Chainia kunmingensis, a New Actinomycete Species Found in Soil

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We propose a new species, *Chainia kunmingensis*. This species is represented by a single isolate from a soil sample obtained in Kunming, Peoples Republic of China. This strain (strain ATCC 35682^T [T = type strain]) has the following morphological and chemical properties typical of the genus *Chainia*: formation of lipid-filled conidia and sclerotia, type I cell wall composition, lack of mycolic acids, type PII phospholipid composition, and deoxyribonucleic acid guanine-plus-cytosine content of 71.3 mol%. Strain ATCC 35682^T differs physiologically from all previously described species and thus is considered a new species.

The actinomycete genus *Chainia* was first described by Thirumalachar almost 30 years ago (15). Subsequent studies showed that members of this genus are closely related to *Streptomyces* in peptidoglycan composition and in the formation of streptomycete-like chains of conidia on the aerial hyphae (7). However, because of their unique sclerotia rich in lipids and because they contain an unusual diamino acid (2,3-diaminopropionic acid), we have argued that this genus deserves to be conserved (11). A total of 12 species have been described to date (reviewed in reference 11).

In the course of our studies of actinomycetes, strain 80-3024^T (T = type strain) was isolated from soil collected in Kunming, Peoples Republic of China (South China). This organism produces sclerotic granules, has a type I cell wall composition, and a type PII phospholipid pattern. Based on our morphological and chemical data, we concluded that strain 80-3024^T should be assigned to the genus *Chainia*. In this paper we describe our morphological and physiological data which support the creation of a new species in this genus. Strain 80-3024 (= ATCC 35682) is the type strain.

MATERIALS AND METHODS

Isolation. Strain 80-3024^T was isolated from soil collected at Dagyanlow's Lake in Kunming, Peoples Republic of China; this soil had been plated out on glucose-asparagine agar and incubated for 2 weeks at 28°C. *Chainia violens* RIA 565 was received from the Institute of Microbiology, USSR Academy of Sciences, Moscow. All other culture sources have been described previously (11).

Morphology. The media used for micromorphological studies were glucose-asparagine agar, Bennett agar, and tap water agar (3, 16, 17). Strain 80-3024^T was examined directly on agar plates by using a light microscope equipped with a long-working-distance condenser.

Cultural characteristics. To determine the gross cultural characteristics of this organism, it was grown on a number of culture media, including glucose-asparagine agar, inorganic salts-starch agar, potato-dextrose agar, Czapek agar, calcium malate agar, oatmeal agar, nutrient agar, and potato agar (3, 16, 17).

Physiology. The methods used for physiological tests were those of Gordon (5), Gordon and Horan (6), and Shirling and Gottlieb (14).

Whole-cell analyses. The procedures of Becker et al. (1) and Lechevalier (8) were used for whole-cell analyses.

Cell wall preparation. Cell walls and sclerotial walls were purified and analyzed by using the method of Lechevalier and Lechevalier (10).

Phospholipid analysis. The phospholipid analysis was carried out by using previously described methods (9).

Guanine-plus-cytosine content. Deoxyribonucleic acid extraction and purification were performed at the American Type Culture Collection; in general, the method of Marmur was used (12). To facilitate lysis of the cells by lysozyme, the cells were suspended in tris(hydroxymethyl)aminomethane-

TABLE 1. Morphological characteristics of *C. kunmingensis* 80-3024^T and *C. antibiotica* IMRU 3750

ISP agar medium ^a	Portion of culture ^b	Strain 80-3024 ^T	Strain IMRU 3750
Yeast extract-malt extract (ISP 2)	AM	Sparse, white	Sparse, grayish white
	VM	Dark orangish yellow (72) ^c	Light grayish yellow or brown (79) to medium yellowish brown (77)
	SP	None	Light yellowish brown
Oatmeal (ISP 3)	AM	None	None
	VM	Colorless to light yellowish tan	Thin, light yellowish tan
	SP	None	None
Glycerol-asparagine (ISP 5)	AM	Yellowish white (92)	None
	VM	Light to yellowish tan to cream	Light yellowish tan
	SP	None	None
Inorganic salts-starch (ISP 4)	AM	Yellowish white (92)	None
	VM	Light yellowish tan	Light grayish yellow to dark yellowish brown (78)
	SP	None	None

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^a ISP, International *Streptomyces* Project.

^b AM, Aerial mycelium; VM, vegetative mycelium; SP, soluble pigment.

^c The numbers in parentheses are ISCC-NBS color numbers (6a).

