Emended Description of the Genus *Pseudonocardia*
Henssen and Description of a New Species
*Pseudonocardia spinosa* Schäfer

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Acropetal hyphal budding and production of blastospores characteristic of the genus *Pseudonocardia* Henssen and designated as *Pseudonocardia*-type were studied by time-lapse photography. The formation of cross walls in the aerial hyphae of *Pseudonocardia thermophila* was reexamined. Not all segments of the articulated hyphae are limited by septa. The new species *Pseudonocardia spinosa* Schäfer is described. The type strain of this species is MB SF-1 (ATCC 25924, CBS 818.70). *P. spinosa* corresponds with the type species of the genus in the acropetal budding of the hyphae, in the formation of blastospores, in the composition of the cell wall (type IV), and in the occurrence of a yellow pigment; it differs mainly in being mesophilic and in possessing spiny spores.

The genus *Pseudonocardia* Henssen 1957 is characterized by the peculiar acropetal budding of the hyphae and spore chains, designated as *Pseudonocardia*-type (6). The budding growth of the hyphae and the production of blastospore chains are not known in any other genus of the Actinomycetales. In *Streptomyces* the spores are formed in basipetal direction or more or less simultaneously within the sporophores (3, 6, 10). The basipetal production of spores in the chains of some oligosporic actinomycetes was demonstrated recently in electron micrographs (5). According to Lechevalier and Solotorovsky (15) and Konev et al. (9), the spore chains in *Micropolyspora* and *Microechinospora*, respectively, are produced successively by a permanent growing point, the youngest one situated at the base of the chain (see also Fig. 2 in reference 6).

Since the manner of spore development is an essential characteristic in the classification of the actinomycetes, a documentation of the type of sporulation by time-lapse photographs seems highly desirable. The difficulties of such studies are mentioned by Lechevalier and Lechevalier (13).

Our results with *Pseudonocardia thermophila* are presented in this paper. In addition, the aerial mycelium was reexamined with the electron microscope.

A new species, *Pseudonocardia spinosa* Schäfer (Latin adjective, *spinosus* meaning spiny), was isolated from soil. It differed in some important respects from the type species, and an emendation of the genus diagnosis is therefore necessary.

The cell wall components of two strains of *P. thermophila* were compared with those of the type strain of *P. spinosa*. Furthermore, a petroleum ether-soluble yellow pigment occurring in both of the species was examined.

**MATERIALS AND METHODS**

For the study of *P. thermophila* the type strain, MB A-18, and a new isolate, MB B-18, were used. (MB is the abbreviation for the Herbarium of the Botanical Institute of the University of Marburg.) Hanging-drop cultures (nutrient-glucose-peptone solution) were first placed in an incubator at 50°C. After 29 hr they were set on a hot stage (Wild M 20 microscope) at the same temperature. The production of segments and aerial spores was studied in 1- and 2-hr intervals.

The new actinomycete *P. spinosa* was found on plates which served for the isolation of *Streptosporangium* species. The strains were isolated by the methods used for these organisms (16). Strain MB SF-1 was isolated from plates which were inoculated with soil samples from Turkey (near Özbag) and strain MB SF-2 was isolated from soil from Iran (Teheran).

The electron micrographs were taken with the Elmiskop I A of Siemens. The following media not mentioned by Schäfer (16) were used, Casamino Acids-peptone-Czapek agar (Couch in lit.): Casamino Acids (Difco), 1 g; peptone (Merck), 2 g; K₂HPO₄, 1 g;
KCl, 0.5 g; MgSO₄·7H₂O, 0.5 g; FeSO₄·7H₂O, 0.01 g; sucrose, 30 g; agar, 15 g; water to 1,000 ml. Soil extract was made from equal volumes of leafy soil and tap water boiled for 2 hr, filtered, cleared by centrifugation, and sterilized for 15 min at 121°C. Artificial soil agar (KEHE) consisted of “AS solution” (17), 1,000 ml; yeast extract (Oxoid), 0.1 g; glucose, 0.01 g; soil extract, 50 g; agar, 15 g. For hanging-drop cultures of P. spinosa, soil extract solution was used.

The analyses of the cell wall components were made after the instructions of Hoare and Work (7), Becker et al. (2), and Lechevalier (11). For this purpose, P. spinosa was cultured for 2 months on Casamino Acids-peptone-Czapek agar and P. thermophila for 4 and 6 days at 50°C on liquid and solid nutrient-sugar medium: peptone, 2.5 g, beef extract, 2.5 g; NaCl 2.5 g; yeast extract, 0.1 g; glucose, 2.5 g; sucrose, 5.0 g; Casamino Acids, 0.1 g; distilled water, 1,000 ml; for solid medium, 15 g of agar was added.

The production of the yellow pigment increased during incubation. The same media were used as for the study of the cell wall components. Both species were grown for 2 months. For the spectrophotometric study, the mycelium was extracted with ethanol and the ethanol was extracted with petroleum ether. The absorption spectra of the pigments were obtained using the Cary model 15 recording spectrophotometer.

