NOTES

Actinoplanete with Cylindrical Sporangia, *Actinoplanes rectilineatus* sp. nov.

MARY P. LECHEVALIER AND H. A. LECHEVALIER

Waksman Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08903

The name *Actinoplanes rectilineatus* sp. nov. is proposed for the organism formerly referred to as *Actinoplanes* sp. 7-10. It forms a very short, macroscopically visible, orange-brown aerial mycelium. Conidia formation has not been observed. Globose, lophotrichous spores with an average of 30 flagella per spore are formed in longitudinal rows in cylindrical sporangia. Diffusible pigments are produced. Nitrates are not reduced. The type strain has been assigned the Waksman Institute of Microbiology IMRU number 3919.

During the course of our studies of actinomycetes, a strain of *Actinoplanes* (strain 7-10) with sporangiospores arranged with *Ampullariella*-like linearity (6, 7) (Fig. 1) was isolated and selected for morphological (12, 15) and physiological (10) studies. This organism was not identifiable with any of the described species. Since this strain of *Actinoplanes* has been one of the most studied members of the genus, nomenclatural orthodoxy, designed to avoid confusion, warrants giving this organism a scientific name, publishing its properties, and depositing it in a culture collection. Strain 7-10 is hereby regarded as belonging to a new species, for which we propose the name *Actinoplanes rectilineatus* (from the Latin *rectus lineatus*, meaning marked with straight lines, an allusion to the disposition of the sporangiospores). This strain, which is the type strain, has been deposited in the culture collection of The Waksman Institute of Microbiology of Rutgers, The State University of New Jersey, under the number IMRU 3919.

Isolation. *A. rectilineatus* IMRU 3919 was isolated in November 1965 from garden soil, collected in Somerset, N.J., that had been plated out on water agar.

Morphology. The organism has the general morphological features that were described by Couch (5) for the genus *Actinoplanes* and later tabulated by Kane (14). The hyphae and spores are mainly gram positive; the sporangia are gram negative. As described previously (12, 15), the globose sporangiospores of this organism are 1.5 to 2.0 μ m in diameter (Fig. 2) and bear a polar tuft of 17 to 40 flagella (mean, 30). The bases of the flagella are hooked (Fig. 3). The linear arrangement of the spores within the

sporangia has been shown previously in sections by Lechevalier et al. (15). The release mechanism of the sporangiospores of this organism was studied by Higgins (10), who also observed that germinating spores do not lose their flagella. A similar observation was made for *Actinoplanes armenicus* by Kalakoutskii and Kuznetsov (13).

Cell wall composition. Higgins and co-workers (12) reported that strain IMRU 3919 has a cell wall of type II containing *meso*-diaminopimelic acid and glycine in addition to glucosamine, muramic acid, alanine, and glutamic acid. The walls also contain an unidentified analogue of diaminopimelic acid that has frequently been found in other strains of *Actinoplanaceae* (1, 19). This strain, like other actinoplanetes, has a whole-cell sugar pattern of type D (16).

Temperature optimum for growth. *A. rectilineatus* 3919 was inoculated on yeast extract-dextrose agar slants and incubated at 10, 23, 28, 37, and 42 C. Growth was most abundant at 23 and 28 C, poor at 10 and 37 C, and absent at 42 C.

Growth on various media.

(i) Czapek agar: Sparse, tan growth; aerial mycelium; no soluble pigment.

(ii) Czapek-dextrose agar: Flat, white colonies; no soluble pigment, sporangia, or aerial mycelium.

(iii) Yeast-Czapek agar: Grayish-tan colonies; no soluble pigment; aerial mycelium.

(iv, v) Potato-carrot and tomato-soy agars: Yellow-tan colonies; no aerial mycelium; light yellow-brown soluble pigment.

(vi) Oatmeal agar: Yellow-brown colonies; aerial mycelium; no soluble pigment.

FIG. 1. Photomicrograph of a water suspension of *A. rectilineatus* sporangia. $\times 1,300$. Note linear arrangement of the sporangiospores. Bar represents 10 μm .

(vii) Tyrosine agar: Yellow-tan young colonies with aerial mycelium becoming wet-looking, shiny, and dark orange; early, pink, soluble pigment produced, which then becomes dark brown.

(viii) Yeast-dextrose agar: Yellow-tan growth; no aerial mycelium; no soluble pigment.

The formulas of the above media may be found in the following references: media i and ii, reference 22; ii, reference 11; iv, reference 8; v, vi, and vii, reference 18; vii, reference 21.

