Rapid Differentiation Between *Nocardia* and *Streptomyces* by Paper Chromatography of Whole-Cell Hydrolysates

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**ABSTRACT**

BECKER, B. (Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J.), MARY P. LECHEVALIER, RUTH E. GORDON, AND H. A. LECHEVALIER. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. **12**:421–423. 1964.—Whole-cell hydrolysates were prepared from 58 strains of *nocardiae* and streptomycetes. Strains morphologically intermediate between the two genera and morphological variants of the same strains were included. Paper chromatograms made from the whole-cell hydrolysates clearly demonstrated meso-diaminopimelic acid as a major constituent of cultures of *Nocardia* spp., and LL-diaminopimelic acid as a major constituent of cultures of *Streptomyces* spp. In cultures of ten strains of *N. madurae* and of three of *N. pelletieri*, meso-diaminopimelic acid predominated, thereby supporting the assignment of these species to the genus *Nocardia*.

The validity of the distinction between the generic forms *Nocardia* and *Streptomyces*, questioned for many years, has been recently stressed by Baldacci (1962). Waksman (1959) considered the two genera to be so dissimilar that he put them in two different families on the dubious assumption that strains of *Nocardia* have a substrate mycelium that fragments into bacillary or cocoid elements, whereas strains of *Streptomyces* have a substrate mycelium that not only does not fragment, but is even nonseptate. Lechevalier, Solotorovsky, and McDermont (1961) took exception to this view and suggested the abolition of the family Streptomycetaceae since it is possible to obtain, from the same strain, mutants that, if Waksman’s criteria were to be taken seriously, would have to be placed not only in different genera but even in different families.

Biochemists, however, have put a new tool in the hands of the morphologists by showing that not all actinomycetes have the same cell-wall composition. The literature on this subject has been reviewed recently by Villanueva, Gascón, and García Acha (1963). Briefly, as summarized by Cummins (1962a), there are two types of cell wall among the aerobic actinomycetes. One could be called the *Streptomyces* type, and contains, as a major component, the LL form of α,ω-diaminopimelic acid and few sugars. Arabinose is never found. The second, which could be called the *Nocardia* type, contains the meso form of diaminopimelic acid as a major constituent, and, among the sugars, arabinose is always present. We have undertaken, therefore, a study of the cell-wall composition of representatives of the many form-genera which have been proposed for actinomycetes (Kuster, 1963; Lechevalier, 1964). The results of this study, which will be published later, have shown that the *Nocardia* type of cell wall is the most common, even though numerous variations of the basic type occur. Most important is that the LL form of diaminopimelic acid is found, as a major constituent of the cell wall, almost exclusively in strains of *Streptomyces*. We believed that an examination of the composition of the cell wall might be very useful in identifying strains that are morphologically intermediate between the nocardiae and streptomycetes. Such an examination, however, is too time-consuming for application to hundreds of strains. Because most of a cell’s diaminopimelic acid is found only in the cell wall, we used the method outlined below to determine the isomeric form of diaminopimelic acid in easily prepared hydrolysates of whole cells instead of cell walls free from cytoplasmic contaminants. To test the value of the method, one of us (R. E. G.) furnished the other authors with cultures of *Nocardia* and *Streptomyces* without any clue as to their identity. “Borderline” cultures from the Institute of Microbiology, Rutgers University (IMRU) collection, as well as variants of certain strains that differed morphologically from their parents, were included. The results indicate, so far, that the method is reliable.

**MATERIALS AND METHODS**

The organisms were grown in shake flasks at 28 or 37 C, and the cells were collected at maximal growth after incubation varying from 24 hr to 2 weeks.

The following media were used: yeast extract-glucose broth (Waksman, 1950); NZ-amime glycerol broth (Lechevalier et al., 1961); soy bean meal broth [soy bean meal, 20 g; commercial glucose, 10 g; NaCl, 5 g; yeast extract BYF-50X (Amber Laboratories, Inc., Milwaukee, Wis.), 5 g; tap water, 1,000 ml]; and nutrient-yeast extract broth [nutrient broth powder (Difco), 8 g; BYF-50X, 5 g; tap water, 1,000 ml]. All media were used as limpid solutions, clarified by filtration whenever necessary. After incubation, the cells were collected by filtration, washed with water and ethyl alcohol, and air-dried at room temperature.

Dried cells (10 mg) were hydrolyzed for 18 hr with 1 ml of 6 x HCl in a sealed Pyrex tube held at 100 C in a sand
bath. After cooling, the tubes were opened and the contents were filtered through paper. The solid material on the paper was washed with 3 drops of distilled water. The liquid hydrolysate was dried three consecutive times on a steam bath to remove most of the HCl. The residue was taken up in 0.3 ml of distilled water, and 20 µl of the liquid were spotted on Whatman no. 1 paper. A spot of 10 µl of 0.01 M meso-diaminopimelic acid was put on the paper to run alongside the sample to serve as a reference standard. The sample of meso-diaminopimelic acid (Mann Research Laboratories, New York, N.Y.) also contained some of the L-isomer of the same compound, thus acting as a standard for both forms of the acid.