RESULTS AND DISCUSSION

Developmental stages in the aerial mycelium of P. thermophila. The successively acropetal formation of segments in the aerial hyphae is seen in Fig. 1 to 3. In Fig. 1, the terminal segment is slightly constricted just behind the tip. Two hours later (Fig. 2), the constriction is more pronounced and the tip is enlarged. After 3 hr (Fig. 3), the development of the new segment is finished.

In Fig. 4 to 7 the corresponding formation of the aerial blastospores is illustrated. In the first stage (Fig. 4), two round spores are seen at the hyphal tip. One hour later (Fig. 5), one more spore was formed. Three hours later (Fig. 6), the terminal spore has enlarged considerably, and the development of a fourth spore is initiated. After 4 hr, the development of the fourth spore is complete (Fig. 7).

The time-lapse photographs confirm our statements previously made about the acropetal budding of the hyphae and spore chains, designated as Pseudonocardia-type. Cross walls cannot be seen by this method. They were earlier demonstrated in electron micrographs (6).

The fact that the new species P. spinosa has a nonseptate mycelium (see below) made us restudy the aerial hyphae of P. thermophila to determine whether articulated hyphae without cross walls occur also in that species. The new electron micrographs revealed considerable variation in the production of septa.

Three possibilities are demonstrated in Fig. 8 to 10. In Fig. 8 the tiny tip of the hyphae is already separated by a wall. Figure 9 shows three segments of which the basal one only is delimited by septa. In Fig. 10, a nonseptate chain of links is seen. Much longer articulated hyphae without cross walls are also frequent. It remains an open question whether such hyphae develop septa later on or whether they give rise to fragmentation spores previously observed by light microscopy (6).

The cross walls of the aerial hyphae look different from the septa in the substrate.

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Fig. 1-3. Development of the segments in aerial mycelium of Pseudonocardia thermophila strain MB A-18; time-lapse photographs. Magnification, ca. x 2,300. Numbers below figures indicate hours.

Fig. 4-7. Production of blastospores in an aerial hypha in Pseudonocardia thermophila strain MB A-18; time-lapse photographs (ca. x 2,300). Numbers below figures indicate hours.
mycelium (compare Fig. 4 in reference 6). An investigation of ultrathin sections is necessary to get more information about the fine structure.

**Cell wall composition and pigment.** The analysis of the whole-cell hydrolysate corresponded in both *Pseudonocardia* species. The three strains studied contained *m*-diaminopimelic acid (DAP), arabinose, galactose, and glucose; *L*-DAP was lacking.

The absorption spectrum of pigment extracts obtained from the three strains studied had a peak at 446, a shoulder at 420, and a plateau at 467 nm (Fig. 20).

*Pseudonocardia spinosa* Schäfer n. sp.

Diagnosis: Gram-positive. Substrate mycelium forms a compact mass composed of obviously nonseptate hyphae of various thicknesses (0.4 to 2.6 μm) constricted at various intervals (Fig. 16). Segments irregular in shape or globose to ovoid. Aerial mycelium white. Hyphae ca. 0.4 to 1.0 μm broad, branched and articulated, without cross walls. The segments act as spores. Spores mostly 2.5 to 4.5 μm long, often thickened at the apex (Fig. 12), loosely covered by tiny spines (Fig. 14). Elongation of the hyphae is by acropetal budding (Fig. 15).

Physiological properties: Extremely slow growth. Colonies become visible after approximately 2 weeks of incubation. Growth continues for half a year at least. In poor media, the organism produces almost exclusively aerial mycelium; in richer media, a golden yellow substrate mycelium covered by scanty to abundant aerial mycelium develops. Melanin or other soluble pigments are not produced.

Asparagin-glycerol-agar: Growth moderate to good; substrate mycelium scanty, yellowish; aerial mycelium abundantly produced.

Ascasimo Acids-peptone-Czapek agar: Growth good; substrate mycelium a compact mass on the agar surface, yellow, covered by aerial mycelium.

Casein-glucose-earth-agar: Growth good; substrate mycelium almost lacking; aerial mycelium abundant.

Cellulose-agar: Growth good; substrate mycelium yellow; aerial mycelium abundant.

Yeast-starch-agar: Growth good; substrate mycelium yellow; by culturing on a medium of half concentration, development of aerial mycelium is increased.

KEHE: Growth moderate to good; substrate mycelium almost lacking; aerial mycelium abundant.

Half-concentrated skim milk-agar. Growth moderate; substrate mycelium yellow; thin cover of scanty aerial mycelium; casein not hydrolyzed.

Starch and milk not hydrolyzed. Good growth between 20 and 30°C. No growth at 37°C. Cell wall composition is of type IV (12).

The type strain is MB SF-1, ATCC 25924 (American Type Culture Collection, Rockville, Md.), CBS 818.70 (Centraalbureau voor Schimmelcultures, Baarn, the Netherlands). The characteristics of the type strain are the same as those of the species, as given above.

Illustrations: Figure 19, colony on agar plate; Fig. 17 and 18, details of the colonies; Fig. 13, aerial mycelium in hanging-drop culture; Fig. 11, 12, 14, and 15, electron micrographs of aerial mycelium; Fig. 16, substrate mycelium.