FIG. 1. Spore chains. (a) Spore chain of strain IMRU 3750 grown on one-fifth-strength yeast-Czapek agar at 28°C for 15 days $\times 1,000$. Bar = 10 μm . (b) Spore chains of strain 80-3024^T grown on half-strength Bennett agar. $\times 1,000$. Bar = 10 μm .

FIG. 2. Section of spore chains of strain 80-3024^T from growth on glucose-asparagine agar after 20 days. $\times 21,000$. Bar = 1 μm .

FIG. 3. Sclerotia of strain 80-3024^T. The strain was grown on a slide of tap water agar at 28°C for 17 days and observed after 15 days. $\times 9,000$. Bar = 1 μm .

FIG. 4. Thin section through a sclerotium. $\times 10,000$. Bar = 1 μm .

sucrose and sonicated three times for 30 s. The purified deoxyribonucleic acid was suspended in a 0.2 M sodium phosphate-ethylenediamine tetraacetate buffer (12) for thermal melting. Three thermal melt runs were performed with a

Gilford model 2400-2 spectrophotometer equipped with a Gilford model 2527 thermoprogrammer. The results of runs on consecutive days were 71.46, 70.61, and 71.82 mol%, giving an average of 71.3 mol%. Deoxyribonucleic acid from

TABLE 2. Differentiating physiological properties of species of *Chainia* (Gordon tests)^a

Strain	Decom- position of xan- thine	Utilization of:			Production of:			Acid produced from:										Growth at:				
		Oxalate	Benzo- ate	Tar- trate	Urease	Nitrate reductase	Adoni- tol	Dulci- tol	i- Eryth- ritol	i-Inosi- tol	d-(+)- Lac- tose	d-(-)- Manni- tol	d-(+)- Meli- biose	α-Me- thyl- gluco- side	d-(+)- Raffi- nose	l-(+)- Rham- nose	d-Sor- bitol	Su- crose	d-(+)- Xylose	10°C	42°C	
<i>C. kunmingensis</i> 80-3024 ¹	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-
<i>C. antibiotica</i> Su7	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
<i>C. antibiotica</i> IMRU 3750	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+
" <i>Chainia au- rea</i> " Su8	-	-	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	-	-	+	-	+
" <i>Chainia flava</i> " Su9	-	+	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	-	+	+	-	+
<i>Chainia fumi- gata</i> Su10	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-	+
<i>C. minuti- sclerotica</i> Su11	-	+	-	+	-	-	-	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+
<i>Chainia nigra</i> Su12	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
<i>C. ochracea</i> Su13	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
<i>Chainia olivacea</i> Su14	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	-
<i>C. olivacea</i> VI7676	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-
<i>C. olivacea</i> 3751	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chainia poon- ensis</i> 3752	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
<i>C. poonensis</i> Su15	+	-	-	-	+	-	+	+	+	+	+	+	-	+	-	+	+	+	-	+	-	-
<i>C. purpurigena</i> Su16	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chainia rosea</i> Su17	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+
<i>Chainia rubra</i> ATCC 17754	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	-	+
<i>C. violens</i> RIA 565	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a See references 5 and 6. -, Negative; +, positive. All strains were positive for glycerol, tyrosine, and maltose. Only strain 3752 was negative for salicin. None grew at 53°C.

TABLE 3. Physiological properties of *Chainia* species (International *Streptomyces* Project tests)^a

Strain	Utilization of:						
	<i>l</i> -(+)- Arabinose	<i>i</i> - Inositol	<i>d</i> -(-)- Mannitol	<i>d</i> -(+)- Raffinose	<i>l</i> -(+)- Rhamnose	Sucrose	<i>d</i> -(+)- Xylose
<i>C. kunningensis</i> 80-3024 ^T	+	—	+	+	+	—	+
<i>C. antibiotica</i> Su7	+	+	+	+	+	+	+
<i>C. antibiotica</i> IMRU 3750	+	+	+	—	+	—	+
" <i>C. aurea</i> " Su8	+	—	+	—	—	—	+
" <i>C. flava</i> " Su9	+	Tr	+	—	+	Tr	+
" <i>C. fumigata</i> Su10	+	—	+	—	+	—	+
<i>C. minutisclerotia</i> Su11	+	Tr	+	—	+	—	+
<i>C. nigra</i> Su12	+	+	+	+	+	+	+
<i>C. ochracea</i> Su13	+	+	+	+	+	+	+
<i>C. olivacea</i> Su14	+	+	+	—	+	—	+
<i>C. olivacea</i> 3751	+	+	+	+	+	+	+
<i>C. olivacea</i> VI7676	+	+	+	Tr	+	+	+
<i>C. poonensis</i> 3752	—	—	—	—	—	+	—
<i>C. poonensis</i> Su15	+	+	+	—	+	—	+
<i>C. purpurogena</i> Su16	+	+	+	+	+	+	+
<i>C. rosea</i> Su17	+	Tr	+	—	+	+	+
<i>C. rubra</i> ATCC 17754	+	—	+	—	+	+	+
<i>C. violens</i> RIA 565	+	+	+	Tr	+	+	+

^a All strains utilized fructose and glucose. +, Utilized; —, not utilized; Tr, slightly more growth than control.

