several chemotaxonomic characteristics and the absence of sporangium-like structures clearly differentiate strain YIM 0006^T from representatives of the phylogenetically close genus *Kibdelosporangium* (Table 1).

On the basis of a combination of phylogenetic distinctness and differences in chemotaxonomic and morphological characteristics, we consider that strain YIM 0006^T represents a novel genus and species, for which the name *Actinomycetospora chiangmaiensis* gen. nov., sp. nov. is proposed.

Description of *Actinomycetospora* gen. nov.

Actinomycetospora (Ac.ti'no.my.ce.to.spo'ra. N.Gr. n. *actinomycesetos* an actinomycete; Gr. fem. n. *spora* a seed and, in bacteriology, a spore; N.L. fem. n. *Actinomycetospora* referring to an actinomycete with spore chains).

Aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes. Substrate mycelium fragments into rod-shaped elements. No aerial mycelium is produced on any medium tested. The cell wall contains meso-diaminopimelic acid. Whole-cell hydrolysates contain

arabinose and galactose (cell-wall chemotype IV). Phosphatidylcholine is the diagnostic phospholipid, with phosphatidylinositol and phosphatidylglycerol. The predominant menaquinone is MK-9(H_4). The type species is *Actinomycetospora chiangmaiensis.*

Description of *Actinomycetospora chiangmaiensis* sp. nov.

Actinomycetospora chiangmaiensis (chiang.mai.en'sis. N.L. fem. adj. *chiangmaiensis* pertaining to Chiang Mai, a city in the north of Thailand in the vicinity of which the type strain was found).

In addition to the characteristics given in the genus description, this species has the following properties. Vegetative mycelium is pale to brilliant orange–yellow in colour. Short spore chains are formed directly from vegetative mycelium. Spores are short and rod-shaped. Spore surfaces are smooth. No soluble pigment is produced. Glucose, fructose, xylose, ribose, rhamnose, sucrose, lactose, sorbitol, glycerol, sodium acetate, asparagine, glycine, histidine and methionine are utilized as sole carbon sources. Acid is not produced from these carbon sources. Gelatin liquefaction, milk coagulation and peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, H2S and melanin production are negative. The major cellular fatty acids are iso-$C_{16:0}$ (29.8%), $C_{16:1}\omega7c$/iso-C_{15} 2-OH (17.6%), $C_{16:0}$ (10.8%) and $C_{16:0}$ 10-methyl (7.2 %). The G+C content of the DNA of the type strain is 69 mol%. The type strain, YIM 0006^T (=CCTCC AA 205017^T =DSM 45062^T), was isolated from soil collected from a tropical rainforest located at Chiang Mai in the north of Thailand.

Acknowledgements

This research was supported by the National Basic Research Program of China (no. 2004CB719601), the National Natural Science Foundation of China (no. 30560001), the Yunnan Provincial International Cooperative Program (no. 2005GH21), the Yunnan Provincial Natural Science Foundation (no. 2004 C0002Q), the Program for New Century Excellent Talents in University and the Centre of Marine Natural Products, which is funded by the Ministry of Science, Economic Affairs and Transport of the state of Schleswig-Holstein (Germany). We thank Dr Jörg Süling, Miss Xiang-Feng Cai and

Mr Yun Chen for technical assistance and discussions.

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Dong, X.-Z. & Cai, M.-Y. (editors) (2001). Determination of biochemical properties. In Manual for the Systematic Identification of General Bacteria, pp. 370–398. Beijing: Science Press (in Chinese).

Embley, M. T., Smida, J. & Stackebrandt, E. (1988). The phylogeny of mycolate-less wall chemotype IV actinomycetes and description of *Pseudonocardiaceae* fam. nov. *Syst Appl Microbiol* **11**, 16–19.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gochnauer, M. B., Leppard,G.G., Komaratat, P., Kates,M., Novitsky, T. & Kushner, D. J. (1975). Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. *Can J Microbiol* **21,** 1500–1511.

Gordon, R. E. (1967). The taxonomy of soil bacteria. In The Ecology of Soil Bacteria, pp. 293–321. Edited by T. R. G. Gray & D. Parkinson. Liverpool: Liverpool University Press.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005). PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33,** W557–W559.

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for

the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.

Henssen, A. (1957). Beitra¨ge zur Morphologie und Systematik der thermophilen Actinomyceten. *Arch Mikrobiol* **26**, 373–414 (in German).

Huang, Y., Wang, L. M., Lu, Z. T., Hong, L., Liu, Z. H., Tan, G. Y. A. & Goodfellow, M. (2002). Proposal to combine the genera *Actinobispora* and *Pseudonocardia* in an emended genus *Pseudonocardia*, and description of *Pseudonocardia zijingensis* sp. nov. *Int J Syst Evol Microbiol* **52**, 977–982.

Jiang, Y., Duan, S. R., Tang, S. K., Cheng, H. H., Li, W. J. & Xu, L. H. (2006). Isolation methods of rare actinomycetes. *Microbiology* (Beijing) **33**, 181–183.

Kämpfer, P., Kroppenstedt, R. M. & Dott, W. (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *J Gen Microbiol* **137**, 1831–1891.

Kämpfer, P., Kohlmeyer, U., Thiemer, B. & Andreesen, J. R. (2006). *Pseudonocardia tetrahydrofuranoxydans* sp. nov. *Int J Syst Evol Microbiol* **56**, 1535–1538.

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.

Kim, S. B. & Goodfellow, M. (1999). Reclassification of Amycolatopsis rugosa Lechevalier et al. 1986 as *Prauserella rugosa* gen. nov., comb. nov. *Int J Syst Bacteriol* **49,** 507–512.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kimura, M. (1983). The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press.

Korn-Wendisch, F., Rainey, F., Kroppenstedt, R. M., Kempf, A., Majazza, A., Kutzner, H. J. & Stackebrandt, E. (1995). *Thermocrispum* gen. nov., a new genus of the order *Actinomycetales*, and description of *Thermocrispum municipale* sp. nov. and *Thermocrispum agreste* sp. nov. *Int J Syst Bacteriol* **45**, 67–77.

Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* **5**, 2359–2367.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In Chemical Methods in Bacterial Systematics (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.

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

Labeda, D. P. (2001). *Crossiella* gen. nov., a new genus related to *Streptoalloteichus*. Int J Syst Evol Microbiol 51, 1575–1579.

Labeda, D. P. & Kroppenstedt, R. M. (2006). *Goodfellowia* gen. nov., a new genus of the *Pseudonocardineae* related to *Actinoalloteichus*, containing *Goodfellowia coeruleoviolacea* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **56**, 1203–1207.

Lacey, J. & Goodfellow, M. (1975). A novel actinomycete from sugarcane bagasse, *Saccharopolyspora hirsuta* gen. et sp. nov. *J Gen Microbiol* **88**, 75–85.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Lechevalier, M. P., Prauser, H., Labeda, D. P. & Ruan, J. S. (1986). Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and Amycolatopsis gen. nov. Int J Syst Bacteriol **36**, 29–37.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Mertz, F. P. & Yao, R. C. (1988). *Kibdelosporangium philippinense* sp. nov. isolated from soil. *Int J Syst Bacteriol* **38,** 282–286.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Nonomura, H. & Ohara, Y. (1971). Distribution of actinomycetes in soil. X. New genus and species of monosporic actinomycetes. J Ferment Technol **49**, 895–903.

Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol **183,** 63–98.

Reichert, K., Lipski, A., Pradella, S., Stackebrandt, E. & Altendorf, K. (1998). *Pseudonocardia asaccharolytica* sp. nov. and *Pseudonocardia sulfidoxydans* sp. nov., two new dimethyl disulfide-degrading actinomycetes and emended description of the genus *Pseudonocardia*. *Int J Syst Bacteriol* **48**, 441–449.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol 4*, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20, 16.

Shearer, M. C., Colman, P. M., Ferrin, R. M., Nisbet, L. J. & Nash, C. H. (1986). New genus of the *Actinomycetales*: *Kibdelosporangium aridum* gen. nov., sp. nov. *Int J Syst Bacteriol* **36**, 47–54.

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

Society for American Bacteriologists (1957). Manual of Microbiological Methods. New York:

McGraw-Hill. Stackebrandt, E., Kroppenstedt, R. M., Jahnke, K. D., Kemmerling, C. & Gürtler, H. (1994). Transfer of *Streptosporangium viridogriseum* (Okuda et al. 1966), *Streptosporangium viridogriseum* subsp. *Kofuense* (Nonomura and Ohara 1969), and *Streptosporangium albidum* (Furumai et al. 1968) to *Kutzneria* gen. nov. as *Kutzneria viridogrisea* comb. nov., *Kutzneria kofuensis* comb. nov., and *Kutzneria albida* comb. nov., respectively, and emendation of the genus *Streptosporangium*. *Int J Syst Bacteriol* **44**, 265–269.

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.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tamura, T., Liu, Z. H., Zhang, Y. M. & Hatano, K. (2000). *Actinoalloteichus cyanogriseus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 1435–1440.

Tatusova, T. A. & Madden, T. L. (1999). BLAST 2 sequences – a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol Lett* **174**, 247–250.

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.

Tomita, K., Nakakita, Y., Hoshino, Y., Numata, K. & Kawaguchi, H. (1987). New genus of the *Actinomycetales*: *Streptoalloteichus hindustanus* gen. nov., nom. rev.; sp. nov., nom. rev. *Int J Syst Bacteriol* **37**, 211–213.

Tomita, K., Hoshino, Y. & Miyaki, T. (1993). *Kibdelosporangium albatum* sp. nov., producer of the antiviral antibiotics cycloviravins. *Int J Syst Bacteriol* **43**, 297–301.

Wang, Y., Zhang, Z. & Ruan, J. S. (1996). A proposal to transfer Microbispora bispora (Lechevalier 1965) to a new genus, *Thermobispora* gen. nov., as *Thermobispora bispora* comb. nov. *Int J Syst Bacteriol* **46**, 933–938.

Xu, P., Li, W. J., Xu, L. H. & Jiang, C. L. (2003). A microwave based method for genomic DNA extraction from actinomycetes. *Microbiology* (Beijing) **30**, 82–84.

Chapter 5 A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov.

Jutta Wiese, Yi Jiang, Shu-Kun Tang, Vera Thiel, Rolf Schmaljohann, Li-Hua Xu*, Cheng-Lin Jiang and Johannes F. Imhoff*

（Int. J. Syst. Evol. Microbiol. 58:1324–1331, 2008）

A novel actinomycete, designated strain YIM 46034^T, was isolated from an evergreen broadleaved forest at Menghai, in southern Yunnan Province, China. Phenotypic characterization and 16S rRNA gene sequence analysis indicated that the strain belonged to the family *Micromonosporaceae*. Strain YIM 46034^T showed more than 3% 16S rRNA gene sequencedivergence from recognized species of genera in the family *Micromonosporaceae*. Characteristic features of strain YIM 46034^T were the production of two types of spores, namely motile spores, which were formed in sporangia produced on substrate mycelia, and single globose spores, which were observed on short sporophores of the substrate mycelia. The cell wall contained *meso*-diaminopimelic acid, glycine, arabinose and xylose, which are characteristic components of cell-wall chemotype II of actinomycetes. Phosphatidylethanolamine was the major phospholipid (phospholipid type II). Based on morphological, chemotaxonomic, phenotypic and genetic characteristics, strain YIM 46034^T is considered to represent a novel species of a new genus in the family *Micromonosporaceae*, for which the name *Planosporangium flavigriseum* gen. nov., sp. nov. is proposed. The type strain of *Planosporangium flavigriseum* is YIM 46034^T (=CCTCC AA 205013^T =DSM 44991^T).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 46034^T is AM232832. Scanning electron micrographs of cells of strain YIM 46034^T grown on：GYM agar are available as supplementary material with the online version of this paper.

The family *Micromonosporaceae* was proposed by Krasil'nikov (1938), and the description of the family has subsequently been emended by Koch *et al*. (1996) and Stackebrandt *et al*. (1997) on the basis of chemotaxonomic data and 16S rRNA gene sequence analysis. These groups of authors included the following genera within the family:*Micromonospora* (Ørskov, 1923), *Actinoplanes* (Couch, 1950), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al*., 1993), *Couchioplanes* (Tamura *et al*., 1994), *Dactylosporangium* (Thiemann *et al*., 1967) and *Pilimelia* (Kane, 1966). The genera *Actinocatenispora* (Thawai *et al*., 2006), *Asanoa* (Lee & Hah, 2002), *Luedemanella* (Ara & Kudo, 2007a, b), *Longispora* (Matsumoto et al., 2003), *Polymorphospora* (Tamura *et al*., 2006), *Salinispora* (Maldonado *et al*., 2005), *Spirilliplanes* (Tamura *et al*., 1997), *Verrucosispora* (Rheims *et al*., 1998) and *Virgisporangium* (Tamura *et* al., 2001) have since been described as additional members of the family.

During the course of a study on actinomycete diversity, a new isolate was obtained from an evergreen broadleaved forest soil sample from Yunnan Province, China, and this is shown here to represent a novel species of a new genus within the family *Micromonosporaceae*.

Strain YIM 46034^T was isolated from soil collected in Menghai, Yunnan Province, China, on fucose-proline medium [per litre distilled water: 5 g fucose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4 \cdot 7H_2O$, 20 g agar, pH 7.2; nalidixic acid (20 mg l^{-1}) and nystatin (100 mg l^{-1}) were added as inhibitors of bacteria and fungi, respectively] incubated at 28 °C.

The phenotypic properties of strain YIM 46034^T were examined by using various standard procedures (Williams et al., 1983). Cultural characteristics were determined following growth on potato-dextrose agar (PDA; Difco), Czapek's agar (30 g sucrose, 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, 20 g agar, pH 7.2–7.4), nutrient agar (10 g peptone, 5 g beef extract, 5 g NaCl, 20 g agar, pH

7.2–7.4), GYM agar (4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g $CaCO_3$, 12 g agar, pH 7.2) and International *Streptomyces* Project media ISP2, ISP3, ISP4 and ISP5 after 25 days at 28 °C according to the methods of Shirling & Gottlieb (1966). Morphology of spores, sporangia, flagella and mycelia was observed after incubation at 28 °C for 15–30 days by light microscopy (BH-2; Olympus), scanning electron microscopy [JSM 5600LV (JEOL) and XL30 ESEM-TMP (Philips)] and transmission electron microscopy (H 800; Hitachi). Negative staining was used to demonstrate flagellation of the spores. Colours and hues were determined according to Kelly (1964). The Gram reaction was performed according to Gregersen (1978) by using KOH for cell lysis. Acid fastness was determined by using carbol–fuchsin solution for cell staining, acid alcohol treatment and counterstaining with methylene blue (Ziehl–Neelsen method).

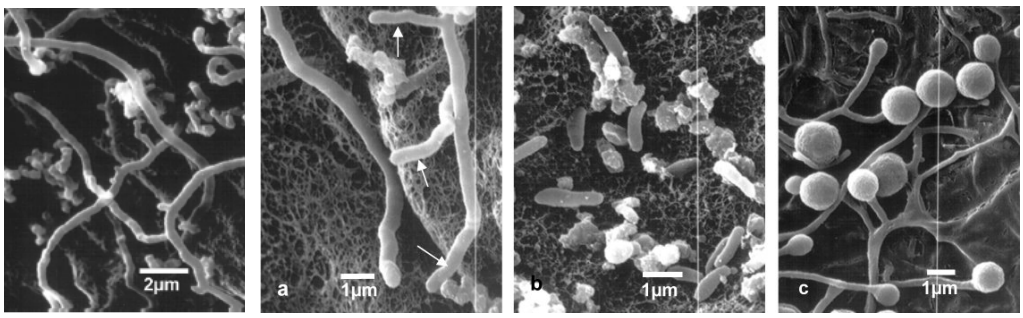


Fig. 1. Scanning electron micrograph of substrate mycelium of a 9-week-old agar culture of strain YIM 46034^T grown on GYM agar. Bar, 2 mm. (a) Finger-like sporangia (arrows) growing directly on substrate mycelium; (b) rod-like motile sporangiospores (flagella not visible); (c) globose, non-motile spores arising from short sporophores of the substrate mycelium.

Biomass for molecular systematic and most of the chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in shaken cultures with yeast extract malt extract broth (ISP2) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987). Cell walls were purified and amino acids in the peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980). Whole-cell sugar composition was analysed according to the methods of Becker *et al*. (1965) and Lechevalier & Lechevalier (1980). Phospholipid analysis was carried out as described

by Lechevalier *et al*. (1981). Menaquinones were determined by using the procedures of Collins *et al*. (1977). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from TSA plates [3% (w/v) trypticase soy broth (BBL), 1.5% (w/v) Bacto agar (Difco)] that had been incubated for 7 days at 28 °C. Fatty acids were extracted, methylated and analysed by using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Table 1. Characteristics of genera in the family *Micromonosporaceae*

Taxa: 1, *Actinocatenispora*; 2, *Actinoplanes*; 3, *Asanoa*; 4, *Catellatospora*; 5, *Catenuloplanes*; 6, *Couchioplanes*; 7, *Dactylosporangium*; 8, *Longispor*a; 9, *Luedemannella*; 10, *Micromonospora*; 11, *Pilimelia*; 12, *Polymorphospora*; 13, *Salinispora*; 14, *Spirilliplanes*; 15, *Verrucosispor*a; 16, *Virgisporangium*; 17, strain YIM 46034^T. +, Present; 2, absent; ND, no data available; m-DAP, *meso*-diaminopimelic acid; Data taken from Thawai et al. (2006), Couch (1950), Lee & Hah (2002), Asano & Kawamoto (1986), Ara & Kudo (2006, 2007a), Yokota *et al*. (1993), Kudo *et al*. (1999), Tamura *et al*. (1994, 1997, 2001, 2006), Thiemann *et al*. (1967), Matsumoto *et al*. (2003), Ørskov (1923), Kane (1966), Maldonado *et al*. (2005) and Rheims *et al*. (1998).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Spore motility	−	+	−	−	+	+	+/−	−	−	−	+	−	−	+	−	+	+/−
Sporangia/ spore vesicles	−	+	−	−	−	−	+	−	+	−	+	−	−	−	−	+	+
Diamino acid	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	L-Lys	L-Lys	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP
Characteristic whole-cell sugar(s)*	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Man, Rha, Rib, Xyl or only Xyl	Man, Rib, Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Man, Rha, Rib, Xyl	Ara, Xyl	Ara, Xyl	Xyl	Ara, Gal, Xyl	Gal, Man, Xyl	Man, Xyl	Ara, Gal, Man, Rha, Xyl	Ara, Xyl
Fatty acid type†	3b	2c	2d	3b	2c	2c	2d	2d	2d	3b	2b	2a	3a	2d	2b	2d	3b
Major menaquinone(s)	9($H_{4,6}$)	9(H_4), 10(H_4)	10($H_{6,8}$)	9($H_{4,6}$) or 10(H_4)	10(H_4), 11(H_4)	9(H_4)	9($H_{4,6,8}$)	10($H_{4,6}$)	9($H_{4,6}$)	9($H_{4,6}$), 10($H_{4,6}$)	9($H_{2,4}$)	9($H_{4,6}$), 10($H_{4,6}$)	9(H_4)	10(H_4)	9(H_4)	10(H_4, H_6,H_8)	9(H_4), 10(H_4)
Phospholipid type‡	II	II	II	II	III	II	II	II	II	II	II	II	II	II	II	II	II
DNA G+C content (mol%)	72	72–73	71–72	70–72	70–73	70–72	72–73	70	71	71–73	ND	71	70–73	69	70	71	71.4

*Ara, Arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylo
†According to the classification of Kroppenstedt (1985).
‡According to the classification of Lechevalier *et al*. (1977).