Biochemical reactions. Casein, tyrosine, gelatin, esculin, and starch are hydrolyzed. Phosphatase is produced. Nitrate reductase is absent on two different media. Acetate, lactate, malate, pyruvate, and succinate are utilized. Acid is formed from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, rhamnose, salicin, trehalose, and xy-

lose. There is no growth on 5% NaCl. Sensitive to lysozyme. No action on hypoxanthine, xanthine, adenine, or urea. No utilization of benzoate, citrate, mucate, oxalate, propionate, or tartrate. No acid from adonitol, dulcitol, erythritol, α -methyl-D-glucoside, raffinose, sorbitol, sucrose, or β -methyl-D-xyloside. Methods for these tests were given previously by Gordon (9) and Lechevalier and Lechevalier (17).

Despite the fact that the cylindrical sporangia and longitudinal arrangement of the spores of this strain are reminiscent of ampullariellae, the globose spores place *A. rectilineatus* in the genus *Actinoplanes* (5, 14). The genus *Ampullariella* is reserved for organisms with rod-shaped spores (3, 6). By virtue of the formation of macroscopically visible aerial mycelium, *A. rectilineatus* is most closely related to *A. armeniacus* (13). However, the aerial mycelium of *A. rectilineatus* is very short, giving the colony

FIG. 2. Electron micrograph of an *A. rectilineatus* spore shadowed with germanium at an angle of 12° . $\times 28,000$. Bar represents $1\ \mu\text{m}$.

FIG. 3. Electron micrograph of an *A. rectilineatus* lysing spore negatively stained with 1% phosphotungstic acid. $\times 150,000$. Note the hook at the base of each flagellum (arrow). Bar represents $0.1\ \mu\text{m}$.

surface a brownish, powdery appearance, and no conidia, such as those reported for *A. armeniacus*, have ever been observed. *A. rectilineatus* differs from *A. armeniacus* in the size of the sporangia, the color of the aerial mycelium, the absence of conidia, the type of flagellation, and the absence of sporangia on Czapek-dextrose agar.

Among the unnamed strains discussed in the literature, *A. rectilineatus* is similar in morphology to *Actinoplanes* sp. of Willoughby (23) but differs from it by the absence of conidia and

the shape of the sporangiospores. It is also similar to strains 20 and 23 of Couch (6), about which we know nothing other than that they have sporangia with longitudinal rows of sporangiospores.

A. rectilineatus differs from all other species of *Actinoplanes* by not reducing nitrate to nitrite (2). Other characteristics of these organisms are compared in Table 1.

Strain IMRU 3919 is the type strain of *A. rectilineatus*. Because the description of *A. rectilineatus* is based on a single strain, it also

TABLE 1. Comparison of the properties of named species of *Actinoplanes*

Species/ reference	Czapek's agar			Sporangia shape/size (μ m)	Sporangiospores		
	Growth mass	Aerial mycelium	Soluble pigment		Arrangement	Shape/size (μ m)	Flagellation
<i>A. philippinensis</i> (4)	Light buff to tawny	None	Pale yellow	Globose (8.4–25)	Coils	(1.0–1.2)	Lophotrichous
<i>A. utahensis</i> (6)	Apricot to salmon- orange	None	None	Irregular (5–18)	Coils	Subglobose (1.0–2.0)	Lophotrichous
<i>A. missouriensis</i> (6)	Ochraceous salmon	None	Pale lavender	Globose (6–14)	Coils	(1.0–1.2)	Peritrichous
<i>A. armeniacus</i> (13)	Sand ^a	White, spiral- shaped chains of conidia	None	Globose (20–50)	Rows	Globose (1.0–1.5)	Peritrichous
<i>A. brasiliensis</i> (20)	Deep orange ^a	None ^a	None ^a	Irregular (3.5–11.5)	Not easily visible	Subspherical to rod- shaped (1.2–2.3)	Lophotrichous
<i>A. italicus</i> (2)	Light straw- berry ^a	None ^a	Deep orange- yellow ^a	Globose (6–11)	Not easily visible	Globose (1.0–2.0)	
<i>A. rectilineatus</i>	White ^a Tan	None ^a Sparse tan, no conidia	None ^a	Cylindrical (8–15)	Straight, longitudinal rows	Globose (1.5–2.0)	Lophotrichous

^a Czapek-dextrose agar.

serves as the description of the type strain. Obviously the species description will be modified as additional strains of this organism are isolated and characterized.