Micrococcus lysodeikticus (Sigma Chemical Co.) was used as a reference.

Thin sectioning. For sectioning, sclerotia grown on glucose-asparagine agar for 3 weeks were fixed in buffered 1% osmium tetroxide for 16 h at 4°C and then washed in 0.5% uranyl acetate for 3 h. Following dehydration with ethanol, they were embedded in epoxy resin by using the method described by Sharples and Williams (13).

RESULTS

Micromorphology. The aerial mycelium and substrate mycelium were well developed, fine, gram positive, non-acid fast, and septate. Chains of conidia in loose spirals were observed on many media, including Bennett agar and yeast extract-malt extract agar (Fig. 1b). In contrast, the conidial coils of *Chainia antibiotica* IMRU 3750, a closely related strain, are very tight (Fig. 1a). A section through a chain of spores is shown in Fig. 2. Observation of the developing sclerotic granules showed them to be composed of incurled hyphal cells on both aerial and substrate hyphae, single or in aggregates, at first enveloped in a hyphal sheath and later becoming free. The mature sclerotia were 3 to 10 µm in diameter (Fig. 3 and 4).

Growth on various media. Strain 80-3024^T grew well on a variety of media, including glucose-asparagine agar, inorganic salts-starch agar, and potato-dextrose agar. A summary of the appearance of strain 80-3024^T on a variety of media is given in Table 1, along with the characteristics of strain IMRU 3750. On Czapek agar, yellow or light brown colonies with little or no aerial mycelium or soluble pigment were produced. On calcium malate agar, nutrient agar, and potato agar growth was yellowish tan or light brown with no aerial mycelium. A yellow soluble pigment was produced. No melanoid pigments were observed on any medium.

Physiological and biochemical reactions. The physiological reactions of strain 80-3024^T and other chainiae are compared in Tables 2 and 3.

Cell wall composition. Strain 80-3024^T had a type I cell wall composition, containing L-diaminopimelic acid, glycine, glutamic acid, alanine, glucosamine, and muramic acid.

Phospholipid composition. Analysis showed that strain 80-3024^T had a type PII phospholipid pattern, containing

phosphatidyl inositol mannosides, phosphatidyl inositol, diphosphatidyl glycerol, and phosphatidyl ethanolamine acylated to both hydroxy and normal fatty acids. The fatty acid pattern was a type 1 pattern (iso and anteiso fatty acids more than 10% of the total).

DISCUSSION

Strain 80-3024^T (= ATCC 35682^T) has a type I cell wall composition and a type PII phospholipid pattern and produces sclerotia; all of these are characteristics typical of the genus *Chainia* (1, 10, 15). No 2,3-diaminopropionic acid was found; however, as this strain has been under cultivation for some years, this character could have been lost, as previously observed in other strains (11).

Stained preparations (Fig. 2 and 4) showed extensive septation like that previously described in chainiae (4), and spores and hyphae were filled with electron-lucent areas, presumably corresponding to the lipids found in another species of *Chainia* (11).

Physiologically, strain 80-3024^T differs from all other strains listed in Table 2 except *C. antibiotica* IMRU 3750 by a minimum of five characters; it differs from *C. antibiotica* IMRU 3750 only in growth at 10 and 42°C. However, a comparison of carbohydrate utilization by using International *Streptomyces* Project tests (Table 3) showed that strain 80-3024^T also differs from strain IMRU 3750 in utilizing raffinose and not utilizing inositol.

In its gross morphological characteristics, strain 80-3024^T is also similar to *Chainia ochracea* Su13 and *Chainia purpurogena* Su16, but differs from strain Su13 in the following physiological characteristics: benzoate and tartrate are not utilized, nitrate reductase is produced, and acid is not formed from adonitol, erythritol, or sorbitol but is formed from raffinose. Strain 80-3024^T differs from strain Su16 by the following characteristics: utilization of oxalate, no utilization of benzoate and tartrate, lack of acid production from adonitol, erythritol, and sorbitol, and lack of growth at 42°C.

Strain 80-3024^T has small sclerotia which are similar to those formed by *Chainia minutisclerotica* Su11, but differs from the latter strain physiologically in six characteristics (Table 2) and in gross morphology, since strain Su11 produces abundant greenish gray aerial mycelium.