Chromosomal DNA of strain YIM 46034^T was extracted as described by Marmur (1961). The DNA G+C content was determined by thermal denaturation (Mandel & Marmur, 1968).

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene of strain YIM 46034^T were carried out according to the procedures described by Xu *et al*. (2003). The 16S rRNA gene sequence of strain YIM 46034^T (1470 nt) was

compared with sequences in the DDBJ, EMBL and GenBank databases by using BLAST searches (Altschul et al., 1997). For initial taxonomic classification of the sequence, the classifier program of the Ribosomal Database Project II (http://rdp.cme.msu.edu/index.jsp) was used. For phylogenetic analysis, sequences of representative species of different families belonging to the Actinobacteria and, in a second step, of all 16 recognized genera in the family *Micromonosporaceae* were used. Neighbour-joining trees (Saitou & Nei, 1987) were calculated by using distances corrected according to the Kimura two-parameter model (Kimura, 1980, 1983) withthe software package MEGA version 3.1 (Kumar *et al*., 2001) after multiple alignment of the data by CLUSTAL_X (Thompson *et al*., 1997). For construction of the maximum-likelihood tree, the online version of PhyML (Guindon *et al*., 2005) was used. The topology of the trees was evaluated by performing a bootstrap analysis (Felsenstein, 1985) of 1000 resamplings. *Nocardiopsis alba* DSM 43377^T was used as outgroup.

To examine the secondary structures of nine variable areas of the 16S rRNA gene (V1–V9) we used the procedures described by Bouthinon & Soldano (1999) and Akutsu (2000). The sequences were cut by using the program CLUSTAL_X and the secondary structures were evaluated and viewed via the programs RNA structure 3.7 (De Rijk & De Wachter, 1997) and RnaViz 2.0 (De Rijk *et al*., 2003).

Cells of strain YIM 46034^T were aerobic, Gram-positive and non-acid-fast. No growth was observed on Czapek's agar, inorganic salts-starch agar (ISP4) or nutrient agar. Cultures grew very slowly, but developed well, within 25 days on yeast extract-malt extract agar (ISP2), oatmeal agar (ISP3), glycerol-asparagine agar (ISP5), PDA and GYM agar. No soluble pigments were produced on all media tested. The substrate mycelium branched extensively, and the colour of colonies on different media was pale grey (ISP5), orange yellow/light yellow (ISP2/PDA), orange (GYM agar) and pale grey–olive (ISP3). No aerial mycelium was observed on these media, except on GYM agar. Colonies on GYM agar were tough and wrinkled, with a

smooth surface. Older cultures (9 weeks) partially developed areas with aerial mycelium at the edge of the colony. A scanning electron micrograph of the substrate mycelium with filament diameters of 0.6–0.7 mm is shown in Fig. 1. Strain YIM 46034^T formed two types of spores. Motile spores were formed in finger-like, short, narrow mentary Fig. S1a in IJSEM Online) produced directly on substrate mycelia. Sporangium envelopes became visible by light microscopy after spore liberation following rupture of the sporangia. Light microscopy also revealed that each sporangium contained a single row of three or more straight or slightly curved rod-shaped spores (1.0–1.560.4–0.5 mm). Cells of similar dimensions were detected by scanning electron microscopy (Supplementary Fig. S1b). Spore motility was demonstrated by light microscopy observation, and transmission electron microscopy revealed that the spores possessed a single polar flagellum. In contrast, non-motile globose spores with a smooth surface and diameters varying between 0.5 and 1.5 mm were also detected at the tip of short sporophores of the substrate mycelium (Supplementary Fig. S1c)

Colony and cell morphology of strain YIM 46034^T were quite similar to those of *Dactylosporangium* species as described by Vobis (1992), especially with regard to the two types of spores produced. Non-motile spores of *Dactylosporangium* species were larger (1.7–2.8 mm in diameter) than those of strain YIM 46034^T. A further difference from *Dactylosporangium* is apparent in the flagellation of the spores. Whereas spores of strain YIM 46034^T possessed only a single flagellum, those of *Dactylosporangium* species are reported to possess a polar or subpolar tuft of flagella (Vobis, 1987).

The physiological, chemotaxonomic and genomic characteristics of strain YIM 46034^T are given in Table 1 and also in the genus and species descriptions below. The major menaquinones were MK-9(H4) (52 %) and MK-10(H4) (48%). The fatty acid profile comprised iso-$C_{15:0}$ (7.3 %), anteiso-$C_{15:0}$ (3.3 %), $C_{15:0}$ (1.2 %), iso-$C_{16:1}$ (1.9%),

iso-$C_{16:0}$ (17.5%), $C_{16:0}$ (2.1%), iso-$C_{17:1}$ω9c (4.2 %), anteiso $C_{17:1}$ ω9c (1.9%), iso-$C_{17:0}$ (5.9%), anteiso-$C_{17:0}$ (19.1%), $C_{17:1}$ ω8c （4.5%), $C_{17:0}$ (5.0 %), $C_{17:0}$ 10-methyl (1.6%), $C_{18:1}$ ω9c (7.0%), $C_{18:0}$ (1.7%), $C_{19:1}$ ω11c/$C_{19:1}$ ω9c (1.9%). Strain YIM 46034^T showed 16S rRNA gene sequence similarities of >97% to recognized representatives of genera belonging to the family *Micromonosporaceae*. Highest levels of similarity were to *Micromonospora aurantiaca* ATCC 27029^T (97.0 %), *Micromonospora halophytica* DSM 43171^T (97.0 %), *Micromonospora purpureochromogenes* DSM 43821^T (96.9 %), *Micromonospora carbonacea* DSM 43168^T (96.7 %), *Virgisporangium ochraceum* YU655-43^T (96.2 %), *Virgisporangium aurantiacum*YU438-5^T (95.9 %) and *Dactylosporangium aurantiacum* IFO 12592^T (95.6 %). Although strain YIM 46034^T showed highest levels of 16S rRNA gene sequence similarity to members of the genus *Micromonospora*, phylogenetic analysis revealed that strain YIM 46034^T formed a distinct lineage within the family *Micromonosporaceae.* It did not cluster within the genus *Micromonospora* but was related to the genus *Virgisporangium* (Fig. 2). The maximumlikelihood tree showed a similar topology (data not shown).

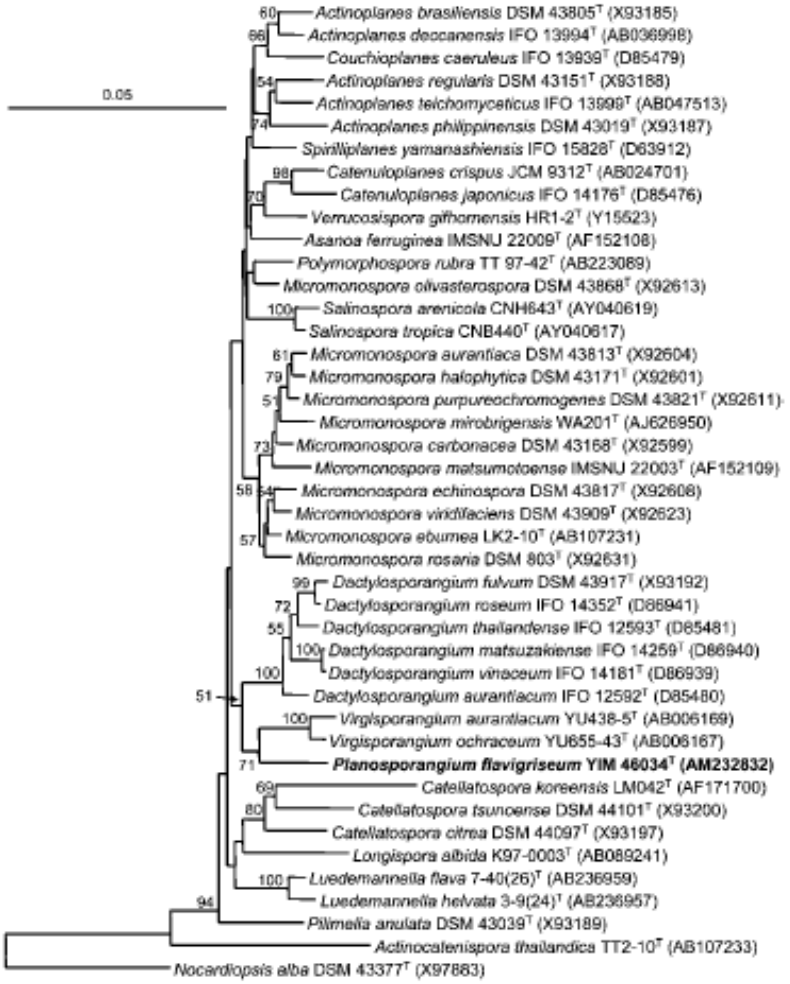


Fig. 2. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship between strain YIM 46034^T and the type strains of species of related genera in the family *Micromonosporaceae*. Numbers on branch nodes are bootstrap percentages (1000 replications; only values .50% are given). Bar, 5% sequence divergence.

Signature nucleotides of the 16S rRNA gene sequence of strain YIM 46034^T were shared in all but one position (1116 : 1184 according to the *Escherichia coli* numbering scheme) with species of the family *Micromonosporaceae* as described by Stackebrandt *et al*. (1997) (Table 2). Other recognized species of the family *Micromonosporaceae* also differ in this position with regard to signature nucleotides (e.g. *Dactylosporangium, Virgisporangium, Salinispora and Actinocatenospora*; Table 2). Within the *Micromonosporaceae* (signature nucleotides according to Stackebrandt *et al*., 1997), strain YIM 46034^T differs from the genera

Micromonospora (positions 1116 : 1184 and 1133 : 1141) as well as from *Virgisporangium* (position 502 : 543), but clusters with the genus *Dactylosporangium* (Table 2). However, signature nucleotides of strain YIM 46034^T at positions based on Koch *et al*. (1996) were different from those of the genus *Virgisporangium* in one position (141 : 222) and from those of *Dactylosporangium* in several positions (141 : 222, 415 : 428, 600 : 638, 601 : 637, 614 : 626, 653, 998), clearly differentiating the novel strain from both of these genera (Table 3).

Table 2. Signature nucleotides of the 16S rRNA gene sequence of members of the family *Micromonosporaceae*

Taxa: 1, *Micromonosporaceae* (Stackebrandt et al., 1997); 2, *Actinoplanes*; 3, *Asanoa*; 4, *Catellatospora*; 5, *Catenuloplanes*; 6, *Couchioplanes*; 7, *Dactylosporangium*; 8, *Longispora*; 9, *Luedemannella*; 10, *Micromonospora*; 11, *Pilimelia*; 12, Salinispora; 13, *Spirilliplanes*; 14, *Verrucosispora*; 15, Virgisporangium; 16, *Polymorphospora*; 17, *Actinocatenispora*; 18, strain YIM 46034^T. R, Purine; Y, pyrimidine.

E. coli position(s)	1, 3, 4, 9, 11, 16	2, 10, 13	5, 6	7, 18	8	12	14	15	17
66: 103	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
127: 234	A–T	A–T	A–T	A–T	A–T	A–T	A–T	A–T	A–T
153: 168	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
502: 543	G–C	G–C	G–C	G–C	A–T	G–C	G–C	A–T	T–G
589: 650	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
747	A	A	A	A	A	A	A	A	G
811	T	T	T	T	T	T	T	T	T
840: 846	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
952: 1229	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
1116: 1184	C–G	C–G	C–G	T–G	C–G	T–G	C–G	T–G	T–G
1133: 1141	G–C	G–G	R–Y	G–C	G–G	G–G	A–T	G–C	G–C

The secondary structure of the variable regions of the 16S rRNA gene sequence of strain YIM 46034T V1 (positions 61–106), V2 (136–227; Fig. 3), V3 (437–497), V4 (588–651), V5 (821–879), V6 (997–1044), V7 (1118–1155), V8 (1241–1296) and V9 (1435–1466) closely matched those of strains and type strains of species of more distantly related genera. The secondary structure of the V2 region of YIM 46034T, for example, is different from that of *M. aurantiaca* ATCC 27029^T, *Virgisporangium ochraceum* CIP 107213^T, *Dactylosporangium fulvum* DSM 43917^T, *Actinocatenispora thailandica* JCM 12343^T, *Catenuloplanes crispus* JCM 9312^T, *Pilimelia anulata* DSM 43039^T and *Polymorphospora rubra* DSM 44947^T (Fig. 3).

Different members of a single genus can display identical V2 secondary structures, as found in, for example, the genus *Micromonospora* (e.g. *M. halophytica* DSM 43171^T, *M. carbonacea* DSM 43168^T, *M. purpureochromogenes* DSM 43821^T; data not shown). Thus, the different V2 secondary structure of strain YIM 46034^T compared with related and recognized species further supports the placement of this novel strain within a new genus.

Table 3. Signature nucleotides of the 16S rRNA gene sequence of strain YIM 46034^T and its most closely related genera modified according to Koch et al. (1996)

Numbers of 16S rRNA gene sequences taken for the determination of signature nucleotides are indicated. S, G or C.

E. coli position(s)	*Dactylosporangium* (*n*=18)	*Virgisporangium* (*n*=4)	Strain YIM 46034^T
141 : 222	G–C	G–C	A–T
129 : 232	T–G	T–G	T–G
415 : 428	A–G	C–G	C–G
441	G	G	G
442 : 491	A–G	A–G	A–G
560	T	T	T
600 : 638	A–T	G–T	G–T
601 : 637	G–C	A–T	A–T
614 : 626	G–T	G–C	G–C
653	T	A	A
998	T	G	G
1002 : 1038	S–S	C–G	G–C

Characteristic properties that differentiate strain YIM 46034^T from related species are given in Table 1. Diagnostic features of YIM 46034^T are the production of motile spores enclosed within sporangia. This clearly differentiates strain YIM 46034^T from species of the genus *Micromonospora*. Whole-cell sugar, fatty acid and menaquinone patterns of strain YIM 46034^T were different from those of recognized *Virgisporangium* species. The menaquinone and fatty acid patterns of strain YIM 46034^T were different from those of members of the genus *Dactylosporangium*. In addition, signature nucleotides (as described by Stackebrandt *et al*., 1997) of strain YIM 46034T were not consistent with any of the described genera of the family *Micromonosporaceae* except *Dactylosporangium* (Table 2). However, the signature nucleotides as defined by Koch *et al*. (1996) clearly distinguished the new isolate

from members of the genus *Dactylosporangium* (Table 3). Furthermore, strain YIM 46034^{T} was not affiliated with any other recognized genus of the family *Micromonosporaceae*. Therefore, we suggest that strain YIM 46034^{T} represents a novel species of a new genus, for which the name *Planosporangium flavigriseum* gen. nov., sp. nov. is proposed.

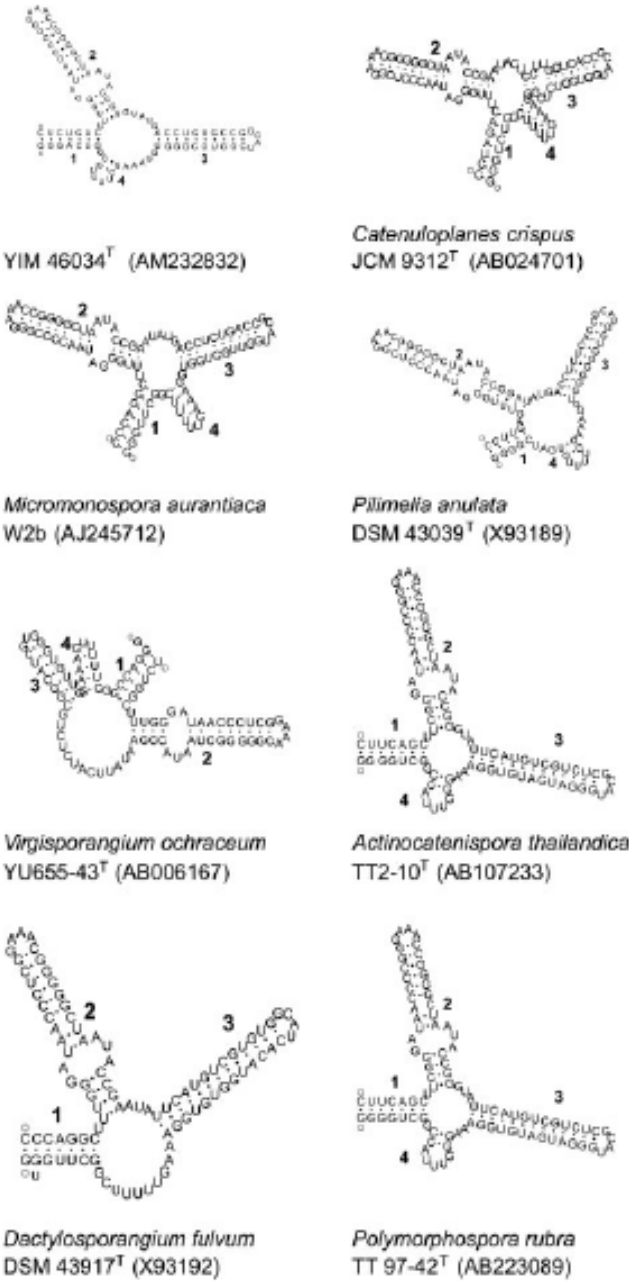


Fig. 3. Secondary structure of the V2 variable region of the 16S rRNA gene sequence of strain YIM 46034^{T} and strains of related species.

Description of *Planosporangium* gen. nov.

Planosporangium (Pla.no.spo.ran'gi.um. Gr. n. *planes* a wanderer; N.L. neut. n. *sporangium* sporangium, spore case; N.L. neut. n. *Planosporangium* wandering sporangium, referring to the production of sporangia with motile spores).

Aerobic, Gram-positive and non-acid-fast. Short and narrow sporangia. Each sporangium contains a single row of three or more straight or slightly curved rod-shaped and motile spores with a single flagellum. A second spore type is represented by globose spores on the tip of short sporophores. The cell wall contains *meso*-diaminopimelic acid and glycine as diagnostic amino acids. Whole-cell hydrolysates contain arabinose and xylose as characteristic sugars (cell-wall chemotype II). Phospholipids are of type II (phosphatidylethanolamine as major component). Major menaquinones are MK-9(H4) and MK-10(H4). Major fatty acids are anteiso- and iso-branched. The type species is *Planosporangium flavigriseum.*

Description of *Planosporangium flavigriseum* sp. nov.

Planosporangium flavigriseum (fla.vi.gri'se.um. L. adj. *flavus* yellow; L. neut. adj. *griseum* grey; N.L. neut. adj. *flavigriseum* yellowish grey, referring to the colour of substrate mycelium of the type strain).