Descending chromatography as advocated by Hoare and Work (1957) was carried out overnight by irrigation with methanol-water-10 N HCl-pyridine (80:17.5:2.5:10, by volume). Amino acids were detected by dipping the papers in a bath of acetonic ninhydrin (0.1 %, w/v), followed by heating for 2 min at 100 C. Diaminopimelic acid spots were olive-green fading to yellow. Other amino acids in the hydrolysate gave purple spots with this reagent and traveled faster than did the diaminopimelic acid, some migrating off the paper during the overnight irrigation. In this system, meso-diaminopimelic acid had an R_{LL-diaminopimelic acid} of 0.8.

Results and Discussion

The following are Streptomyces strains that contained LL-diaminopimelic acid as a major constituent. Strain numbers are those of the IMRU collection, except as otherwise indicated. S. albus strains 758, 1205, 3004, and 618 (ATCC); S. aureofaciens strains 3617, 3708, and 3709; S. citreus strains 678, 951, and 3574; S. fradiae strains 3535, 3535-R, 3535-3, and 3535-7B; S. griseus strains 712, 1247, and 3008; S. lavendulae strains 467, 467W, and 3440-14; S. rimosus strains 3082, 3558, and 3669; S. somaliensis strains 632 and 719; and Streptomyces spp. strains 3319, 3417, and 3678. Macroscopic differences between S. fradiae strains 3535 and 3535-7B, and between S. rimosus strains 3558 and 3669, are illustrated in Plate 6 of Gordon and Mihm (1962).

Nocardia strains that contained meso-diaminopimelic acid as a major constituent are as follows: N. asteroides strains A5, 102, Emmons 9991 (C. W. Emmons, National Institutes of Health, Bethesda, Md.); SL-107-B (R. S. Sandhu, University of Delhi, India); N. brasiliensis strains 774-A, 774-B, and 3487; N. caviae strain 424; N. coeliaca strains 1105, 1124, and 3520; N. farcinica strain 1242; N. madurae strains 413, 507, 953, 1091, 1136, 1190, 1294, 1253, 3632-A, and 1070 (National Collection of Type Cultures); N. pelletieri strains 408, 408-W, and 513; N. vaccinii strain 3500; and Nocardia spp. strains 710, 714, 715, and 717.

Examination of the paper chromatograms never left any doubt whether a given strain should be labeled a Streptomyces or a Nocardia. Among the human pathogens, strains of N. madurae and N. pelletieri contained meso-diaminopimelic acid as a major constituent. Cummins (1962b) noted that strains of N. pelletieri have a cell wall that contains both the LL and the meso form of diaminopimelic acid, with a preponderance of the meso form. Our results on whole cells were essentially in agreement with those of Cummins, because we also found the meso form of the acid as a major component. Cummins noted, however, that the cell wall of these organisms did not contain any arabinose but had some glycine. On the basis of these results, Cummins (personal communication) was inclined to place the species pelletieri and madurae in the genus Streptomyces rather than in the genus Nocardia. We do not necessarily object to Cummin's point of view. Indeed it may very well be that the species pelletieri and madurae are intermediate between the nocardiae and streptomyces. We feel that, until more basic facts are known about these organisms, the presence of one or the other form of diaminopimelic acid as a major constituent is a convenient criterion for the separation of the genus Streptomyces from the genus Nocardia. Our method has the advantage of having been based on a characteristic that can be rapidly evaluated without ambiguity. Two strains of another human pathogen, S. somaliensis, on the other hand, contained LL-diaminopimelic acid as a major constituent.

Another interesting group of strains were those listed as S. griseus 712 and Nocardia spp. 710, 714, 715, and 717. These strains were received as isolates from animals with infections of the respiratory tract. Strain 712 was a typical S. griseus and its identification was not difficult. The other strains seemed akin to S. griseus in certain biochemical properties, but in varying degrees. The diaminopimelic acid determination sharply separated the typical S. griseus from the atypical forms. Here again, we might be tempted to speculate on the presence of an intergeneric bridge.

Cummins and Harris (1958) suggested that the nocardiae be placed in the Mycobacteriaceae on the basis of their cell-wall composition. Studies now in progress on the chemical composition of the cell wall of numerous form genera of actinomycetes not studied by Cummins cause us to refrain at this time from making any suggestion about the standing of the families of the actinomycetes. The rapid test described here could also very likely be applied to the differentiation of strains belonging to the genera Nocardia and Actinomyces. In borderline cases, this method could be of use, since Cummins (1962a, b) and Cummins and Harris (1958) have shown that cultures of Actinomyces spp. have no diaminopimelic acid in their cell wall, whereas cell walls of the nocardiae contain the meso form of this acid.

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**LITERATURE CITED**