We regard the organism described here as a new species, for which we propose the name *Chainia kunmingensis* sp. nov. (kun. ming. en'sis. M.L. adj. *kunmingensis* pertaining to Kunming, a province of South China).

Description of *Chainia kunmingensis* sp. nov. Small sclerotia (5 to 25 μ m) formed in and on the surface of agar media. Coiled chains of conidia also occasionally formed in the aerial hyphae. Colonies usually yellowish tan to dark orange yellow, depending on the medium, with or without yellowish white aerial mycelium. Soluble pigments little to none. Type I cell wall and type PII phospholipid pattern. The guanine-plus-cytosine content of the deoxyribonucleic acid is 71.3 mol% (thermal denaturation method). Cultures hydrolyze adenine, casein, hypoxanthine, tyrosine, xanthine, starch, and esculin but not gelatin and urea. Phosphatase and nitrate reductase are produced. Acetate, lactate, malate, pyruvate, succinate, citrate, mucate, oxalate, and propionate are utilized but benzoate and tartrate are not. Acid is formed from arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, α -methyl-D-glucoside, raffinose, rhamnose, salicin, sucrose, trehalose, xylose, and β -methyl-D-xyloside. Acid is not formed from adonitol, dulcitol, erythritol, or sorbitol. Lysozyme-sensitive. Grows well at 10 and 37°C; no growth observed at 42°C. Arabinose, fructose, glucose, mannitol, raffinose, rhamnose, and xylose are utilized; sucrose and inositol are not.

The type strain is strain 80-3024 (= ATCC 35682).

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LITERATURE CITED

1. Becker, B., R. E. Gordon, H. A. Lechevalier, and M. P. Lechevalier. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl. Microbiol.* 12:421-423.
2. Frontali, C., L. R. Hill, and L. G. Silvestri. 1965. The base composition of deoxyribonucleic acids of *Streptomyces*. *J. Gen. Microbiol.* 38:243-250.
3. Furumai, T., M. Hamada, A. Matsumae, Y. Mikami, A. Seino, and A. Shimazu. 1975. Culture media for actinomycetes. The Society for Actinomycetes, Tokyo, Japan.
4. Ganju, P. L., and M. R. S. Iyengar. 1974. Micromorphology of some sclerotial actinomycetes and development of their sclerotia. *J. Gen. Microbiol.* 82:35-48.
5. Gordon, R. E. 1967. The taxonomy of soil bacteria, p. 293-321. In T. R. G. Gray and D. Parkinson (ed.), *The ecology of soil bacteria*. Liverpool University Press, Liverpool.
6. Gordon, R. E., and A. C. Horan. 1968. A piecemeal description of *Streptomyces griseus* (Krainsky) Waksman and Henrici. *J. Gen. Microbiol.* 50:223-233.
- 6a. Kelly, K. L., and D. B. Judd. 1976. Color universal language and dictionary of names. NBS special publication 440. National Bureau of Standards, Washington, D.C.
7. Lechevalier, H. A., and M. P. Lechevalier. 1967. Biology of actinomycetes. *Annu. Rev. Microbiol.* 21:71-100.
8. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. *J. Lab. Clin. Med.* 71:934-944.
9. Lechevalier, M. P., C. De Bièvre, and H. A. Lechevalier. 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem. Syst. Ecol.* 5:249-260.
10. Lechevalier, M. P., and H. A. Lechevalier. 1980. The chemotaxonomy of actinomycetes, p. 227-291. In A. Dietz and D. W. Thayer (ed.), *Actinomycete taxonomy*. Society for Industrial Microbiology special publication 6. Society for Industrial Microbiology, Arlington, Va.
11. Lechevalier, M. P., H. A. Lechevalier, and C. E. Heintz. 1973. Morphological and chemical nature of the sclerotia of *Chainia olivacea* Thirumalachar and Sukapure of the order *Actinomycetales*. *Int. J. Syst. Bacteriol.* 23:157-170.
12. Marmur, J. 1961. A procedure for the isolation of DNA from microorganisms. *J. Mol. Biol.* 3:208-218.
13. Sharples, G. P., and S. T. Williams. 1974. Fine structure of the globose bodies of *Dactylosporangium thailandense* (Actinomycetales). *J. Gen. Microbiol.* 84:219-222.
14. Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* 16:313-340.
15. Thirumalachar, M. J. 1955. *Chainia*, a new genus of the Actinomycetales. *Nature (London)* 176:934-935.
16. Tuite, J. 1969. Plant pathological methods. Burgess Publishing Co., Minneapolis.
17. Waksman, S. A. 1967. *The actinomycetes*. Ronald Press, New York.