Displays the following properties in addition to those given in the genus description. No growth on Czapek's agar, inorganic salts-starch agar (ISP4) or nutrient agar. Slow but good growth after 25 days on PDA. The substrate mycelium is extensively branched with pale grey, orange–yellow, orange or pale grey–olive colour, depending on media and culture conditions. Diffusible pigments are not formed. The surface of spores is smooth. D-Glucose, Dgalactose, D-mannose, D-arabinose, D-xylose, D-ribose, Drhamnose, sucrose, maltose, melibiose, cellobiose, raffinose, mannitol, sorbitol and galactitol are utilized, but no acid is produced from these carbon sources. Fructose, lactose, inositol, erythritol, sodium acetate, ammonium acetate, sodium citrate, urea, L-glycine, L-histidine and Lmethionine are not utilized. Negative for

gelatin liquefaction, milk coagulation and peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, production of H2S and melanin. Major fatty acids are anteiso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{17:1}\omega8c$; lesser components (>10% of total) are iso-$C_{15:0}$, $C_{18:1}\omega9c$, iso-$C_{17:0}$ and $_{C17:0}$; minor components (>5% of total) are anteiso-$C_{15:0}$, $C_{15:0}$, iso-$C_{16:1}$, $C_{16:0}$, iso-$C_{17:1}\omega9c$, anteiso-$C_{17:1}\omega9c$, iso-$C_{17:0}$, $C1_{7:0}$ 10-methyl, $C_{18:0}$ and $C_{19:1}\omega llc/C_{19:1}\omega9c$. The G+C content of the genomic DNA of the type strain is 71.4 mol%.

The type strain, YIM 46034^T (=CCTCC AA 205013^T =DSM 44991^T), was isolated from an evergreen broadleaved forest soil at Menghai, southern Yunnan Province, China.

Acknowledgements

This research was supported by the National Basic Research Program of China (project no. 2004CB719601), the Center of Marine Natural Products, which is financed by the Ministry of Science, Economic Affairs and Transport of the State of Schleswig-Holstein (Germany), the National Natural Science Foundation of China (project no. 30560001), the Yunnan Provincial Natural Science Foundation (project no. 2004C0002Q) and the Program for New Century Excellent Talents in University. We thank Xiang-Feng Cai and YunChen for their technical assistance.

References

Akutsu, T. (2000). Dynamic programming algorithms for RNA secondary structure prediction with pseudoknots. *Discrete Appl Math* **104**, 45–62.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Ara, I. & Kudo, T. (2006). Three novel species of the genus *Catellatospora*, *Catellatospora chokoriensis* sp. nov., *Catellatospora coxensis* sp. nov. and *Catellatospora bangladeshensis* sp. nov, and transfer of *Catellatospora citrea* subsp. *methionotrophica* Asano and Kawamoto 1988 to *Catellatospora methionotrophica* sp. nov., comb. nov. *Int J Syst Evol Microbiol* **56**, 393–400.

Ara, I. & Kudo, T. (2007a). *Luedemannella* gen. nov., a new member of the family *Micromonosporaceae* and description of *Luedemannella helvata* sp. nov. and L*uedemannella flava* sp. nov. *J Gen Appl Microbiol* **53**, 39–51.

Ara, I. & Kudo, T. (2007b). *Luedemannella* gen. nov. In List of new names and new combinations previously effectively, but not validly, published, Validation List no. 116. *Int J Syst Evol Microbiol* **57**, 1371–1373.

Asano, K. & Kawamoto, I. (1986). *Catellatospora*, a new genus of the *Actinomycetales*. Int J Syst Bacteriol 36, 512–517.

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

Bouthinon, D. & Soldano, H. (1999). A new method to predict the consensus secondary structure of a set of unaligned RNA sequences. *Bioinformatics* **15**, 785–798.

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

Couch, J. N. (1950). *Actinoplanes*. A new genus of the *Actinomycetales*. *J Elisha*

Mitchell Sci Soc **66**, 87–92.

De Rijk, P. & De Wachter, R. (1997). RnaViz, a program for the visualisation of RNA secondary structure. *Nucleic Acids Res* **25**, 4679–4684.

De Rijk, P., Wuyts, J. & De Wachter, R. (2003). RnaViz 2: an improved representation of RNA secondary structure. *Bioinformatics* **19**, 299–300.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol* **5**, 123–127.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005). PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33,** W557–W559.

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.

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

Kane, W. D. (1966). A new genus of *Actinoplanaceae, Pilimelia, with a description* of two species, *Pilimelia terevasa* and *Pilimelia anulata*. *J Elisha Mitchell Sci Soc* **82**, 220–230.

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.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16,** 111–120.

Kimura, M. (1983). The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press.

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.

Krasil'nikov, N. A. (1938). Ray Fungi and Related Organisms – Actinomycetales. Moscow: Akademii Nauk SSSR.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In Chemical Methods in Bacterial Systematics, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Kudo, T., Nakajima, Y. & Suzuki, K. (1999). *Catenuloplanes crispus* (Petrolini et al. 1993) comb. nov.: incorporation of the genus *Planopolyspora Petrolini* 1993 into the genus *Catenuloplanes* Yokota et al. 1993 with an amended description of the genus *Catenuloplanes*. *Int J Syst Bacteriol* **49**, 1853–1860.

Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of

actinomycetes. In Actinomycete Taxonomy (Special Publication no. 6), pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

L**echevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981).** Phospholipids in the taxonomy of actinomycetes. Zentralbl Bakteriol Hyg Abt 1 (Suppl. 11), 111–116.

Lee, S. D. & Hah, Y. C. (2002). Proposal to transfer Catellatospora ferruginea and '*Catellatospora ishikariense'* to *Asanoa* gen. nov. as *Asanoa ferruginea* comb. nov. and *Asanoa ishikariense* sp. nov., with emended description of the genus *Catellatospora*. *Int J Syst Evol Microbiol* **52,** 967–972.

Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., Ward, A. C., Bull, A. T. & Goodfellow, M. (2005). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae. Int J Syst Evol Microbiol* **55**, 1759–1766.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbancetemperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymo*l **12B**, 195–206.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Matsumoto, A., Takahashi, Y., Shinose, M., Seino, A., Iwai, Y. & Omura, S. (2003). *Longispora albida* gen. nov., sp. nov., a novel genus of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **53,** 1553–1559.

Ørskov, J. (1923). Investigation into the Morphology of the Ray Fungi.Copenhagen: Levin & Munksgaard.

Rheims, H., Schumann, P., Rhode, M. & Stackebrandt, E. (1998). *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol* **48**, 1119–1127.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20, 16.

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.

Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994). A genus of the order *Actinomycetales*, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caerulens* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caerulens* subsp. *azurens* subsp. nov. *Int J Syst Bacteriol* **44**, 193–203.

Tamura, T., Hayakawa, M. & Hatano, K. (1997). A new genus of the order *Actinomycetales*, *Spirilliplanes* gen. nov., with description of *Spirilliplanes yamanashiensis* sp. nov. *Int J Syst Bacteriol* **47**, 97–102.

Tamura, T., Hayakawa, M. & Hatano, K. (2001). A new genus of the order

Actinomycetales, *Virgisporangium* gen. nov., with descriptions of *Virgisporangium ochraceum* sp. nov. and *Virgisporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1809–1816.

Tamura, T., Hatano, K. & Suzuki, K. (2006). A new genus of the family *Micromonosporaceae*, *Polymorphospora* gen. nov., with description of *Polymorphospora rubr*a sp. nov. *Int J Syst Evol Microbiol* **56,** 1959–1964.

Thawai, C., Tanasupawat, S., Itoh, T. & Kudo, T. (2006). *Actinocatenispora thailandica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **56**, 1789–1794.

Thiemann, J. E., Pagani, H. & Beretta, G. (1967). A new genus of the A*ctinoplanaceae: Dactylosporangium* gen. nov. *Arch Mikrobiol* **58**, 42–52.

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.

Vobis, G. (1987). Sporangiate Actinoplaneten, Actinomycetales mit aero-aquatischem Lebenszyklus. *Forum Mikrobiol* **11,** 416–424 (in German).

Vobis, G. (1992). The genus *Actinoplanes* and related genera. In The Prokaryotes, 2nd edn, pp. 1029–1060. Edited by A. Balows, H. G. Tru¨per, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129,** 1743–1813.

Xu, P., Li, W. J., Xu, L. H. & Jiang, C. L. (2003). A microwave based method for genomic DNA extraction from actinomycetes. *Microbiology* [English translation of Microbiology (Beijing)] **30**, 82–84.

Yokota, A., Tamura, T., Hasegawa, T. & Huang, L. H. (1993). *Catenuloplanes japonicus* gen. nov., sp. nov., nom. rev., a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **43**, 805–812.

Chapter 6 *Promicromonospora flava* sp. nov. isolated from sediment of the Baltic Sea

Yi Jiang, Jutta Wiese, Yan-Ru Cao, Li-Hua Xu, Johannes F. Imhoff and Cheng-Lin Jiang

(Int. J. Syst. Evol. Microbiol. 59, 2009, in press)

A Gram-positive, non-spore-forming actinomycete strain CC 0387^T, was isolated from a sediment sample from the Baltic Sea, Germany. A polyphasic classification of the strain and comparative 16S rRNA gene sequencing showed that the strain belongs to the genus *Promicromonospora*, and displaying more than 3 % sequence divergence with all species of the genus *Promicromonospora* with availably published names. The strain did not produce aerial mycelium. Substrate mycelia were yellowish white to pale orange yellow and fragmented into bacillary or coccoid elements. The cell wall contained lysine and alanine. Whole cell hydrolysates contained galactose, glucose, rhamnose and ribose. The polar lipid profile consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), unknown phospholipid (PL) and unknown glycolipid (GL). The predominant menaquinone was MK-9(H_4) (86%). G+C content of DNA was 71.87 mol %. Based on morphological, chemotaxonomic, phenotypic and genetic characteristics, strain CC 0387^T (=CCTCC AA 208024^T =DSM 21481^T) is proposed as a novel species of the genus, and named as *Promicromonospora flava* sp. nov.

The 16S rRNA gene sequence of strain CC 0387^T has been deposited in EMBL under the accession number AM 992980.

The genus *Promicromonospora* was proposed by Krasil'nikov *et al*. 1961. Members of the genus are characterized by the production of substrate mycelia that fragment into bacillary or coccoid elements, no branch acids, the major menaquinone is MK-9 (H_4); G+C of DNA is 70 to 75 %. After reclassification, the species of *Promicromonospora* are *Promicromonospora aerolata* (Busse *et al*. 2003), *Promicromonospora citrea* (Krasil'nikov *et al*. 1961), *Promicromonospora sukumoe* (Takahashi *et al*. 1987), *Promicromonospora vindobonensis* (Busse *et al*. 2003), and *Promicromonospora kroppenstedtii* (Alonso-Vega *et al*. 2008). In the program on actinomycete diversity of the Baltic Sea, Germany, polyphasic taxonomy of strain CC 0387^T and comparative studies with related species and genera were carried out, and a novel species of the genus *Promicromonospora* is proposed.

Strain CC 0387^T was isolated from a sediment sample collected from the Baltic Sea, Germany, on fucose-proline medium [fucose 5 g, proline 1 g, $(NH_4)_2SO_4$ 1 g, NaCl 1 g, $CaCl_2$ 2 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, agar 20 g, natural marine water of the Baltic Sea 1000 ml, pH 7.2; nalidixic acid 20 mg/L, nystatin 100 mg/L, as the inhibitors of bacteria and fungi] incubated at 28 °C for 21 days. The strain was maintained on Medium YIM 83 (Difco marine broth 37g, peptone 2g) at 28 °C for 5 days.

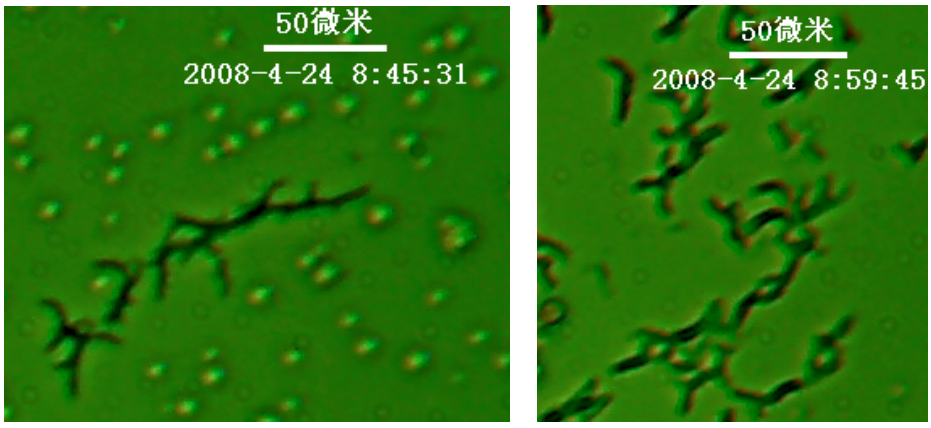


Fig. 1. Morphological characteristic of CC 0387^T

Morphology of mycelia was observed by light microscopy (Olympus microscope BH-2) and LY-SUPPER HP CCD IMAGING after incubation at 28 °C for 3 and 7 days. Cultural characteristics were determined after growth on six media for 14 days at 28 °C and by methods used in the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966). The results were recorded following incubation at 28 °C for 14 days. Colors and hues were determined according to Kelly (1964). Strain CC 0387^T was examined for a range of phenotypic properties using standard procedures (Williams *et al.*, 1983). Enzyme activities were determined by API ZYM System test kits (biomèrieux).

Biomass for molecular systematic and most of the chemotaxonomic studies was obtained after incubation of cultures in yeast extract-malt extract broth (ISP 2; Shirling & Gottlieb, 1966) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987) and NaCl 20g/L at 28 °C for 5 days with shaking. Cell walls were purified, and amino acids of peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980; Jiang *et al.*, 2001). Analysis of whole-cell sugar composition followed procedures described by Becker *et al.* (1965) and Lechevalier & Lechevalier (1980). Phospholipid analysis was carried out as described by Lechevalier *et al.* (1981). Menaquinones were determined by using the procedure of Collins *et al.* (1977). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from TSA plates [trypticase soy broth (BBL), 3% (w/v); Bacto agar (Difco), 1.5% (w/v)] that had been incubated for 5 days at 28 °C. The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990).

The chromosomal DNA of strain CC 0387^T was extracted as described by Marmur (1961). The DNA G+C content was determined by the HPLC method (Tamaoka & Komagata, 1984) with an Agilent 1000 LC system (IRIS Technologies).

Table 1. Enzyme activities of strain CC 0387^T by API ZYM assays

Enzyme	Reaction	Enzyme	Reaction
Control		Acid phosphatase	-
Alkaline phosphatase	+	Naphthol-AS-BI-phosphohydrolase	+
Esterase (C4)	-	α -galactosidase	+
Esterase lipase (C8)	+	β -galactosidase	+
Lipase (C14)	+	β -glucuronidase	-
Leucine arylamidase	+	α -glucosidase	+
Valine arylamidase	+	β -glucosidase	+
Cystine arylamidase	+	N-acetyl- β -glucosaminidase	+
Trypsin	-	α -mannosidase	-
α -chymotrypsin	-	α -fucosidase	+
Catalase	+	Oxidase	-

Phylogenetic analysis based on 16S rRNA sequences was carried out using the procedures previously described (Jiang *et al*., 2007).

Strain CC 0387^T was aerobic and Gram-positive. No aerial mycelium was produced on all 6 media tested. Substrate mycelia fragmented into non-motile, coccoid, Y-shaped, V-shaped or curve bacillary elements. No sessile spores or other spore-like elements were observed. Cultures developed weakly on Czapek's agar (Dong & Cai, 2001) but developed well on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), nutrient agar (Dong & Cai, 2001) and YIM 83. The substrate mycelium on these media was yellowish white and pale orange yellow.

Physiological and biochemical characteristics, utilization of carbon and nitrogen

sources and acid production by strain CC 0387^T is described in the description of *Promicromonospora flava* sp. nov. Enzyme activities are shown in Table 1.

The cell wall of strain CC 0387^T contained lysine and alanine. Whole cell hydrolysates contained ribose, galactose, glucose, rhamnose. The polar lipid profile consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), unknown phospholipid (PL) and unknown glycolipid (GL). The predominant menaquinone was MK-9(H_4) (86%) with minor amounts of MK-8(H_4) 6.65%, MK-9(H_0) 2.64% and MK-9(H_2) 4.80%. Composition of fatty acids and G+C content of genomic DNA are described in description of *Promicromonospora flava* sp. nov.

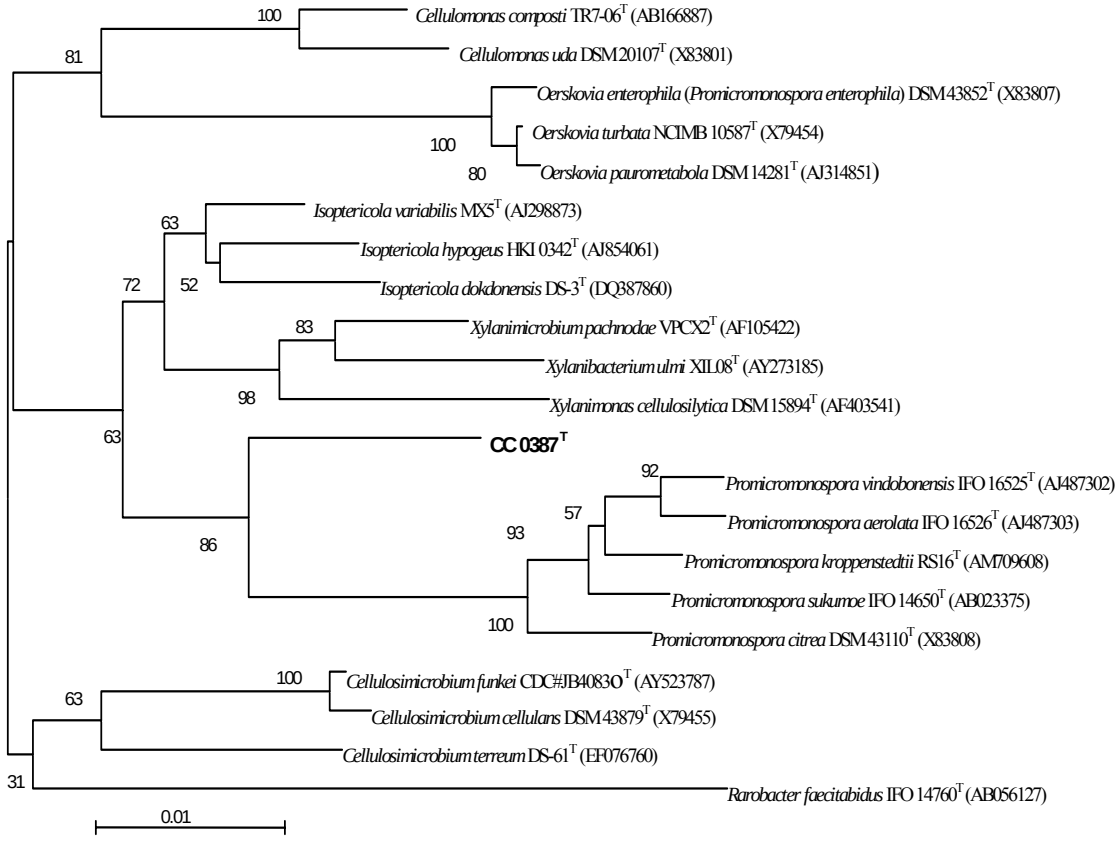


Fig. 2. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of CC 0387^T and species of related genera of the family *Promicromonosporaceae*. Numbers on branch nodes are bootstrap percentages (1000 replication). Bar, 1% sequence divergence.