We wish to thank Michael Higgins for providing Fig. 2 and Magda Gagliardi for excellent technical assistance.

This study was supported by National Science Foundation grant GB 41631.

REPRINT REQUESTS

Address reprint requests to: Dr. H. A. Lechevalier, Waksman Institute of Microbiology, Rutgers University, New Brunswick, N.J. 08903.

LITERATURE CITED

- Becker, B., M. P. Lechevalier, and H. A., Lechevalier. 1965. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl. Microbiol.* 13:235–243.
- Beretta, G. 1973. *Actinoplanes italicus*, a new red-pigmented species. *Int. J. Syst. Bacteriol.* 23:37–41.
- Buchanan, R. E., and N. E. Gibbons (ed.). 1974. *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Couch, J. N. 1950. *Actinoplanes*, a new genus of the *Actinomycetales*. *J. Elisha Mitchell Sci. Soc.* 66:87–92.
- Couch, J. N. 1955. A new genus and family of the *Actinomycetales*, with a revision of the genus *Actinoplanes*. *J. Elisha Mitchell Sci. Soc.* 71:148–155.
- Couch, J. N. 1963. Some new genera and species of the *Actinoplanaceae*. *J. Elisha Mitchell Sci. Soc.* 79:53–70.
- Couch, J. N. 1964. The name *Ampullaria* Couch has been replaced by *Ampullariella*. *Int. Bull. Bacteriol. Nomencl. Taxon.* 14:137.
- Cross, T., M. P. Lechevalier, and H. Lechevalier. 1963. A new genus of the *Actinomycetales*: *Microellobosporia* gen. nov. *J. Gen. Microbiol.* 31:421–429.
- Gordon, R. E. 1967. The taxonomy of soil bacteria, p. 293–321. *In* T. R. G. Gray and D. Parkinson (ed.), *Ecology of soil bacteria*. Liverpool University Press, Liverpool.
- Higgins, M. L. 1967. Release of sporangiospores by a strain of *Actinoplanes*. *J. Bacteriol.* 94:495–498.
- Higgins, M. L., and M. P. Lechevalier. 1969. Poorly lytic bacteriophage from *Dactylosporangium thailandensis* (*Actinomycetales*). *J. Virol.* 3:210–216.
- Higgins, M. L., M. P. Lechevalier, and H. A. Lechevalier. 1967. Flagellated actinomycetes. *J. Bacteriol.* 93:1446–1451.
- Kalakoutskii, L. V., and V. D. Kuznetsov. 1964. A new species of the genus *Actinoplanes* Couch. *Microbiology USSR* 33:553–560.
- Kane, W. D. 1966. A new genus of *Actinoplanaceae*: *Pilimelia*. *J. Elisha Mitchell Sci. Soc.* 82:220–230.
- Lechevalier, H. A., M. P. Lechevalier, and P. E. Holbert. 1966. Electron microscopic observation of the sporangial structure of strains of *Actinoplanaceae*. *J. Bacteriol.* 93:1228–1235.
- Lechevalier, M. P., and H. A. Lechevalier. 1968. Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes, p. 311–316. *In* H. Frauser (ed.), *The actinomycetales*. Gustav

- Fischer, Jena.
17. Lechevalier, M. P., and H. A. Lechevalier. 1974. *Nocardia amarae* sp. nov., an actinomycete common in foaming activated sludge. *Int. J. Syst. Bacteriol.* 24:278-288.
 18. Shirling, E. B., and D. Gottlieb. 1966. Methods of characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* 16:313-340.
 19. Szaniszló, P. J., and H. Gooder. 1967. Cell wall composition in relation to the taxonomy of some *Actinoplanaceae*. *J. Bacteriol.* 94:2037-2047.
 20. Thiemann, J. E., G. Beretta, C. Cornelli, and H. Pagani. 1969. Antibiotic A/672 isolated from a new species of *Actinoplanes*: *Actinoplanes brasiliensis* nov. sp. *J. Antibiot.* 22:119-125.
 21. Waksman, S. A. 1950. The actinomycetes—their nature, occurrence, activities and importance. *Chronica Botanica Co.*, Waltham, Mass.
 22. Waksman, S. A. 1967. The actinomycetes. A summary of current knowledge. *Ronald Press*, New York.
 23. Willoughby, L. G. 1966. A conidial *Actinoplanes* isolate from Blelham Tarn. *J. Gen. Microbiol.* 44:69-72.