Source: Soil.

Remarks. The organism was found on agar plates prepared for the isolation of *Streptosporangium* species where it formed tufts of white aerial mycelium about 0.5 to 1 mm in diameter (Fig. 19). The separation from the accompanying bacteria proved to be very diffi-
Fig. 11–19. *Pseudonocardia spinosa* (strain SF-1 with exception of Fig. 12). Fig. 11. Aerial hypha with developing side branches (×10,000). Fig. 12. Chain of spores with spiny walls; strain SF-2 (×9,000). Fig. 13. Acropetal budding of aerial hyphae in hanging-drop culture; the links showing the beginning of the striation (×2,500). Fig. 14. Spiny spores in higher magnification (×15,000). Fig. 15. Acropetal budding in detail, the segments with a globular body (×9,000). Fig. 16. Irregularly swollen segments of the substrate mycelium (×8,000). Fig. 17. Aerial hyphae with developing branches on KEHE agar (×800). Fig. 18. Zig-zag-shaped aerial hyphae on KEHE agar (×800). Fig. 19. Tufts of aerial mycelium on KEHE agar (×200).
cult. Success was finally obtained on cellulose agar. Cellulose, however, is not essential for growth. The organism develops equally well on the same medium lacking cellulose powder.

On rich media, the substrate mycelium forms a compact mass of irregularly swollen hyphae (Fig. 16). This fact obscured the study of the hyphal structure. Similar substrate hyphae were formed in hanging-drop cultures. No septa have been observed.

The white tufts of the aerial mycelium (Fig. 19) may simulate at a cursory glance a streptomycete colony with straight, branched chains of long spores. A closer examination of the plates by direct study with the light microscope, however, reveals the peculiar acropetal budding of the aerial hyphae (Fig. 13, 15, 17), characteristic for the genus *Pseudonocardia*. The aerial mycelium is entirely articulated. Nonconstricted hyphae, common in *Streptomyces*, do not occur. The links are not separated by septa. A globular body is seen in each segment (Fig. 14, 15).

*P. spinosa* differs from *P. thermophila* mainly in the spiny spores (Fig. 12, 14), the obviously complete lack of septa in the substrate and aerial mycelium, the more branched blastospore chains, the extremely slow growth, and the mesophilic properties of the former. Furthermore, the zig-zag shape of the aerial hyphae is less pronounced (Fig. 18), and no fragmentation spores were observed with *P. spinosa*. Old aerial hyphae often become transversely striated; the beginning of this is seen in Fig. 13.

Emended description of *Pseudonocardia Henssen*.

Diagnosis: Hyphal growth by acropetal budding, production of blastospores, and cell wall composition type IV.

Substrate hyphae often zig-zag-shaped or densely packed and irregularly swollen. Fragmentation spores are occasionally formed.

The segments of the aerial hyphae directly act as spores; the blastospores are typical for the genus; fragmentation spores may also be produced. Spores are smooth walled or spiny. Gram-positive, not acid-fast. Growth good on a variety of media under aerobic (or anaerobic) conditions; mesophilic or thermophilic.

Remarks. In the imperfect fungi, the type of development of conidia turned out to be a fundamental criterion for classification (8, 18). To us the type of development of the spores of actinomycetes seems equally important as a tool for the classification of these organisms. In *Pseudonocardia* the spore chains arise in the same way as the blastospores in fungi. The nonseptate spore chains in *P. spinosa* resemble, e.g., those of *Monilia cinerea* (reference 1, Fig. 138B), whereas the septated spore chains of *P. thermophila* are like those of *Cladosporium* sp. (reference 1, Fig. 6A; reference 8, Fig. 7). *Pseudonocardia* is the only known genus of the actinomycetes producing such blastospore chains; unique also is the acropetal budding of the aerial hyphae bearing the sporophores. Such hyphae are produced abundantly under certain conditions (6). In the first investigations of the genus, only spore chains arising directly from the substrate mycelium were noticed (4). Until now, this is the only method of spore formation which has been observed in *P. spinosa*.

Time-lapse photographs are important for the documentation of the type of spore development. They are, however, not necessary for diagnostic purposes. It may be stressed here that the budding of aerial hyphae or the presence of spore chains is easily recognized by the trained eye. Agar plates need only be studied directly under the microscope. The cultures contain all stages of the budding process (Fig. 17 and 13).

Our findings concerning the cell wall composition of *P. thermophila* confirmed the results reported previously by Lechevalier and Lechevalier (12). These authors designated a cell wall containing *m*-DAP, arabinose, and galactose as type IV. The acropetal budding of our new organism pointed clearly to its close relationship to *Pseudonocardia*; the same type
of cell wall composition further justified the inclusion of this organism in *Pseudonocardia*. The articulate aerial hyphae of *P. spinosa* resemble somewhat the constricted mycelium of *Sporichthya* (14). The development of this unusual member of the actinomycetes, however, follows other lines. Elongated portions become secondarily divided, and no budding occurs.

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**LITERATURE CITED**