The sequence of the 16S rRNA gene (1612 nucleotides) of strain CC 0387^T was determined. Accession number in EMBL is AM992980. Phylogenetic analysis including sequences of different representative members of the family *Promicromonosporaceae*. The database search (BLAST) and neighbor-joining tree showed that strain CC 0387^T belongs to the genus *Promicromonospora* (Krasil'nikov *et al*. 1961), and displayed more than 3 % sequence divergence with all availably published species of the genus *Promicromonospora*: *Promicromonospora sukumoe* IFO 14650^T (96.86% similarity) (Takahashi *et al*., 1987), *Promicromonospora aerolata* IFO 16526^T (96.54% similarity) (Busse *et al*., 2003), *Promicromonospora citrea* DSM 43110^T (96.56% similarity) (Krasil'nikov *et al*., 1961), *Promicromonospora vindobonensis* IFO 16525^T (96.55% similarity) (Busse *et al*., 2003), *Promicromonospora kroppenstedtii* RS16^T (96.56% similarity) (Alonso-Vega *et al*., 2008) (Fig. 1). The similarities between CC 0387^T and the 5 availably published species were lower than the similarities among the 5 species. The Maximum Likelihood tree supported this result (data not shown). CC 0387^T was isolated on a medium prepared with natural marine water, grew well in media containing 2 to 5 % of NaCl. CC 0387^T did not produce acid from any of 25 carbon sources tested, which distinguishes it from all other species of *Promicromonopspora*. Fatty acid components of the 5 availably published species of the genus *Promicromonospora* and strain CC 0387^T were different, especially content of several major fatty acids, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso and $C_{17:0}$ anteiso. The proportion of $C_{15:0}$ anteiso in CC 0387^T was 57.20 %., whereas in other *Promicromonospora* species it is below 51.1 %. $C_{15:0}$ iso in CC 0387^T is 16.26%, whereas it is more than 30.8% （Table 2）. To date, 18 genera actinobacteria, *Rhodococcus, Micromonospora, Streptomyces, Nocardia, Tsukamurella, Corynebacterium, Dietzia, Gordonia, Mycobacterium, Dermacoccus, Kokuria, Williamsia, Salinispora, Aeromicrobium, Salinibacterium, Verrucosispora, Aureobacterium* and *"Marinomyces"* have been isolated and characterized (Jiang *et al*., 2007) from marine samples. No member of the genus *Promicromonospora* was isolated yet from marine samples. Therefore CC 0387^T is proposed as a novel species of the genus *Promicromonospora*.

Table 2. Chemotaxonomic characteristics of 5 availably published species of the genus *Promicromonospora* and strain CC 0387 [T].

Species: 1, *Promicromonospora vindobonensis*, V45[T]; 2, P*romicromonospora aerolata*, V54A[T]; 3, P*romicromonospora sukumoe* IFO 14650[T]; 4, P*romicromonospora citrea* IFO 12397 [T]; 5, *Promicromonospora kroppenstedtii*; 6, CC 0387 [T]. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; GL, glycolipids; PGL, phosphoglycolipids; Ala, alanine; Glu, glutamic acid; Lys, Lysine; Gly, glycine; rib, ribose; gal, galactose; glu, glucose; rha, rhamnose; ND, not determined; -, non; (), %.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
Menaquinone composition	MK-9(H_4)(92) ; MK-9(H_2)(4); MK-9(H_6)(4)	MK-9(H_4)(93); MK-9(H_2)(7)	MK-9(H_4); MK-9; MK-9(H_2); MK-9(H_6)	MK-9	MK-9(H_4)(64) ; MK-9(H_6)(15) ; MK-8(H_4)(10); MK-9(H_2)(2)	MK-9(H_4) (86); MK-8(H_4) (7); MK-9(H_2) (5); MK-9 (3)
Polar lipids	DPG; PG; unknown glycolipids; phosphoglycolipids	DPG; PG; 1 unknown PL	PI; DPG	PG; PI; unidentified glucosamin-phosphlioid	PG; DPG; PI; GL; PGL	DPG; PG; PL; GL
Cell-wall comosition	Glu:Gly:Ala:Lys (0.98:0.69:2.88:1.0) (A3α)	Glu:Gly:Ala:Lys (1.05:0.43:3.4:1.0) (A3α)	Lys (A3α)	Lys-Ala (A3α)	Ala; Glu; Lys (2.3:2.0:1.0) (A4α)	Lys-Ala (A3α)
Cell-wall sugars	rha; gal; glu	rha; gal; glu	ND	gal	gal; rha	rib; gal; glu; rha
Fatty acids:						
$C_{13:0}$	1.0	-	-	-	0.17	-
$C_{14:0}$	1.5	0.8	1.3	0.6	1.78	1.87
$C_{14:0}$ iso	-	0.3	0.9	1.2	1.46	2.34

$C_{15:1}$ iso G	-	0.4	3.4	-	0.54	-
$C_{15:1}$ anteiso A	-	0.4	4.4	-	1.21	-
$C_{15:0}$ iso	36.7	30.8	36.6	40.6	39.7	16.26
$C_{15:0}$ anteiso	46.5	51.1	38.0	38.9	43.2	57.20
$C_{15:0}$	-	-	1.6	1.0	0.95	-
$C_{16:0}$ N alcohol	-	-	0.4	-	-	-
$C_{16:0}$ iso	2.2	2.2	3.9	4.0	4.41	9.08
$C_{16:0}$	6.0	3.8	2.4	0.8	1.93	3.89
Summed feature 5	2.0	1.4	-	-	-	-
$C_{17:0}$ iso	1.3	1.5	2.0	3.1	1.21	0.87
$C_{17:0}$ anteiso	3.8	6.7	4.6	9.7	3.4	8.48
$C_{18:1}$ ω 9c	-	0.5	-	-	-	-
$C_{18:3}$ ω 6c (6,9,12)	-	-	0.4	-	-	-

*Determined according to Busse *et al*. (2003) and Alonso-Vega *et al*. (2008).

Description of *Promicromonospora flava* sp. nov. (fla'va L.fem. adj. *flavus* yellow; M.L.adj. *flava* being yellowish organism)

Aerobic, Gram-positive. No aerial mycelium is produced on all 6 media tested. Substrate mycelia fragment into non-motile, coccoid, Y- shaped, V-shaped or curve bacillary elements. No sessile spores or other spore-like elements are observed. Grows weakly on Czapek's agar but develops well on ISP 2, ISP 3, ISP 4, ISP 5 and nutrient agar . The substrate mycelium on different media is yellowish white and pale orange yellow. Grows well in media containing 2 to 5 % NaCl. Gelatine liquefaction, milk coagulation and peptonization, starch hydrolysis, growth on cellulose, production of H_2S and melanin are negative. Nitrate reduction is positive. D-glucose,

ribose, sucrose, lactose, starch, trehalose, inositol, xylitol, and xanthine are utilized as sole carbon sources. No acid is produced from these carbon sources. D-Fructose, galactose, D-mannose, D-arabinose, xylose, rhamnose, maltose, melibiose, raffinose, sorbose, dextrin, mannitol, sorbitol, erythritol, esculin, sodium DL-malate, succinic acid, and glycine, histidine, lysine are not utilized. The cell wall contains lysine and alanine. Whole cell hydrolysates contain ribose, galactose, glucose, rhamnose. The phospholipids are consist of diphosphatidylglycerol, phosphatidylglycerol, unknown phospholipid and unknown glycolipid. The predominant menaquinone is MK-9(H_4). The components of major fatty acid profile are $C_{15:0}$ anteiso (57.20%) and $C_{15:0}$ iso (16.26%). The G+C content of genomic DNA is 71.87 mol % (HPLC). The type strain CC 0387^T (=CCTCC $AA208024^T$ = DSM 21481^T) was isolated from a sediment sample collected from the Baltic Sea, Germany.

Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (No. 30560001), International Cooperative Key Project of Ministry of Science and Technology (2006DFA33550), The Yunnan Provincial International cooperative Program (No. 2005GH21), the Yunnan Provincial Natural Science Foundation (No. 2004 C0002Q), The Center of Marine Natural Products, which is financed by the Ministry of Science, Economic Affairs and Transport of the State of Schleswig-Holstein(Germany). We thank Dr. Zhang Yu-qin, Miss Cai Xiang-Feng and Mr. Chen Yun for their technical assistance.

References

Alonso-Vega, P., Santamaría, R. I., Martínez-Molina E.& Trujillo M. E. (2008).

Promicromonospora kroppenstedtii sp. nov., isolated from sandy soil.*Int J Syst Evol Microbiol* **58,**1476 - 1481.

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

Busse, H. J., Zlamala,C., Buczolits, S., Lubitz, W. Kämpfer, P. & Takeuchi M. (2003). *Promicromonospora vindobonensis* sp. nov. and *Promicromonospora aerolata* sp. nov., isolated from the air in the medieval 'Virgilkapelle' in Vienna. *Int J Syst Evol Microbiol* **53,** 1503–1507 1211–1216.

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

Dong, X. Z. & Cai, M. Y. (2001). *Manual of systematics and identification of general bacteria.* Beijing: Science Press.

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* 65, 501-509.

Jiang, L. Y., Li, M. G., Li, W. J., Cui, X. L., Xu, L. H. & Jiang, C. L. (2001). Study on the application of quantitative analysis of cell-wall amino acids in actinomycetes. *Acta Microbiol Sin* **41,** 270-277.

Jiang, Y., Wiese, J., Tang,S. K. Xu, L. H. Imhoff, J. F. & Jiang C. L. (2007a). *Actinomycetospora chiangmaiensis* gen. nov., sp. nov., a new member of the family Pseudonocardiaceae. *Int J Syst Evol Microbiol* **58,** 408–413.

Jiang Y., Wiese, J., Xu, L. H., Imhoff, J. F. and Jiang C. L. (2007b). Marine actinobacteris, an important source of novel secondary metabolites with bioactivities. *Chinese J Antibiotics* **32**, 705-722.

Kalakoutskii, L. V., Agre, N. S., Prauser, H. & Evtushenko, L. I. (1989). Genus *Promicromonospora* Krasil'nikov, Kalakoutskii and Kirillova 1961a, 107AL. In Bergey's Manual of Systematic Bacteriology, vol. 4, pp. 2392–2395. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

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.

Krassilnikov, N. A., Kalakoutskii, L.V. & Kirillova, N. F. (1961). A new genus of ray Fungi—*Promicromo-nospora* gen.nov. *Izv. Akad. Nauk SSSR Ser. Biol*. **1:**107-112.

Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete taxonomy*, Special publication no. 6, pp. 227-291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.

Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. *Zentralbl Bakteriol Hyg Abt 1 Suppl* **11**, 111-116.

Takahashi, Y., Tanaka, Y., Iwai, Y. & ōmura, S. (1987). *Promicromonospora sukumoe* sp. nov., a new species of the Actinomycetales. *J Gen Appl Microbiol* **33**, 507–519.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3,** 208-218.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1-6.

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

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25,** 125–128.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129,** 1743-1813.

Chapter 7 Discussion

Up to now, about over 4,000 species of actinomycetes (actinobacteria)and 11,000 compounds produced by them were discovered from nature. Today discovery of new actinomycetes and new compounds is very difficulty indeed. In order to find new actinomycetes and provide new sources for discovery of new bioactive metabolites in this research, several key points are discussed as following.

1. Habitats of Actinomycetes

At least 90 % of microorganisms are not cultured and described yet based on the research results home and abroad. The question is how to obtain these uncultured microorganisms. Yunnan has been called “plant and animal kingdom” and is one of the richest areas of biodiversity in the world. There are 15,000 higher plants in tropical, subtropical, temperate zones and snow mountain of Yunnan, China. There is also a rich diversity of actinomycetes (Jiang *et al*., 1996).

Microorganisms under extreme environments have been gained much attention owing to their production of various natural compounds and to special mechanism adapting to extreme environments (Antranikian *et al*., 1993). Research work on Actinomycetes of extreme environments was very few before 1990 (Jiang *et al*., 1993). There are wide saline and alkaline soils, salt lakes and snow-mountains in Xinjiang, Qinghai and Gansu Provinces, China. A vast amount of strains of 24 genera of *Actinomycetes* were isolated from soil and sediment samples collected from saline and alkaline soil and lakes of three provinces. One novel family, *Yaniaceae* (Li *et al*., 2005), and six genera, *Yania, Streptomonospora* (Cui *et al*., 2001), *Jiangella* (Song *et al*., 2005), *Myceligenerans* (Cui *et al*., 2004), *Alkalibacillus* (Che *et al*., 2005) and *Naxibacter* (Xu *et al*., 2005), and many species were described and published in recent years. This indicates that there is a rich diversity of actinomycetes in saline and alkaline soil and lakes in northwestern China (Jiang *et al*., 2006).

A special issue of Antonie van Leeuwenhoek published the research achievements on marine actinomycetes until 2005. Before the 1980', the research progress on marine actinomycetes was slow. Today 18 genera of actinomycetes, *Nocardia, Corynebacterium, Dietzia, Gordona, Mycobacterium, Micromonospora, Streptomyces, Rhodococcus, Dermacoccus, Kocuria, Tsukamurella*， *Williamsia*， *Salinospora* (Mincer et al. 2002)，*Aeromicrobium* (Bruns et al. 2003), *Salinibacterium* (Han et al. 2003)，*Verrucosispora* (Riedlinger et al. 2004), *Aureobacterium* (Takami et al. 1997) and *'Marimomyces'* (Jensen et al. 2004) were isolated and described from marine habitats. This is only 10 % of the total of 180 genera of actinomyctes.

The characterization results of 204 of selected strains based on partial 16S rRNA gene sequences indicate that they belong to 33 genera including two new genera 16 new species or new subspecies, and there are rich diversity of actinomycetes in tropical rain forest and saline and alkaline soil.

Therefore primeval tropical rain forest in Yunnan and Hainan Island, saline and alkaline soil and lakes in Xinjiang, Qinghai and Gansu, China, and marine habitats, may be excellent habitats for the discovery of unknown actinomycetes.

2. Isolation methods

For many years, Nonomura's, Goodefellow's and Jiang's laboratories have made important contributions to and developments of actinomycete isolation methods. In order to find more new actinomycetes, our interest was not in the general diversity, but the discovery of un-known actinomycetes. The aim of actinomycete isolation methods is to accelerat the growth of unknown actinomycetes if possible, but to restrain the growth of fungi, other bacteria as well as known and familiar actinomycetes at the same time.

One problem in actinomycete isolation is the contamination easily of agar plates by

bacteria and fungi because growth of actinomycetes is rather slow. HV agar and mycose-proline agar for isolation of rare actinomycetes, and histidin-raffinose agar for 'rare streptomycetes' have proven to yield better results. Media containing mixture of salts (Na, K and Mg in different rates) are useful for isolation of actinomycetes from saline soils and salt lakes. In a medium with agar as single added nutrient many actinomycetes from saline soil samples can be isolated. Pathom-aree *et al*. (2006) isolated thirty-eight presumptive actinomycetes from Challenger Deep sediments (10,898 m) in the Mariana Trench by using 20 selective isolation media in the dilution plate methods. Their results indicated that most strains (22/38 strains, 58%) were isolated with raffinose–histidine agar plates, a medium designed for the isolation of 'rare' streptomycetes (Vickers *et al*. 1984). Goodfellow *et al*.(1984) (a private communication) designed over 20 media for isolation of members of *Amycolatopsis*. It is shown from the results that diversity of target is the richest in Gauze's medium 2. So it is difficult to generally judge the suitability of a medium. It is good for one sample and one purpose, but it may be not good for other samples and other purposes. Based on our experience, it is better to change the nutrient elements and proportion of the isolation medium, especially carbon sources, at any moment and everywhere. In this research, we always obtained better results by adding mixture vitamins (Hayakawa *et al.* 1987) into the isolation media.

Also the addition of nystatin and cycloheximide to isolation media in order to inhibit the growth of fungi gave better result for the isolation of actinomycetes. We recommend the use of potassium dichromate，which is a better inhibitor for fungi and bacteria. But adding anti-bacterial agents into isolation media of actinomycetes has to be made with caution because these agents may inhibit also the growth of target actinomycetes.

The isolation methods have become an important research target and are continually improved for various isolation purposes.

3. Molecular systematics

Actinomycete (actinobacterium) systematics is a part of bacterial systematics. Actinomycete systematics comes through a far-flung course of classical, chemical and molecular systematic. Waksman, the Lechevalier´s and Stackebrandt made important contributions to actinomycete systematics. Molecular systematics, especially the phylogenetic analysis based on whole sequences of the 16S rRNA gene is most important in modern actinomycete systematics.

Which strains are worth to identify?

1. Similarity of partial sequences (about 500bp) of the 16S rRNA gene of the considered strain is lower than 97 % with available species;
2. The strain grows well at 15 % $CaCl_2$, or 20% to 25% of other salts, pH >12, pH <3, >70°C, and <4°C.
3. The strain produces new bioactive metabolites.

How to demarcate a new species and genus?

The border of 16S rRNA gene sequence similarity value of a species is <97-98% (of a genus it is <94 %) and of DNA homology value lower than 70 % for the species distinction according to Xu et al (2007). But the border of different genus is different from. The border of among members of *Pseudonocardia* is lower than 94.5% (Huang et al., 2001). Data from the following Table show that strains with 99% 16S rDNA sequence similarity still have a 20 to 30 % possibility being recognized as a new species. This indicates that a strain with 97-98% sequence similarity to closely related species should be compared to the next relatives by DNA-DNA hybridization. Below 97 % sequence similarity of related strains it is generally accepted that they are recognized as different species, if additional phenotypic properties support this distinction.

Table 7-1 Relationship between 16S rDNA sequence similarity and possibility of new species

16S rDNA sequence similarity	% possibility of new species
99%	20~30
98.5%~99%	50
98%~98.5%	70~80
97%~98%	90
95%~97%	100
95%	100

4. Two new genera and two new species were described in chapter 3 to 6 here. Main characteristics of Strain YIM 0006^T were short spore chains formed directly on substrate mycelium, cell wall contained *meso*-DAP, whole-cell sugar pattern consisted of arabinose and galactose, PE, PI and PG were main phospholipids, MK-9(H_4) was predominant menaquinone and 16S rRNA sequence similarity with closed genera of the family *Pseudonocardiaceae* was below 95.24%. These features were defferent from other genera of the family. So it was proposed as a new genus of the family *Pseudonocardiaceae,* and named *Actinomycetospora chiangmaiensis*, gen. nov., sp. nov. Diagnostic features of strain YIM 46034^T were production of single spores and sporangia directly on substrate mycelia, the cell wall contained *meso*-diaminopimelic acid and glycine, whole cell hydrolysates contained arabinose and xylose, PE was the main phospholipid. The presented Neighbor-joining tree shows, that within the *Micromonosporaceae* the strain does not affiliate with the *Micromonospora* or with any described genus of this family, but branches deeply with an uncertain position related to the genera *Virgisporangium* and *Dactylosporangium* (bootstrap values <50). The Maximum Likelihood tree supported this result. Low 16S rRNA gene sequence similarity values were found by comparison of YIM46034^T to validly described representatives of these genera *Virgisporangium ochraceum* YU655-43^T (96.2%, similarity), *Virgisporangium aurantiacum* YU438-5^T (95.9%), *Dactylosporangium aurantiacum* IFO 12592^T (95.6%). So it was proposed as a new genus of the family *Micromonosporaceae,* and named *Planosporangium flavogriseum* gen nov., sp. nov.

It is indicated from the results of complete genome sequence that a streptomycete contained 20 at least gene clusters to synthesis secondary metabolites (Omura *et al*. 2001, Bentley *et al*. 2002). Above new genera and species in this research should contain new genes to synthesis new secondary metabolites. Maybe it is possible to find new compounds from these new genera and species.

5. In order to find un-known actinomycetes for providing the new sources for discovery of new leader compounds.

A. Test samples should be collected from primeval and special or extreme habitats.

B. Actinomycetes should be isolated by using new, specific and variable procedures.

C. Isolates are characterized by using fast identifying methods, especially a part of sequencing of 16S rRNA genes.

References

Aoyagi, T., Hatsu, M., Imada, C., Naganawa, H., Okami, Y. & Takeuchi, T. (1992). Pyrizinostatin: a new inhibitor of pyroglutamyl peptidase. *J Antibiotics* **45**，1795–1796.

Attwell, R. W. & Colwell, R. R. (1984). Thermoactinomycetes as terrestrial indicators for marine and estuarine waters. In: Ortiz-Ortiz L, Bojalil LF, Yakoleff V (eds) Biological, biochemical and biomedical aspects of actinomycetes. Academic Press, Orlando, pp 441–452

Berdy, J. (2005). Bioactive microbial metabolites, a personal view. *J Antibiotics* **58,** 1-26.

Bergey's Manual of Systematic Bacteriology, Second Edition, Release 5.0 May 2004.

Blunt, J. W., Copp, B. R., Munro, M. H. G., Northcote, P. T. & Prinsep, M. R. (2003). Marine natural products. *Nat Prod Rep* **20,** 1–48.

Blunt, J. W., Copp, B. R., Munro, M. H. G., Northcote, P. T. & Prinsep, M. R. (2006). Marine natural products. *Nat Prod Rep* **23**, 26–78.

Bentley, S. D., Chater, K. F., Cerdeno-Tarraga, A.-M., Challis, G. L., Thomson, N. R., James, K. D., Harris, D. E., Quail, M. A., Kieser, H., Harper, D., Bateman, A., Brown, S., Chandra, G., Chen, C. W., Collins, M., Cronin, A., Fraser, A., Goble, A., Hidalgo, J., Hornsby, T., Howarth, S., Huang, C.-H., Kieser, T., Larke, L., Murphy, L., Oliver, K., O'Neil, S., Rabbinowitsch, E., Rajandream, M.-A., Rutherford, K., Rutter, S., Seeger, K., Saunders, D., Sharp, S., Squares, R., Squares, S., Taylor, K., Warren, T., Wietzorrek, A., Woodward, J., Barrell, B. G., Parkhill J. & Hopwood, D. A. (2002). Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). *Nature* **417**, 14-147.

Bruns, A., Philipp, H., Cypionka, H. & Brinkhoff, T. (2003). *Aeromicrobium marinum* sp nov., an abundant pelagic bacterium isolated from the German Wadden Sea. *Antonie van Leeuwenhoek* **53**，1917–1923.

Bull, A. T., Stach, J. E. M., Ward, A. C., & Goodfellow. M. (2005). Marine actinobacteria: perspectives, challenges, future directions. *Antonie van Leeuwenhoek* **87,** 65-79.

Colquhoun, J.A., Mexson, J., Goodfellow, M., Ward, A. C., Horikoshi, K. & Bull, A. T. (1998). Novel rhodococci and other mycolate actinomycetes from the deep sea. *Antonie van Leuwenhoek* **74**，27–40.

Cross, T. (1981). Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *J Appl Bacteriol* **50,** 379-423.

Deming, J.W. & Yager, P. L. (1992). Natural bacterial assemblages in deep-sea sediments: towards a global view. In: Rowe G.T. and Pariente V. (eds), Deep-sea food chains and the global carbon cycle. Kluwer Academic Publishers, Dordrecht, pp. 11–27.

Feling, R. H., Buchanan, G. O., Mincer, T. J., Kauffman, C. A., Jensen, P. R. & Fenical, W. (2003). Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. *Angew Chem Int Ed* **42**, 355–357.

Goodfellow, M. & Haynes, J. A. (1984). Actinomycetes in marine sediments. In: Ortiz–Ortiz L, Bojalil LF, Yakoleff V (eds) Biological, biochemical and biomedical aspects of actinomycetes. Academic Press, Orlando, pp 453–472

Fiedler, H. P., Bruntner, C., Bull, A. T., Ward, A. C., Goodfellow, M., Potterat, O., Puder, C. & Mihm, G. (2005). Marine actinomycetes as a source of novel secondary metabolites. *Antonie van Leeuwenhoek* **87**, 37–42.

Gao, B. & Gupta, R. S. (2005). Conserved indels in protein sequences that are characteristic of the phylum Actinobacteria. *Int J Syst Evol Microbiol* **55,** 2401–2412.

Gochnauer, M. B., Leppard, G. G., Komarat, P., Kates, M., Novitsky, T. & Kushner, D. J. (1975). Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. *Can J Microbiol* **21**, 1500-1511.

Han, S. K., Nadashkovskaya, O. I., Mikhailov, V. V., Kim, S. B. & Bae, K. S. 2003. *Salinibacterium amurskyense* gen.nov., sp nov., a novel genus of the family Microbacteriaceae from the marine environment. *Int J Syst Evol Microbiol* **53,** 2061–2066.

Hatayama, K., Shoun, H., Ueda, Y. & Nakamura. A. (2005). *Planifilum fimeticolagen*.nov. and sp.nov. and *Planifilun fulgidum* sp.nov., novel members of the family 'Thermoactinomycetaceae' isolated from compost. *Int J Syst Evol Microbiol* **55,** 2101~2104.

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65,** 501-509.

Huang, Y., Wang, L. M., Lu, Z. T., Hong, L., Liu Z. H., Tan, G. Y. A. & Goodfellow, M. 2002). Proposal to combine the genera *Actinobispora* and *Pseudonocardia* in an emended genus *Pseudonocardia*, and description of

Pseudonocardia zijingensis sp. nov. *Int J Syst Evol Microbiol* **52**, 977-982.

Imada, C. & Simidu, U. (1988). Isolation and characterization of an α-amylase inhibitor producing actinomycete from marine environment. *Nippon Suisan Gakkaishi* **54**, 1839–1845.

Imada, C. & Simidu, U. (1992). Culture conditions for an α-amylase inhibitor-producing marine actinomycete and production of the inhibitor ‘‘amylostreptin’’. *Nippon Suisan Gakkaishi* **58**, 2169–2174.

Imada, C. (2005). Enzyme inhibitors and other bioactive compounds from marine actinomycetes. *Antonie van Leeuwenhoek* **87**, 59–63.

Jensen, P. R., Dwight, R. & Fenical, W. (1991). Distribution of actinomycetes in near-shore tropical marine sediments. *Appl Environ Microbiol* **57**, 1102–1108.

Jensen, P. R. & Fenical, W. (2003). Salinosporamide A: A Highly Cytotoxic Proteasome Inhibitor from a Novel Microbial Source, a Marine Bacterium of the New Genus Salinospora. *Angew Chem Int Ed* **42**, 355 − 357.

Jensen, P. R., Mincer, T. J., Williams, P. G., & Fenical W. (2005). Marine actinomycete diversity and natural product discovery. *Antonie van Leeuwenhoek* **87,** 43-48.

Jiang, C. L. & L. H. Xu (1996). Diversity of soil actinomycetes in Yunnan, China. *Appl Env Microbiol* **62,** 244-248.

Jiang Yi, Li Wen-Jun, Xu Ping, Tang Shu-Kun & Xu Li-Hua: Study on actinomycete diversity under salt and alkaline environments. *Acta Microbiologica Sinica* (Chinese) **46**：191～195，2006.

Kurtböke, D. Ipek. (2005). Actinophages as indicatore of actinomycete taxa in marine environments. *Antonie van Leeuwenhoek* **87,** 19-28.

Lechevalier, H.A. & Lechevalier, M. P. (1967). Biology of the actinomycetes. *Annu Rev Microbiol* **21,** 71-100.

Liu, Z. H., & Jiang, C. L. (2005). Modern Biology and Biotechnology of Actinomycetes (Chinese), Beijing, Science Press.

Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., Ward, A. C. & Goodfellow, M. (2005). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **55**, 1759-1766.

Mincer, J., Kauffman, C. A., Paul, R., Vickers, J. C., Williams, S. T. & Ross, G. W. (1984). Principles and application in aquatic microbiology. Heukelekian H. et al.

(eds), John Wiley & Sons, Inc., New York.

Mincer, T. J., Jensen, P. R., Kauffman, C. A. & Fenical, W. (2002). Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl Environ Microbiol* **68,** 5005–5011.

Moore, B. S., Kalaitzis J. A. & Xiang, L. K. (2005). Exploiting marine actinomycete biosynthetic pathways for drug discovery. *Antonie van Leeuwenhoek* **87**, 49-57.

Maldonado, L. A., Stach, J. E. M., Pathom-aree, W., Ward, A. C. Bull, A. T. & Goodfellow, M. (2005). Diversity of cultivable actinobacteria in geographically widespread marine sediments. *Antonie van Leeuwenhoek* **87**, 11-18.

Montalvo, N. F., Mohamed, N. M., Enticknap J. J., & Hill, R. T. (2005). Novel actinobacteria from marine sponges. *Antonie van Leeuwenhoek* **87**, 29-36.

Nonomura, H., & Ohara Y. (1957). Distribution of actinomycetes in soil. II. *Microbispora* ,a new genus of *Strepomycetaceae*. *J Ferment Technol* **35,** 307-311.

Nonomura, H. & Ohara Y. (1960). Distribution of actinomycetes in soil. IV. The isolation and classification of the genus *Microbispora*. *J Ferment Technol* **38**, 401-405.

Nonomura, H. & Ohara Y. (1960). Distribution of actinomycetes in soil.V. The

isolation and classification of the genus *Streptosporangium*. *J Ferment Technol* **38**, 405-409.

Nonomura, H. & Ohara Y. (1969). Distribution of actinomycetes in soil. VI. A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil (Part 1). *J Ferment Technol* **47,** 463-469.

Nonomura, H. & Ohara Y. (1969). Distribution of actinomycetes in soil. VII. A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil (Part 2). Classification of the isolates. *J Ferment Technol* **47,** 701-709.

Nonomura, H. & Ohara Y. (1971). Distribution of actinomycetes in soil. VIII. Green-spore group of *Microtetraspora*, its preferential isolation and taxonomic characteristic, *J Ferment Technol* **49,** 1-7.

Nonomura, H. & Ohara Y. (1971). Distribution of actinomycetes in soil. X. New species of the genus *Microbispora* and *Microtetraspora* and their isolation methods. *J Ferment Technol* **49,** 887-894.

Nonomura, H. & Ohara Y. (1971). Distribution of actinomycetes in soil. X. New genus and speices of monosporic actinomycetes, *J Ferment Technol* **49,** 895-903.

Nonomura, H. & Ohara Y. (1971). Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier et al. *J Ferment Technol* **49,** 904-912.

Nonomura, H. & Ohara Y. (1974). A new species of actinomycetes, *Thermomonospora mesouviformis* sp.nov. *J Ferment Technol* **52,** 10-13.

Nonomura, H., S. Iino & M. Hayakawa. (1979). Classification of actinomycetes of genus *Ampullariella* from soils of Japan. *J Ferment Technol* **57,** 79-85.

Nonomura, H. & Hayakawa, M. (1988). New methods for the selective isolation of soil actinomycetes. In Biology of Actinomycetes'88, Tokyo. 288-293

Ortiz, L., **Bojalil, L. F. & Yakoleff, V. (eds) (1984).** Biological, biochemical and biomedical aspects of actinomycetes. Academic Press, Orlando, pp 553–561

Omura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H., Osonoe, T., Kikuchi, H., Tadayoshi，S., Sakaki, Y. & Hattori, M. (2001). Genome sequence of an industrial microorganism Streptomyces avermitilis: Deducing the ability of producing secondary metabolites. *PNAS* **98**, 12215-12220.

Pathom-aree, W., Stach, J. E. M., Ward, A. C., Horikoshi, Koki., Bull A. T. & Goodfellow, M. (2006). Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. *Extremophiles* **10,** 181–189.

Rheims, H., Schumann, P., Rohde, M. & Stackebrandt, E. 1998. *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst. Bacteriol* **48**, 1119-1127.

Riedlinger, J., Reicke, A., Krismer, B., Zahner, H., Bull, A. T., Maldonado, L. A., Goodfellow, M., Bister, B., Bischoff, D., Süssmuth, R. D. & Fiedler, H-P. (2004). Abyssomicins, inhibitors of para-aminobenzoic acid pathway produced by the marine *Verrucosispora* strain AB-18–032. *J Antibiot* **57,** 271–279.

Shiomi, K., Iinuma, H., Naganawa, H., Hamada, M., Hattori, S., Nakamura, H. & Takeuchi, T. (1990). New antibiotic produced by *Micromonospora globosa*. *J Antibiot* **43**, 1000–1005.

Stach, E.M. & Bull, A. T. (2005). Estimating and comparing the divesity of marine actinobacteria. *Antonie van Leeuwenhoek* **87,** 3-9.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. (1997). Proposal for a New Hierarchic Classification System, *Actinobacteria* classis nov. *Int J Syst Evol Microbiol* **47,** 479–491.

Taechowisan, T., Peberdy, J. F. & Lumyong, S. (2003). Isolation of endophytic actinomycetes from selected plants and their antifungal activity. *World J Microbiol Biotechnology* **19,** 381-385.

Takami H., Inoue A., Fuji F. & Horikoshi K. 1997. Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS Microbiol Lett* **152**, 279–285.

Vickers, J. C., Williams, S. T. & Ross, G. W. (1984). A taxonomic approach to selective isolation of streptomycetes from soil. In: Ortiz–Ortiz L, Bojalil LF, Yakoleff V (eds) Biological, biochemical and biomedical aspects of actinomycetes. Academic Press, Orlando, pp 553–561.

Ward, A. C., Horikoshi, K. & Bull, A. T. (2006). Diversity of actinomycetes isolated from Challenger Deep sediment (10898 m) from the Mariana Trench. *Extremophiles* **10**, 181-189.

Waksman S. A. 1950. The *Actinomycete*s. Their nature, occurrence, activities, and importances. Chronica Botanica Co. Waltham, Mass.

Xu, L. H., C. L. Jiang & G. Y. Guo (1987). The investigation on actinomycete populations and resources in some area in Yunnan. III. Actinomycete population in Xishuangbanna. ACTA Microbiologica Sinica, **27**, 173-177.

Xu, L. H. & C. L. Jiang. (1996). A study on diversity of aquatic actinomycetes in lakes of the middle plateau, Yunnan, China.. *Appl Env Microbiol* **62**, 249-253.

Xu, L. H., Li, W. J., Lou, K., Liu Z. H. & Jiang, C. L. (2007). Actinomycete systematics. Academic Press, Beijing.

Curriculum Vitae

Personal Information

Name	Yi Jiang
Citizenship	Chinese
Address	Leibniz-Institut für Meereswissenschaften IFM-GEOMAR Düsternbrooker Weg 20 D-24105 Kiel Germany
Born	10th of August 1978, in Kun Ming, P.R. China
Marital status	single

University History:

2004-2009	PhD student at the Leibniz Institute for Marine Sciences IFM-GEOMAR, Kiel; in the research division three, Marine Microbiology. Supervisor: Prof. J.F. Imhoff. Thesis title: Systematic Research in Actinomycetes with some Bioactivities
2001-2004	M.Sc. student at the University of Yun Nan, P.R. China, at the Yun Nan Institute of Microbiology, Lab of Actinomycetal Taxonomy. Supervisor: Prof. Li-Hua Xu and Dr. Wen-Jun Li Thesis title: Screening and systematic research on actinomycetes producing bioactiv metabolites
2001	Special course in High Throughput Screening (HTS) at the National Center for Pharmaceutical Screening, Institute of Materia Medica Chinese Academy of Medical Sciences & Peking Union Medical College, P.R. China. Supervisor: Prof. Dr. Du Guan Hua
1997-2001	Student at the Yun Nan University, P.R. China. Major subject: Biotechnology B.Sc. thesis conducted in the Yun Nan Institute of Microbiology, Lab of Actinomycetal Taxonomy. Supervisor: Dr. Zhang Li-Min

School History:

1994-1997	Highschool in Kun Ming No. 10 Middle school, Yun Nan Province, P. R. China Degree: High School certificate for starting University
1991-1994	Middle School in Yun Nan University Second School in Kun Ming, Yun Nan Province, P.R. China
1986-1991	Primary School appertained to Yun Nan Normal University in Kun Ming; Yun Nan Province, P.R. China.

List of Publications:

2009

40. **Jiang Yi**, Cao Yan-Ru, Jutta Wiese, Lou Kai, Zhao Li-xing, Johannes F. Imhoff and Jiang Cheng-lin: A new approach of research and development on pharmaceuticls from actinomycetes. J. Life Science US, 2009, in press.
39. Zheng Dan, **Jiang Yi**, Han Li, Lou Kai, Duan Ruo-Ling, Xu Li-Hua and Huang Xue-Shi: A study on secondary metabolites of an actinomycete strain. Chinese J. Medical chemistry, 2009, in press**.**
38. **Jiang Yi**, Jutta Wiese, Cao Yan-Ru, Xu Li-Hua, Johannes F. Imhoff and Jiang Cheng-Lin: *Promicromonospora flava* sp. nov., isolated from sediment of the Baltic Sea. Int J Syst Evol Microbiol, 2009, in press.
37. Cao Yan-Ru, **Jiang Yi**, Xu Li-Hua and Jiang Cheng-Lin: *Sphaerisporangium flaviroseum* sp. nov. and *Sphaerisporangium album* sp. nov., two new species of the genus *Sphaerisporangium* isolated from forest soil in China. Int J Syst Evol Microbiol, 2009, in press.
36. Cao Yan-Ru, **Jiang Yi**, Wu Jin-Yuan, Xu Li-Hua and Jiang Cheng-Lin: *Actinopolymorpha alba* sp.nov. isolated from a rhizosphere soil. Int J Syst Evol Microbiol, 2009, in press.
35. Cao Yan-Ru, **Jiang Yi** and Xu Lih-ua:Actinomycete composition and bioactivities in Grand Shangri-La. Acta Microbiol. Sinica, 49:105-109, 2009:

2008

34. Li Yi-qing, Huang Xue-shi, Keishi Ishida, Armin Maier, Gerhard Kelter, **Jiang Yi**, Gundel Peschel, Klaus-Dieter Menzel, Li Ming-gang, Wen Meng-liang, Xu Li-hua, Susanne Grabley, Heinz-Herbert Fiebig, Jiang Cheng-lin, Christian Hertweck and Isabel Sattler: Plasticity in gilvocarcin-type *C*-glycoside pathways: discovery and antitumoral evaluation of polycarcin V from *Streptomyces polyformus*. Org Biomol Chem. **6**: 3601–3605, 2008
33. Valentina Gargiulo, Cristina De Castro, Rosa Lanzetta, **Jiang Yi,** Xu Li-Hua, Jiang Cheng-Lin, Antonio Molinaro, Michelangelo Parrilli: Structural elucidation of the capsular polysaccharide isolated from Kaistella flava. Carbohydrate Research 343：2401-2405, **2008.**
32. Cao Yan-Ru, **Jiang Yi**, Chen Yi-guang, Tan Shu-kun, Qin Sheng, Zhao Guo-zhen and Xu Li-hux: Diversity of actinomycetes in Wuling Mountain. Acta Microbiol. Sinica, 48: 952-958，**2008.**
31. Jutta Wiese, **Jiang Yi,** Tang Shu-Kun, Vera Thiel, Xu Li-Hua, Johannes F. Imhoff and Jiang Cheng-Lin**:** A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov. Int J Syst Evol Microbiol, 58: 1324–1331，**2008.**
30. **Jiang Yi,** Jutta Wiese, Tang Shu-Kun, Xu Li-Hua, Johannes F. Imhoff and Jiang Cheng-Lin: *Actinomycetospora chiangmaiensis* gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*. Int J Syst Evol Microbiol. 58: 408-413, **2008.**

29. **Jiang Yi,** Xu Ping, Lou Kai, Xu Li-Hua1and Liu Zhi-Heng: Problem and Countermeasure on Development of Pharmaceuticals from Actinomycete Resources. Microbiology, 35(2): 272-274, **2008.**

2007

28. Yang Ying, Jiang Yi, Yin Min, Xu Ping, Luo Tao and Xu Lihua: Synthetic gemone of secondary metabolites of actinomycetes. J. Microbiol. 27(6): 68-71, 2007.
27. Chen Yi-guang, **Jiang Yi**, Li Wen-jun, Cui Xiao-long and Xu Li-hua: Microbial diversity and screening of antitumor activity of actinomycete strains from saline and alkaline environments in the Qinghai Province, P. R. China. Acta Microbiologica Sinica, 47(5): 757-762, **2007.**
26. **Jiang Yi,** Jutta Wiese, Xu Li-Hua, Johannes F. Imhoff and Jiang Cheng-Lin: Marine actinobacteria, an important sources of novel secondary metabolites with bioactivities. J. Chinese Antibiotics, 32(12): 705-722, **2007.**
25. Tang Shu-kun, **Jiang Yi**，Zhi Xiao-yang, Lou Kai, Li Wen-jun and Xu Li-hua: Isolation methods of halophilic actinomycetes, Microbiology, 34(2): 390-392, **2007.**
24. He Jian, Ernst Roemer, Corinna Lange, Xueshi Huang, Armin Maier,Gerhard Kelter, **Jiang Yi**, Xu Li-hua, Klaus-Dieter Menzel,Susanne Grabley, Heinz-Herbert Fiebig, C Jiang heng-Ling and Isabel Sattler: Structure, Derivatization, and Antitumor Activity of New Griseusins from *Nocardiopsis* sp. *J. Med. Chem.*. 50, 5168-5175, **2007.**
23. Alb Silipo, Antonio Molinaro, Jiang Cheng-Lin, **Jiang Yi,** Xu Ping, Xu Li-Hua, Rosa Lanzetta and Michelangelo Parrilli: The O-chain structure from the LPS of the bacterium Naxibacter alkalitolerans YIM 31775^{T}. Carbohydrate Research 342: 757–761, **2007.**
22. **Yi Jiang,** Shu-Kun Tang, Jutta Wiese, Li-Hua Xu, Johannes F. Imhoff, and Cheng-Lin Jiang：*Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces***.** Int J Syst Evol Microbiol. 57: 2694-2698, **2007.**
21. **JiangYi**, Tang Shukun, Zhang Yuqin, Lou Kai and Xu Lihua: Bioactive metabolites from actinomycetes. Microbiology. 34(1):188-190, **2007**.
20. Peng Yun-Xia, **Jiang Yi,** Duanshu-Rong, Li Wen-Jun and Xu Li-Hua: Selective isolation methods of rare actinomycetes. J. Yunnan Univ. 29(1): 86-89, **2007.**

2006

19. **Jiang Yi,** Tang Shu-Kun, Wang Yong-Xia, Li Wen-Jun and Xu Li-Hua: Isolation methods of marine actinomycetes. Microbiology, 33(6): 153-155, **2006.**
18. Chen Yi-Guang, **Jiang Yi,** Cui Xio-Long, Li Wen-Jun, Tan Sh-Kun, Wen Meng-Liang, Jiang Cgeng-Lin and Xu Li-Hua: Isolation and phylogenetic analysis of one moderately halophile YIM 80186 with strong anticancer activity. J. Yunnan Univ. 28(5): 444-449, **2006.**

17. **Jiang Yi,** Tang shu-Kun, Xu Li-Hua, J F Imhoff, Liu Zhi-Heng and Jiang Cheng-Lin: Alanysis of secondary structure of the family *Yaniaceae*. Microbiology, 33(5):173-179, 2006.
16. Chen H. H., Yang Y., **Jiang Y.**, Tang S. K. and Xu L. H.: Isolation methods of plant endo-phytic actinomycetes. Microbiology, 33(4):182-185, **2006.**
15. Chen Guo-Zhong, **Jiang Yi,** Tang Shun-Kun, Li Wen-Jun and Xu Li-Hua: Application of analysis for variable regions of secondary structure of 16S rRNA in actinomycete classification. Microbiology, 33(2)：184－187，**2006.**
14. **Jiang Yi,** Li Wen-Jun, Xu Ping, Tang Shu-Kun and Xu Li-Hua: Study on actinomycete diversity under salt and alkaline environments. Acta Microbiologica Sinica. 46:191-195，**2006.**
13. Xu P., Li W. J., Tang S.K., **Jiang Y.,** Gao H.Y., Xu L.H. and Jiang C.L.: *Nocardia lijiangensis* sp. nov., a novel actinomycete strain isolated from soil in China. Syst. Appl. Microbiol. 29 (4), 308-314, **2006.**
12. Zhang Yu-Qin, Li Wen-Jun, Zhang Ke-Yun, Tian Xin-Peng, **Jiang Yi,** Xu Li-Hua, Jiang Cheng-Lin and Lai Ren**:** *Massilia dura* sp. nov., *Massilia albidiflava* sp. nov., *Massilia plicata* sp. nov. and *Massilia lutea* sp. nov., isolated from soils in China. Int. J. Syst. Evol. Microbiol.. 56: 459-463, **2006.**
11. **Jiany Yi,** Duan Shu-Rong, Tang Shu-Kun, Cheng Hua-Hong, Li Wen-Jun and Xu Li-Hua: Isolation methods of rare actinomycetes. Microbiology, 33(1)：181－183，**2006.**

2005

10. **Jiang Yi**, Yang Ying, Chen Hua-Hong, Li Wen-Jun, Xu Li-Hua and Liu Zhi-Heng: Plant endophytic microorganism resources. Microbiology，32(6):146-147, **2005.**
9. Xu Ping , Li Wen-Jun, Tang Shu-Kun, **Jiang Yi**, Chen Hua-Hong, Xu Li-Hua and Jiang Cheng-Lin：*Nocardia polyresistens* sp. nov. Int. J. Syst. Evol. Microbiol. 55: 1465-1470, **2005.**
8. Li-Hua Xu, **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang and Jiang Cheng-Lin**:** *Streptomyces roseoablus* sp. nov., an antimicrobial actinomycetes from soil in Yunnan, China**.** Antonie van Leeuwenhoek 87:215-220, **2005.**
7. **Jiang Yi**, Li Wen-Jun, Li Ming-Gang, Wen Meng-Liang, Du Guan-Hua, Xu Li-Hua and Jiang Cheng-Lin*: Streptomyces nanningensis* sp. nov., a new streptomycete with inhibitor activity against acetylcholinesterase**.** Antonie van Leeuwenhoek 87:189-194, **2005.**

2004

6. Li Wen-Jun, **Jiang Yi,** Kroppenstedt R. M., Xu Li-Hua and Jiang Cheng-Lin: *Nocardia alba* sp. nov., a novel actinomycete strain isolated from soil in China. System. Appl. Microbiol. 27: 308-312, **2004.**
5**. Jiang Yi,** Li Wen-jun, Wu Wen-long, Peng Yun-xia, Li Ling-feng, Yang Hui-min, Xu Li-hua and Jiang Cheng-lin: Polyphasic classification of

three new species of the genus *Streptomyces.* J. Yunnan Univ. 26：215-223, **2004.**

4. **Jiang Yi,** Du Guan-hua, Sun Qiu, Peng Yun-xia, Li Ling-feng, Xu Li-hua: High throuphput screening of bioactive substances from actinomycetes. J. Yunnan Univ. 26(4): 364-366，**2004.**
3. Wang D., Li W. J., Zhang Y. Q., **Jiang Y.,** Xu L. H. and Jiang C. L: A study on polyphasic taxonomy of one antifungal actinomycete strain YIM 31530^{T}. J. Yunnan Univ. 26(3):265-269, **2004.**
2. Li W. J., **Jiang Y.,** Wang D., Kroppenstedt R. M.., Xu L. H. and Jiang C. L: Isolation and identification of one actinomycete strain with strong activity restraining No synthesize enzyme. J. Yunnan Univ. 26(3):256-260, **2004.**
1. **Jiang Yi,** Li Wen-jun, Cui Xiao-long, Zhang Hua, Xu Li-Hua and Jiang Cheng-lin: Polyphasic classification of *Streptomyce bannaenesis* sp. nov. J. Yunnan Univ. 26(2):179-182, **2004.**

Book:

Xu Li-Hua, Li Wen-jun, Liu Zhi-heng and Jiang Cheng-lin: 《Actinomycete systematic》, Chapter 8, 9, 12, 13. ed. by **Jiang Yi,** Academic Press, Beijing, 2007.

Prize:

Li Wen-jun, Zhang Yu-qin, Tang Shu-kun, Zhi Xiao-yang, **Jiang Yi** et al: The study on actinomycete taxonomy, **First Grade**, Yunnan Provincial Prize for Natural Science, Yunnan Provonce Goverment, 2008.

Zhou Zhi-yu, Xu Li-hua, **Jiang Yi**, Huang Bi-ping, Duan Ruo-ling, Liang Zeng-tai and Wang Jian-ming: Industrial production and application of potassium bacterium fertilizer, **Second Grade**, Kunming City Prize for Science and Technology, Kunming Government, Sept 3, 2007.

Patents:

8. Name: Streptimonosporamycin

Inventors: Li Ming-Gang, Wen Meng-Liang, **Jiang Yi**, Li Yi-Qing, Xu Li-Hua and Jiang Cheng-Lin
Activities: Anti-oxidation
Number of Application: 02142361.X
Date of Application: September 19, 2002
Patent Number: ZL 02 1 42361.X
Authorization Date: June 29, 2005

7. Name: *Streptomyces nanningemsis*

Number of Strain: YIM33098
Inventors: Xu Li-Hua, **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang, and Jiang Cheng-Lin
Activities: Inhibitions against AChE
Number of Application: 03124071.2
Date of Application: 2003,4,29
Patent Number: ZL 03 1 24071.2
Authorization Date: June 21, 2006

6. Name: *Streptomyces pleomorphus*

Number of Strain: YIM32065
Inventors: Jiang Cheng-Lin, **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang, and Xu Li-Hua
Activities: Inhibitions against AChE
Number of Application: 03124238.3
Date of Application: 2003, 4, 29
Patent Number: ZL 03 1 24238.3
Authorization Date: 2006,7,26

5. Name: *Streptomyces roseoalbus*

Number of Strain: YIM33634
Inventors: Xu Li-Hua, **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang, and Jiang Cheng-Lin
Activities: Anti-fungi (*Botrytis* sp., *Pyricularia oryzae, Sphaerotheca fuliginea*,etc）
Number of Application: 03124239.1
Date of Application: 2003, 4, 29
Patent Number: ZL 03 1 24239.1
Authorization Date: July 12, 2006

4. Name: *Streptomyces griseoalbus*

Number of Strain: YIM30823
Inventors: Jiang Cheng-Lin, **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang and Xu Li-Hua
Activities: Insecticide against *Aphis medicaginis, Leneania separata and Tetranychus cinnabarius*
Number of Application: 03124240.5
Date of Application: 2003,4,29
Patent Number: ZL 03 1 24240.5
Authorization Date: April 26, 2006

3. Name: *Streptomyces antifungus*

Number of Strain: YIM35551
Inventors: **Jiang Yi**, Li Wen-Jun, Wen Meng-Liang, Li Ming-Gang, Xu Li-Hua, and Jiang Cheng-Lin
Activities: Anti-fungi (*Botrytis* sp., *Pyricularia oryzae, Gibberella saubinetii*, etc)
Number of Application: 03124237.5
Date of Application: 2003,4,29
Patent Number: ZL 03 1 24237.5
Authorization Date: June 21, 2006

2. Name: *Nocardia alba*

Number of Strain: YIM30243
Inventors: Li Wen-Jun, **Jiang Yi**, Wen-Meng-Liang, Li Ming-Gang, Xu Li-Hua, Jiang Cheng-Lin
Bioctivity: Inhibition against NO synthetase
Number of Application: 03124075.5
Date of Application: 2003,4,29
Patent Number: ZL 03 1 24075.5
Authorization Date: June 21, 2006

1. Name *Kribbella antibiotica*

Noumber of Strain: YIM31530
Inventors: Li Wen Jun, **Jiang Yi**, Wen-Meng-Liang, Chen Qi-Gang, Li Ming-Gang, Xu Li-Hua, Jiang Cheng-Lin
Bioactivity: Anti-*botrytis sp., Rhizoctonia solani, Pyricularia oryzae etc.*
Number of Application: 03124068.2
Date of Application: 2003,4,29
Patent Number: ZL 03 1 24068.2
Authorization Date: June 21, 2006

Yi Jiang

Medium list

Media of Actinomycete Isolation

1, Chitin agar (Hsu and Lockwood, 1975. for isolation of aquatic act)

Colloidal chitin 2g (solution in 10％NaOH); K_2HPO_4 0.7g; KH_2PO_4 0.3g; $MgSO_4 \cdot 7H_2O$ 0.5g; $FeSO_4$ 0.1g; $ZnSO_4$ 0.001g; $MnCl_2$ 0.001g; agar 20g., pH 7.0.

2, HV agar (Hayakawa 1990, Actinomycetologica 4:95-102, 1990): Humic acid 1.0g, Na_2HPO_4 0.5g, KCl 1.7g, $MgSO_4 \cdot 7H_2O$ 0.05g, $FeSO_4 \cdot 7H_2O$ 0.01g, $CaCl_2$ 1g, distilled water 1 liter, agar 18g, vitamins mixture (0.5mg each of thiamine-HCl (B1), riboflavin, niacin, pyridoxin, Ca-pantothenate, inositol, p-aminobenzoic acid, and 0.25mg of biotin) , pH 7.2.

3, Mycose-proline agar: mycose 5g，proline 1g，$(NH_4)_2SO_4$ 1g, NaCl 1g, $CaCl_2$ 2g, K_2HPO_4 1g, $MgSO_4 \cdot 7H_2O$ 1g, agar 20g, pH 7.2.

4, Modified Glycerol-Asparagine agar: glycerol 10g, asparagine 1g, $K_2HPO_4 \cdot H_2O$ 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, $CaCO_3$ 0.3g, agar 20g, pH 7.2.

5, Modified Gauze's medium 2: glucose 10 g; peptone 5 g; tryptone 3 g; NaCl 5 g; distilled water 1 liter; agar 15 g; pH 7.0.

6, Starch-casein：Starch 10g, casein 3g, KNO_3 2g, $MgSO_4 \cdot 7H_2O$ 0.05g, K_2HPO_4 2g, $CaCO_3$ 0.02g, $FeSO_4$ 10mg, $NaCl_2$ 150g, agar 20g, pH 7.2.

7, Modified Starch-casein: Glucose 10g, casein 0.3g, KNO_3 2g, $MgSO_4 \cdot 7H_2O$ 0.05g, K_2HPO_4 2g, $CaCl_2$ 1g, $FeSO_4$ 10mg, Mixture of salts ($MgCl_2$, NaCl, KCl) 150g, distilled water 1 liter; agar 20 g, pH 7.2.

8, M1 medium：Starch 10g，yeast ext. 4g，peptone 2g，agar 18g, sea water1000ml, pH 7.2.

9, M2 medium：Glycerol 6ml，arginine 1g，K_2HPO_4 0.5g, $MgSO_4$ 0.5g，agar 18g，sea water1000ml, pH 7.2.

10, M3 medium: glucose 2g，agar 18g，sea water 1000ml, pH 7.2.

11, Histidin-raffinose agar

Histidin 1g, raffinose 5g, $K_2HPO_4 \cdot 3H_2O$ 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, Rifampin 25mg, agar 20g, PH 7.7.

12, Modified Gauze's （Medium 2: Glucose 10 g; peptone 5 g; tryptone 3 g; NaCl 5 g; distilled water 1 liter; agar 15 g; pH 7.0; supplemented with filter sterilised solutions of cycloheximide (50 mg/L), nalidixic acid (10mg/L), novobiocin (10 mg/L) and nystatin (100 mg/L).

***Trace salt**: $FeSO_4 \cdot 7H_2O$ 0.2g; $MnCl_2 \cdot H_2O$ 0.1g; $ZnSO_4 \cdot 7H_2O$ 0.1g; distilled water 100ml.

Reproduction medium (slant medium)

YIM 38 medium: malt ext. 10g, yeast ext. 4g, glucose 4g, trace salts 1 ml, vitamins mixture of HV (Hayakawa & Nonomura, 1987) [0.5mg each of thiamine-HCl (B1), riboflavin, Niacin, pyridoxin, Ca-pantothenate, inositol, p-aminobenzoic acid, and 0.25mg of biotin], distilled water 1 liter; agar 20 g.

YIM 381, For marine actinomycetes (2007,1,18)

Marine Broth 20g, glucose 2, malt wxt. 2g, peptone 1g, B-vitamins [0.5mg each of thiamine-HCl(B1), riboflavin, Niacin, pyridoxin, Ca-pantothenate, inositol,p-aminobenzoic acid, and 0.25mg of biotin], pH 7.2 (may add trace salt 1ml)

YIM 382，For Actinobispora and other Actinomycetes

yeast ext 4g; glucose 4g; malt ext 5g; peptone 2g; B-vitamins [0.5mg each of thiamine-HCl(B1), riboflavin, Niacin, pyridoxin, Ca-pantothenate, inositol,p-aminobenzoic acid, and 0.25mg of biotin], pH 7.2(may add trace salt 1ml)

Media for Cultural characteristics

1, Czapek's agar

Sucrose 30g; $NaNO_3$ 2g; K_2HPO_4 1g; $MgSO_4 \cdot 7H_2O$ 0.5g; KCl 0.5g; $FeSO_4$ 0.01g; agar 20g; pH7.2-7.4. 8^b 30min.

2, Calcium malate agar

Glucose 20g; Cacium malate 10g; NH_4Cl 0.5g; agar 15g; pH7.2-7.4. 8^b 30min.

3, Glycerol asparagines (ISP 5)

L-asparagine 1g; glycerol 10g; K_2HPO_4 1g; trace salt 1ml; agar 20g; PH 7.2-7.4.

4, Yeast ext-Malt ext agar (ISP 2)

Yeast ext 4g; glucose 4g; malt ext 10g; agar 20g. pH 7.3.

5, Peptone-yeast ext-Fe agar (ISP 6)

Peptone 15 g; tryptone peptone 5g; Ammonium citrate 0.5g; Sodium thiosulohate 0.09g, K_2HPO_4 1g; yeast ext 1g; agar 15g. pH 7-7.2.

6, Tyrosine agar (ISP 7)

Glycerol 15g; L-asparagine 1g; L-tyrosine 05g; K_2HPO_4 0.5g; NaCl 0.5g; $MgSO_4 \cdot 7H_2O$ 0.5g; $FeSO_4 \cdot 7H_2O$ 0.01g; trace salt 1ml; agar 20g. pH 7.2.

7, Oatmeal agar

Oatmeal 20g (boring 20min, filtered) trace salt 1ml; agar 20g. pH7.2.

8, Potato ext agar

Potato 200g (1000ml water, 80℃ 1hr. filtrated); glucose 10g-20g; agar 20g. pH 7.4.

9, Inorganic salt-starch agar (ISP 4)

Sol starch 10g; K_2HPO_4 1g; $MgSO_4 \cdot 7H_2O$ 1g; NaCl 1g; $(NH4)_2SO_4$ 2g; $CaCO_3$ 2g; Trace salt 1ml; agar 20g. pH 7-7.4.

Media for Physiology and Biochemistry

1, Gelatin liquefication

Peptone 5g; glucose 20g; gelatin 200g

2, Milk test

Milk+0.02% $CaCO_3$, 4000rpm/min 30min. remove fatty

3, Hydrolysis of starch

Starch 10g; $MgSO_4$ 1g; KNO_3 1g; K_2HPO_4 0.3g; NaCl 0.5g; agar 15g; pH7.2.

5, Production of H_2S

Peptone 10g; Fe-citrate 0.5g; agar 15g. pH 7.2. 8^b 30min.

6, Production of melanin

Yeast ext 1g; L-tyrosine 1g; NaCl 8.5g; agar 15g.

7, Degradation of cellulose

$MgSO_4$ 0.5g; K_2HPO_4 0.5g; KNO_3 1g; NaCl 0.5g; filter paper strip.

8, Basic medium for utilization of carbon

$(NH_4)_2SO_4$ 2.64g; KH_2PO_4 2.38g; K_2HPO_4 5.65g; $MgSO_4 \cdot 7H_2O$ 1g; $CuSO_4 \cdot 5H_2O$ 0.0064g; $FeSO_4 \cdot 7H_2O$ 0.0011g; $MnCl_2 \cdot 4H_2O$ 0.0079g; $ZnSO_4 \cdot 7H_2O$ 0.0015g; Bromocresol purple 0.4％ 1.5ml to detect production of acid from Carbon source.

9, **Reduction of NO_3**

Peptone 10mg; beef ext 3g; NaCl 5g; KNO_3 1g; pH 7-7.4.

Agent A: p-aminobenzene sulfonic acid 0.5g； 10％ nitric acid 150ml.

B：diphenylamine 0.5g in 100ml fulfuric acid，water 100ml.

10, For pH requirement

Glucose 10g; L-asparagin 0.5g; K_2HPO_4 5.45g; pH 4.5.

11, Urease

1) Medium: peptone 1g; NaCl 5g; glucose 1g; KH_2PO_4 2g; phenol red 0.12g (or 6ml 1:500 solution); agar20g; pH6.8-6.9 (pale yellow) 30％ urea, 55℃ put into medium, urea=2％.

2) Observation culture for 20hr.

Abbreviations

A_2pm （LL-DAP）, diaminopimelic acid

Ara, arabinose

DPG, diphosphatidyl glycerol

Gal, galactose

Glu, glucose;

GluNU, Phospholipids of unknown structure containing glucosamine

Gly, glycine

Mad, madurose

Man, mannose

Meso-DAP, meso- diaminopimelic acid

MK-, menaquinone

ND, not determined

PC, phosphatidyl choline

PE, phosphatidyl ethanolamine

PG, phosphatidyl glycerol

PI, phosphatidyl inositol

PIM (PIM’S), phosphatidyl inositol mannosides

PME, phosphatidyl methyl ethanolamine

Rha, rhamnose

Rib, ribose

Xyl, xylose

Actinostreptospora chiangmaiensis gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*

Yi Jiang,[1,2] J. Wiese[1], S.-K. Tang[2], L.-H. Xu[2], J. F. Imhoff [1] and C.-L. Jiang[2]

1. Zentrum für Marine Wirkstoffe und IFM-GEOMAR, D-24105 Kiel.

2. Yunnan Institute of Microbiology, Kunming, 650091, P.R. China

Introduction

A novel actinomycete strain YIM 0006^T was isolated from a soil sample of tropical rain forest in northern Thailand. The strain was characterized by using a polyphasic taxonomic approach. The organism had morphological and chemotaxonomic properties consistent with the family *Pseudonocardiaceae*. Phylogenetic analysis and the phenotypic characteristics showed that strain YIM 0006T can be phylogenetically distinguished from all genera of the family *Pseudonocardiaceae*. Distinct feature of YIM 0006^T are: absence of aerial mycelium, short spore chains were directly formed on substrate mycelium, cell wall contains *meso*-DAP, whole-cell sugar pattern consists of arabinose and galactose (cell wall chemotype IV), phosphatidyl choline and phosphatidyl inositol are main phospholipids, MK-9(H_4) is the predominant menaquinone and the G+C content of genomic DNA is 69.0 mol %. A new genus with the name *Actinostreptospora* gen. nov. is proposed. The type species of this new genus is *Actinostreptospora chiangmaiensis* sp. nov., and the type strain is YIM 0006^T (= CCTCC AA 205017^T = DSM 45062^T).

Results

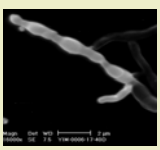


Fig. 1. Scanning electron micrograph of fragments of vegetative mycelium and short spore chains of strain YIM 0006T on HV medium (Hayakawa, 1987) after incubation for 60 days.

Table 1. Cultural characteristics of strain YIM 0006^T

Medium	Growth	Substrate mycelium Presence	Color
Czapek's agar	Poor	+	Light orange-yellow
Glycerol-asparagines agar (ISP 5)	Poor	+	Pale orange-yellow
Inorganic salts-starch agar (ISP 4)	Poor	+	Light orange-yellow
Yeast extract-malt extract agar (ISP 2)	Good	+	Brilliant orange-yellow
Oatmeal agar (ISP 3)	Poor	+	Pale orange-yellow
Nutrient agar	Poor	+	Pale orange-yellow

Table 2. Differential characteristics of YIM 0006^T and related genera

Abbreviations: DAP, diaminopimelic acid; Ara, arabinose; Gal, galactose; Glu, glucose; Mad, madurose; Man, mannose; Rib, ribose; Rha, rhamnose; DPG, diphosphatidyl glycerol; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PIM, phosphatidyl inositol mannosides; PME, phosphatidyl methyl ethanolamine; GluNU, N-acetylglucosamine-containing phospholipids. ND: Not determined

Genus	Morphological characteristics	Cell wall type[1]	Sugar pattern[2]	Phospholipids	Major menaquinone(s)	Fatty acid type[3]	G+C mol%
Actinoalloteichus	Long spore chain	III	Rib, Man, Glu, Gal	PE, PME	MK-9(H_2, H_4), 8(H_4)	2d	73
Actinopolyspora	Long spore chain	IV	Ara, Gal	PC	MK-9(H_4), 10(H_4)	2c	64
Amycolatopsis	Long spore chain	IV	Ara, Gal	PE, PME	MK-9(H_2), 8(H_4)	3f	60-69
Crossiella	Rod-shaped vegetative mycelium and sclerotium-like pseudosporangia	IV	Ara, Gal	PE, PME	MK-9(H_4)	2a	74
Goodfellowia	Ovoid conidia, fragmentation	*meso*-DAP	Gal, Rib	PC	MK-9(H_4), 10(H_4)	3a	68
Jiangella	Fragmentation	I	Glu, Rib	PIM, PI, DPG	MK-9(H_4)	1a	70
Kibdelosporangium	Sporangium-like structure	IV	Ara, Gal	PE, PI	MK-9(H_2 H_4 H_6), MK-8(H_4 H_6)	3c	66
Kutzneria	Sporangium	III	Ara, Gal	PE	MK-9(H_2 H_4 H_6), MK-8(H_4), 10(H_4)	3c	70
Lechevalieria	Rodshaped elements Aerial mycelium	III	Gal, Man	PE	MK-9(H_4)	2a	68.6
Prauserella	Short spore chain	IV	Ara, Gal	PE	MK-9(H_4)	2b	65-70
Pseudonocardia	Long spore chain	IV	Ara, Gal	PC	MK-8(H_2, H_4)	2b	79
Saccharomonospora	Single spore on aerial mycelium	IV	Ara, Gal	PE	MK-9(H_4)	2a	69-74
Saccharopolyspora	Beadlike spore chain	IV	Ara, Gal	PC	MK-9(H_4)	2d	77
Streptoalloteichus	Long hyphae and sporangia, motile spore	IV	Gal, Man	PE	MK-10(H_4, H_6)	3e	ND
Thermobispora	Spore chain with two spores on aerial mycelium	*meso*-DAP	Mad, Gal	GluNu	MK-9(H_0)	1b	70
Thermocrispum	Pseudosporangium, long chain, fragmentation	IV	Ara, Gal	PE	MK-9(H_4)	3f	69-73
YIM 0006^T	Short spore chain on substrate mycelium, fragmentation	IV	Ara, Gal	PC, PI	MK-9(H_4)	2b	69

1 According to the classification of Lechevalier & Lechevalier (1970). All organisms have the *meso*-diaminopimelic acid isomer.
2 According to the classification of Kroppenstedt (1985).
3 According to the classification of Lechevalier & Lechevalier (1970).

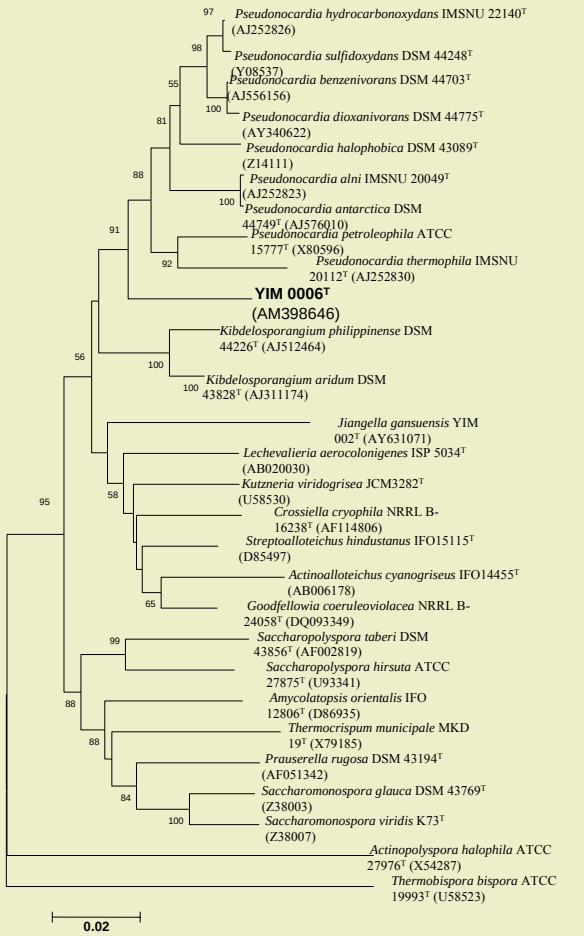


Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of YIM 0006^T and representative species of 16 genera of the family *Pseudonocardiaceae*. Numbers on branch nodes are bootstrap percentages (1000 resamplings, only values over 50% are given). Bar, 2% sequence divergence.

Conclusion

Description of *Actinostreptospora* gen. nov.

Actinostreptospora (Ac.ti.no.strep.to spo'ra. G. n. *actis, actinos* ray; Gr. adj. *streptos* pliant, bent; Gr. n. *spora* a seed; L. M. L. fem. n. *Actinostreptospora* referring to an actinomycete with spore chains). Aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes. Substrate mycelia are short and fragment into rod-shaped elements. No aerial mycelia are produced. Cell wall contains *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of arabinose and galactose. Main phospholipids are phosphatidyl choline (PC) and phosphatidyl inositol (PI). G+C content of genomic DNA is 69 mol %. Type species is *Actinostreptospora chiangmaiensis* sp. nov.

Description of *Actinostreptospora chiangmaiensis* sp. nov.

Actinostreptospora chiangmaiensis (chiang.mai.en'sis. M.L. fem. adj. *chiangmaiensis* pertaining to Chiang Mai, a city in the north of Thailand in the vicinity of which the type strain was found). Based on the phylogenitc analysis of the 16S rDNA the strain belongs to the family *Pseudonocardiaceae* (Fig. 2). The growth on most media is poor (Table 1). No aerial hyphae are produced on all tested media. Vegetative mycelium is short and fragments into rod-shaped elements (Fig. 1), pale orange-yellow to brilliant orange-yellow in color. Short spore chains are formed directly from vegetative mycelium. Spores are short rod-shaped and 0.3-0.6×0.8-1.2 µm in dimension. Spore surfaces are smooth. No soluble pigment is produced. Gelatine liquefaction, milk coagulation and peptonization, starch hydrolization, nitrate reduction, growth on cellulose, H2S and melanin production are negative. Glucose, fructose, xylose, ribose, rhamnose, sucrose, lactose, sorbitol, glycerol, sodium acetate, asparagine, glycine, histidine and methionine are utilized. Acid is not produced from these carbon sources. Galactose, arabinose, mannose, raffinose, inositol, mannitol and sodium citrate are not utilized. The cell wall contains *meso*-diaminopimelic acid. Whole-cell hydrolysates contain arabinose and galactose (cell wall chemotype IV). Main phospholipids are phosphatidylcholine (PC) and phosphatidylinositol (PI). The predominant menaquinones are MK-9(H4). The predominant fatty acids compositions are iso C14:0 (1.1%), iso C15:0 (3.2%), iso C16:1 H (2.6%), iso C16:0 (29.8%), C16:1ω7c/iso-C15 2-OH (17.6%), C16:0 (10.8%), C16:0 10 methyl (7.2%), iso C17:0 (2.5%), anteiso C17:0 (5.5%), C17:1ω8c (4.4%), C17:0 (1.6%), C17:0 10 methyl (1.3%), C18:1ω9c (2.3%) and C18:0 (3.7%) (fatty acid type 2b). G+C content of genomic DNA is 69 mol % (Table 2). The type strain (YIM 0006^T = CCTCC AA 205017^T = DSM 45062^T) was isolated from a soil sample collected from tropical rain forest located at Chiang Mai in the north of Thailand (pH 5.5 to 6.5).

Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (No. 30560001), the Yunnan Provincial International Cooperative Program (No. 2005GH21), the Yunnan Provincial Natural Science Foundation (No. 2004 C0002Q), the Program for New Century Excellent Talents in University and the "Zentrum für Marine Wirkstoffe", which is founded by the Ministerium für Wirtschaft, Wissenschaft und Verkehr des Landes Schleswig-Holstein (Germany). We thank Dr. Vera Thiel, Dr. Jörg Süling, Dr. Rüdiger Stöhr, Dr. Rolf Schmaljohann, Xiang-Feng Cai and Yun Chen for assistance and discussions.

Kontakt:
Leibniz-Institut für Meereswissenschaften
IFM-GEOMAR
Düsternbrooker Weg 20
D-24105 Kiel

Zentrum für Marine Wirkstoffe
Am Kiel Kanal 44
24106 Kiel

Tel. +49 431 600-4450
Fax +49 431 600-4452
E-mail: jimhoff@ifm-geomar.de

Lab of Actinomycetal Taxonomy
Yunnan Institute of Microbiology
Yunnan University, Kunming, 650091
P. R. China

Tel. +86 871 5034139/5035253
Fax +86 871 5173878
E-mail: lihxu@ynu.edu.cn

List of 230 primary identified strains

1 List of primarily identified strains with anti-cancer activities (26 strains)
(Similarity with closest relative species)

YIM 31529，*Streptomyces scabrisporus* 416/420 (99%)
YIM 31531， *Streptomyces cirratus* 793/798 (99%)
YIM 31622，*Streptomyces fimbriatus* 1421/1443, (98.47%)
YIM 31729，*Nocardiopsis dassonvellei* 648/648 (100%)，
YIM 31786， *Streptosporangium amethystogenes* 643/644 (99%)，
YIM 32001，*Streptomyces galilaeus* 645/650 (99%)
YIM 32021，*Streptosporangium roseum* PB，544/545 (99%)
YIM 32201， *Streptomyces galilaeus* 645/650 (99%)
YIM 32242，*Streptomyces mirabilis* 647/650 (99%)
YIM 32342， ***Streptomyces flavoablus*** **sp. nov.**
YIM 32647， *Nocardia fluminea* 640/650 (98%)
YIM 33363，*Streptomyces aureus* 645/650 (99%)
YIM 33422， ***Streptomyces setonii*** **ATCC25497 D63872 (99.93%)**
YIM 33742，*Leifsonia xyli* subsp. *Xyli* 644/650 (99%)
YIM 34044，*Streptomyces tauricus* 570/570 (100%)
YIM 34065，*Streptomyces aureus* 660/678 (97%),
YIM 34077, *Streptomyces cinereoruber subsp. Fructofermentans* 650/650 (100%)
YIM 34138，*Streptomyces griseus* 640/650 (98%)
YIM 34416，*Streptomyces griseus* 640/650 (98%)
YIM 34452， *Micromonospora endolithica* 1398/1426 (98%)
YIM 34620， *Bacillus vallismortis* 650/650 (100%)，
YIM 34672，*Streptomyces setonii* 1517/1519 (99%)
YIM 35475，*Nocardia fluminea* 1442/1464 (98%)
YIM 35495，*Streptomyces resistomycificus* 639/648 (98%)
YIM 35514， *Streptomyces heliomycini* 637/650 (97.85%)
YIM 37293， ***Nocardia alboflava*** **sp. nov.**

2. List of identified strains with PKS genes (22 strains)
(Similarity with closest relative species)

YIM 33309，*Nonomuraea kuesteri*(647/650 ,99%)
YIM 33353，*Nonomuraea kuesteri*(647/650 ,99%)
YIM 33361，*Nocardia polyresistens* sp. nov.
YIM 33378， *Nocardia lijiangensis* sp. nov.
YIM 34712，*Micromonospora chalcea* 646/648 (99%)
YIM 34787，*Streptomyces mirabilis* 646/650 (99%)
YIM 34825，*Rhodococcus tukisamuensis* 673/686 (98%)
YIM 35371，*Streptomyces galilaeus* 632/640 (98%)
YIM 35572，*Streptomyces argenteolus* 637/640 (99%)

YIM 35581, *Streptomyces resistomycificus*(593/600, 98%)
YIM **36090，*Streptomyces aureus*(593/600 , 98%)**
YIM 36109，*Nocardia cummidelens*(563/571 ,98%)
YIM 36122，*Streptomyces fimbriatus*，1421/1443 (98.47%)
YIM 36723，*Nocardia jejuensis* (99.5%)
YIM 36851，*Micromonospora echinospora*(589/600 ,98%)
YIM 36856，*Nocardia niigatensis*(1449/1465， 98.9%)
YIM 37217，*Micromonospora echinospora* (644/651 ,98%)
YIM 37218，*Nocardia niigatensis*(1413/1428, 98.94%)
YIM 37225，*Nocardia fluminea* (587/598 ,98%)
YIM 37251, *Streptosporangium vulgare* 565/576 (98%)
YIM 37272, *Duganella violaceinigra* 1389/1439 (99.57%)
YIM 80280, *Nocardia alba* (1425/1445, 98%)

3 List of identified strains with some enzyme inhibitors (32 strains) (Similarity with closest relative species)

3.1 Anti-oxidation

YIM 31292，*Streptomyces venezuelae* (689/696 ,98%)
YIM 31570，*Streptomyces fimbriatus* (723/732 ,98%)
YIM 31646，*Streptomyces cyaneus* (644/650,99%)，
YIM 32199，*Kitasatospora kifunense* (650/650,99%)，
YIM 32311，*Streptomyces purpeofuscus* (1467/1475, 99%)
YIM 32374，*Streptomyces hygroscigopicus*(645/650,99%)，
YIM 32791，*Streptomyces caelestis* (645/650,99%)

3.2 AchE inhibitor

YIM 31339, *Streptomyces avermectinius*, (1461/1461, 100%)
＝32196，*Streptomyces avermectinius* (650/650, 100%)
YIM 32065=33176, *Streptomyces pleomorphus sp. nov.*
YIM 32200，*Streptomyces phaeochromogenes*，(783/795, 98%)
YIM 32212，*Streptomyces flavogriseus (*649/650, 99%)
YIM 32213，*Sreptomyces galbus* (640/650,98%)，
YIM 32215，*Streptomyces lincolnensis* (596/600,99%)，
YIM 32220，Streptomyces tauricus (640/650, 98%)
YIM 33098, *Streptomyces nanningensis* sp.nov

3.3 AGE inhibitor

YIM 32234，*Streptomyces xianggelilaensis* sp. nov.

3.4 α-Glucosidease inhibitor

YIM 31531，*Streptomyces cirratus* (793/798, 99%)
YIM 32252，*Sreptomyces galbus* (640/650, 98%)，
YIM 32255，*Streptomyces scaibiei* (649/650, 99%)，
YIM 32240，*Streptomyces lavendulae* (648/650, 99%)
YIM 32452，*Kitasatospora kifunensis* (650/650, 100%)
YIM 33532，*Streptomyces nodosus*，*(*649/650, 99%)

YIM 33588，*Kitasatospora kifunense* (650/650,100%)

YIM 32452=33588=32199，*Kitasatospora kifunense* (650/650, 99%-100%α)，

YIM 32213=32252，*Sreptomyces galbus* (640/650, 98%)，

3.5 NO synthetase inhibitor

YIM 30243, *Nocardia alba* sp. nov.

3.6 Anti-microbes

YIM 31634, *Streptomyces roseoalbus* sp.nov.

4 List of identified strains from salt and alkaline habitats (14 strains)

(Similarity with closed species)

J 0034, *Micromonospora olivasterospora* 642/650 (98%)

J 0038, *Micromonospora mirobrigensis* 1482/1508 (98%)

J 0039, *Micromonospora echinospora* 643/650 (98%)

J 0042, *Streptomyces ferralitis* 636/647 (98%)

J 0043, *Streptomyces peucetius* 649/650 (99%)

YIM 80052, *Nocardiopsis alba* , 98.63%

YIM 80154, *Nocardiopsis umidischolae, 98.53%*

YIM 80186, *Nocardiopsis metallicus, 99.0%*

YIM 80188, *Nocardiopsis umidischolae, 98.53%*

YIM 80189; *Nocardiopsis umidischolae, 98.53%*

YIM 80280, *Nocardia alba, 98%*

YIM 90012, *Nocardiopsis dassonvillei, 100%*

YIM 90018, *Streptomyces sclerotialus*, 98.0%,

YIM 90039, *Nocardiopsis alba*, 98.5%

5 List of characterized rare actinomycete strains with similar species (110 strain) (Similarity with closed species)

YIM 0006, *Actinomycetospora chiangmeiensis* gen. nov., sp. nov.,

YIM 0019, *Nonomuraea salmonea*

YIM 0026, *Micromonospora auratinigra*

YIM 0027, *Cryobacterium aff. psychrophilum*

YIM 0032, *Chelatococcus asaccharovorans*

YIM 45066, *Aerococcus viridans* 585/591 (98%)

YIM 45067, *Arthrobacter agilis* (1452/1470, 98.77%)

YIM 45068, *Rhodococcus fascians* (547/548 ,99%

YIM 45070, *Kocuria palustris* (540/540 ,100%)

YIM 45102, *Nocardia pseudovaccinii* 649/650 (99%)

YIM 45103, *Methylobacterium aquaticum*(1333/1376, 96.87%)

YIM 45140, *Nocardia flavorosea* (746/755 ,98%)

YIM 45191, *Rhodococcus opacus* 648/650 (99%)

YIM 45192, *Rhodococcus opacus* (538/538 ,100%),

YIM 45193, *Nocardia flava* sp. nov.

YIM 45197, *Micromonospora purpureochromogenes* subsp. *hongheensis*

subsp. nov.

YIM 45198, *Nocardia alba*(1401/1427, 98.17%)
YIM 45352, *Nocardia nova*(747/753 ,99%)
YIM 45353, *Nocardia alba* 1417/1438 (98%)
YIM 45362, *Glycomyces harbinensis* 639/650 (98%)
YIM 45364, *Nocardiopsis umidischolae* (746/752 ,99%)
YIM 45489, *Glycomyces harbinensis*(1406/1432, 98.18%)
YIM 45492, *Streptomyces caviscabies*(1429/1456, 98.14%)
YIM 45494, *Streptomyces setonii* ATCC25497 D63872 (1420/1444,98.44%)
YIM 46011, *Pseudonocardia yunnanensis* 639/650 (98%)
YIM 46034, *Planosporangium flavogriseum* gen. nov.
YIM 46083, *Dietzia maris* PB (750/750 , 100%)
YIM 46159, *Promicromonospora sukumoe* 646/650 (99%)
YIM 46257, *Promicromonospora sukumoe* 644/650 (99%)
YIM 46258, *Streptomyces ferralitis* 633/635 (99%)
YIM 46260, *Promicromonospora sukumoe* 552/556 (99%)
YIM 46315, *Nocardia vinacea* (1433/1462 ,97.75%)
YIM 46393, *Bacillus pumilus* 650/650 (100%)
YIM 46399, *Promicromonospora sukumoe* 643/646 (99%)
YIM 46459, *Rhodococcus erythropolis* 633/650 (97%)
YIM 46539, *Nocardia inohanensis* 645/650 (99%)
YIM 46644, *Micromonospora endolithica* (1436/1462 ,98.1%)
YIM 46785, *Micrococcus luteus* (1446/1474, 98.1%)
YIM 46787, *Rhodococcus coprophilus* 650/650 (100%)
YIM 47631, *Streptomyces alboflavus*, 707/720 (98%)
YIM 47634, *Nocardia flavorosea*, 747/750 (99%)
YIM 47635, *Streptomyces chrestomyceticus*, 746/750 (99%)
YIM 47637, *Nocardia pseudovaccinii*, 747/750 (99%)
YIM 47638, *Nocardia pseudovaccinii*, 747/750 (99%)
YIM 47639, *Chelatococcus asaccharovorans*, 742/745 (99%)
YIM 47640, *Methylobacterium radiotolerans*, 750/750 (100%),
YIM 47641, *Streptomyces aculeolatus*, 747/750 (99%)
YIM 47643, *Methylobacterium radiotolerans*, 749/750 (99%)
YIM 47645, *Rhodococcus fascians*, 742/750 (98%),
*Rhodococcus yunnanensis*1432/1449 (98%)
YIM 47648, *Nocardia asteroids*, 733/745 (98%)
YIM 47649, *Paenibacillus daejeonensis1*423/1451 (98%)
YIM 47650, *Promicromonospora sukumoe*, 743/750 (99%)
YIM 47651, *Streptomyces aculeolatus* 746/750 (99%)
YIM 47652, *Streptosporangium vulgare*, 749/750 (99%)
YIM 47654, *Nonomuraea kuesteri*, 716/720 (99%)
YIM 47655, *Nocardia inohanensis*, 742/750 (98%)
YIM **47657, *Chryseobacterium taichungense* 642/671 (95%)**
YIM 47658, *Nocardia asteroids*, 730/744 (98%)

YIM 47659, *Nocardia asteroids*, 739/744 (99%)
YIM 47661, *Rhodococcus equi*, 737/750 (98%)
YIM 47662, *Mycobacterium septicum*, 750/750 (100%)
YIM **47663,** *Streptomyces agglomeratus*, 727/750 (96%),
***Streptomyces violaceogriseus* sp. nov.**
YIM 47664, *Nocardia pseudovaccinii*, 747/750 (99%)
YIM 47665, *Nocardia pseudovaccinii*, 747/750 (99%)
YIM 47666, *Nocardia pseudovaccinii*, 749/750 (99%)
YIM 47667, *Nonomuraea kuesteri*, 747/750 (99%)
YIM 47668, *Nocardia pseudovaccinii*, 745/750 (99%)
YIM 47669, *Nonomuraea kuesteri*, 728/730 (99%)
YIM **47670*, Methylobacterium suomiense*, 646/698 (92%),**
Methylobacterium aminovorans, **99.35%**
YIM 47671, *Isoptericola variabilis*, 741/748 (99%)
YIM 47672, *Streptomyces hainanensis* sp. nov.
YIM 47674, *Methylobacterium radiotolerans*, 749/750 (99%)
YIM 47675, *Streptosporangium vulgare*, 749/750 (99%)
YIM 47676, *Mycobacterium septicum*, 748/750 (99%)
YIM 47677, *Streptomyces kunmingensis*, 739/746 (99%)
YIM 47678, *Streptomyces aculeolatus*, 750/750 (100%)
YIM 47679, *Nonomuraea kuesteri* 1472/1495 (98%),
YIM 47681, *Streptomyces galbus*, 745/750 (99%)
YIM 47682, *Nocardia pseudovaccinii*, 734/750 (97%),
*Nocardia pseudovaccinii1*424/1445 (98%)
YIM 47683, *Bacillus drentensis*, 701/701 (100%)
YIM 47686, *Nocardia araoensis*, 750/752 (99%)
YIM 47687, *Streptosporangium amethystogenes*, 745/745 (100%)
YIM 47688, *Streptosporangium vulgare*, 745/750 (99%)
YIM 47689, *Nocardia pseudovaccinii*, 736/750 (98%),
*Nocardia pseudovaccinii1*424/1445 (98%)
YIM 47690, *Streptosporangium vulgare*, 719/720 (99%)
YIM 47691, *Bacillus muralis*, 745/752 (99%)
YIM 47692, *Nonomuraea kuesteri*, 747/750 (99%)
YIM 47693, *Nocardia beijingensis*, 745/750 (99%)
YIM 47694, *Streptosporangium vulgare*, 746/750 (99%)
YIM 47695, *Rhodococcus fascians*, 749/750 (99%)
YIM 47696, *Nocardia takedensis*, 737/750 (98%)
YIM 47697, *Micromonospora chalcea*, 748/751 (99%)
YIM 47698, *Rhodococcus zopfii*, 741/750 (98%)
YIM 47699, *Bacillus fusiformis*750/750 (100%)
YIM 47700, *Arthrobacter agilis*, 715/720 (99%)
YIM 47701, *Actinobispora yunnanensis*, 737/752 (98%)
YIM 47702, *Actinobispora yunnanensis*, 737/752 (98%)
YIM 47703, *Streptomyces pulveraceus*, 748/750 (99%)

YIM **47705,** *Pseudonocardia hydrocarbonoxydans,* 732/750 (97%),
***Pseudonocardia flava* sp. nov.**
YIM 47706, *Nonomuraea kuesteri*, 747/750 (99%)
YIM 47707, *Gordonia sputi*, 749/750 (99%)
YIM 47708, *Gordonia sputi*, 749/750 (99%
YIM 47709, *Micromonospora aurantiaca,* 742/750 (98%)
YIM 47710, *Streptosporangium viridalbum,* 748/750 (99%)
YIM 47711, *Dactylosporangium auranticum,* 740/751 (98%)
YIM 47712, *Bacillus pumilus,* 750/750 (100%)
YIM 47713, *Catellatospora tsunoense,* 730/750 (97%)
YIM 47714, *Actinomadura rugatobispora,* 735/741 (99%)
YIM 47715, *Streptosporangium roseum,* 748/750 (99%)
YIM 47716, *Streptosporangium vulgare,* 748/751 (99%)

6 List of characterized marine strains with similar species (26 strains)

CC 0385 *Rhizobium undicola,* 1423/1487 (95%)
CC 0386 *Rhizobium undicola,* 1432/1481 (96%)
CC 0387 Promocromonospora flava, new species
CC 0396 *Myceligenerans xiligouense,*569/571 (99%)
CC 0420 *Bacillus hwajinpoensis*, 99.3%
CC 0421 *Rhodococcus fascians,* 566/567 (99%)
CC 0422 *Promicromonospora aerolata,*568/577 (98%)
CC 0440 *Cellulomonas variformis*, 98.2%
CC 0444 *Agrococcus jenensis,* 100%
CC 0446 *Chromohalobacter salexigens,* 533/562 (94%)
CC 0447 *Bacillus megaterium,* 98.3%
CC 0498 *Phyllobacterium trifolii* PETP02^{T} AY786080 (97%)
CC 0520 *Zobellia uliginosa =F.uliginosum,* (99.5%)
CC 0522 *Bacillus barbaricus,* 504/505 (99%)
CC 0523 *Exiguobacterium aurantiacum*, 98.1%
CC 0524 *Microbacterium foliorum,* 534/538 (99%)
CC 0526 *Kocuria rosea*, 99.1%
CC 0549 *Bacillus alcalophilus,* 97.2%
CC 0558 *Nocardiopsis dassonvillei subsp. dassonvillei* 1351/1354 (98%)
CC 0559 *Agrobacterium tumefaciens,* 543/555 (96%)
CC 0560 *Isoptericola halotolerans,* 99.5%
CC 0565 *Pseudomonas pseudoalcaligenes*, 99.3%
CC 0 576 *Mycobacterium confluentis,* 1477/1502 (98%)
CC 0580 *Actinomadura livida,* 1513/1524 (99%)
CC 0574 *Amycolatopsis palatopharyngis,* 566/576 (98%)
CC 0575 *Bacillus aquaemaris,* 98.3%

Acknowledgment

I want to express my gratitude to my mentors, Prof. Dr. Johannes F. Imhoff, Prof. Xu Li-Hua and Dr. Jutta Wiese for their all-around guidance, indoctrination, and continued support, to guide me during the thesis. I will embalm their benefaction forever.

I would also like to express my thankfulness to all colleagues and schoolmates, Dr. Vera Thiel, Dr. Jörg Süling, Dr. Rüdiger Stöhr, Dr. Rolf Schmaljohann, Dr. Xu Ping，Dr. Li Wen-Jun, Dr. Tang Shu-Kun, Dr. Chen Hua-Hong, Miss Yang Ying, Miss Cai Xiang-Feng, Mr. Chen Yun for their technically help.

Thanks to Tim Staufenberger for the translation.

I am grateful for the help and guidance of Prof. A. Bull, Prof. M. Goodfellow and Prof. Liu Zhi-Heng in research idea, methods and references.

I am special grateful of directors and staffs of the Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR and Yunnan Institute of Microbiology, Yunnan University for supplying very good research conditions.

This research work was supported by the “Zentrum für Marine Wirkstoffe”, which is founded by the Ministerium für Wissenschaft, Wirtschaft und Verkehr des Landes Schleswig-Holstein (Germany), the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (No. 30560001), the Yunnan Provincial International Cooperative Program (No. 2005GH21), the Yunnan Provincial Natural Science Foundation (No. 2004 C0002Q)，and the Program for New Century Excellent Talents in University. I would like to thank these German and Chinese foundations very much.

I am not able to use language to express my gratefulness to my parents for their constant support and meticulously solicitude.

Danksagung

Ich möchte meine Dankbarkeit gegenüber meinen Mentoren, Prof. Dr. Johannes F. Imhoff, Prof. Xu Li-Hua und Dr. Jutta Wiese für ihre umfassende Anleitung, ihren Rat und ihre anhaltende Unterstützung, die mich durch meine Doktorarbeit geführt haben, ausdrücken. Ich werde mich an ihre Wohltätigkeit stets erinnern.

Weiterhin möchte ich meinen Kollegen und Freunden, Dr. Vera Thiel, Dr. Jörg Süling, Dr. Rüdiger Stöhr, Dr. Rolf Schmaljohann, Dr. Xu Ping, Dr. Li Wen-Jun, Dr. Tang Shu-Kun, Dr. Chen Hua-Hong, Frau Yang Ying, Frau Cai Xiang-Feng und Herrn Chen Yun für ihre Hilfe bei technischen Fragestellungen danken.

Danke an Tim Staufenberger für die Übersetzungen.

Ich bin sehr dankbar für Hilfe und Rat von Prof. A. Bull, Prof. M. Goodfellow und Prof. Liu Zhi-Heng bei Forschungsansätzen, Methoden und Hinweisen.

Ich bin besonders den Direktoren und Angestellten des Leibniz-Instituts für Meereswissenschaften, IFM-GEOMAR, und dem Yunnan Institut für Mikrobiologie, Yunnan Universität, für die guten Arbeitsbedingungen zu Dank verpflichtet.

Diese Arbeit wurde unterstützt vom Zentrum für Marine Wirkstoffe, finanziert durch das Ministerium für Wissenschaft, Wirtschaft und Verkehr des Landes Schleswig-Holstein (Germany); dem National Basic Research Program of China (No. 2004CB719601), der National Natural Science Foundation of China (No. 30560001), dem Yunnan Provincial International Cooperative Program (No. 2005GH21), der Yunnan Provincial Natural Science Foundation (No. 2004 C0002Q) und dem Program for New Century Excellent Talents in University. Diesen deutschen und chinesischen Stiftungen möchte ich danken.

Meine große Dankbarkeit meinen Eltern gegenüber für ihre andauernde Unterstützung und Sorge liegt jenseits dessen, was sich mit Worten ausdrücken lässt.

ERKLÄRUNG

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt habe und, dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle in Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Mikrobiologie erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

_____

(Yi Jiang)