MORPHOLOGICAL STUDIES IN THE GENUS *NOCARDIA*

V. SEPTATION IN *Nocardia rubra* AND *Jensenia canicruria*[1]

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Bisset and Moore (J. Gen. Microbiol., **3,** 387, 1949) reported that septation in members of the genus *Nocardia* differed from that in soil diphtheroids. They found that soil diphtheroids were unicellular upon examination of cell-wall stained preparations, but with the same staining method *Nocardia* sp. were multicellular. Using uni- versus multi-cellularity as a basis, these authors proposed a new genus, *Jensenia,* to include the unicellular soil diphtheroids, thus separating them at the genus level from the multicellular genus, *Nocardia.* Webb *et al.* (J. Bacteriol., **67,** 498, 1954) reported that multicellular filaments were produced before fragmentation by *Nocardia corallina* and considered multicellular bacilli and coccoids as involution forms due to environmental influences.

Since Bisset and Moore attached considerable importance to septation as a taxonomic criterion in these forms, this phenomenon was studied further in our laboratory, in an effort to distinguish *Jensenia canicruria* (Bisset and Moore, J. Gen. Microbiol., **4,** 280, 1950) from *Nocardia rubra* (received as *Proactinomyces rubra* (Casabó) Bald.). The culture of *J. canicruria* used in this study was kindly supplied by Dr. Francis E. Clark, who obtained it from Dr. Bisset's laboratory.

The two organisms were grown on nutrient agar slants for 18 hr at room temperature and harvested by suspending the surface growth in sterile distilled water. Properly diluted suspensions were then used for preparations for cell-wall staining following the method described by Webb (J. Bacteriol., **67,** 252, 1954) and for electron microscope specimens. The collodion supported suspensions for the latter studies were shadow-cast with platinum-palladium alloy.

Electron microscope studies revealed no differences in degree of septation in *J. canicruria* and *N. rubra* (figures 1 to 4), either in young filaments (figures 1 and 2) or in bacillary cells (figures 3 and 4). Studies of the cell-wall stained preparations with the light microscope showed that in properly stained smears no filaments, bacilli, or coccoids of either organism were multicellular (figures 5 and 6). Septa may occasionally occur in young filaments in both organisms, but are only frequently and regularly produced as the organism approaches the age when rapid fragmentation of filaments takes place (McClung, Trans. Kansas Acad. Sci., **58,** 50, 1955). Further study of cell-wall stained preparations revealed that when decolorization with Congo red was incomplete, structures appeared (figure 7) which give the impression of multicellularity in *N. rubra* (compare figure 7 with figure 9 of Bisset and Moore, J. Gen. Microbiol., **3,** 387, 1949). McClung (J. Bacteriol., **59,** 589, 1950) observed large numbers of lipid inclusions in *N. rubra* which may account for this apparent multicellular appearance in young filaments.

On the basis of these studies, the frequency of septation resulting in unicellular or multicellular forms appears to be an inadequate criterion for differentiating between the genera *Jensenia* and *Nocardia.* This is especially true since septation is related to the stage in the life-cycle of the organisms (McClung, Trans. Kansas Acad. Sci., **58,** 50, 1955). There is also evidence in Nocardia that septation, time of fragmentation, and length of filaments are considerably influenced by media composition and other environmental factors (Webb *et al.*, J. Bacteriol., **67,** 498, 1954).

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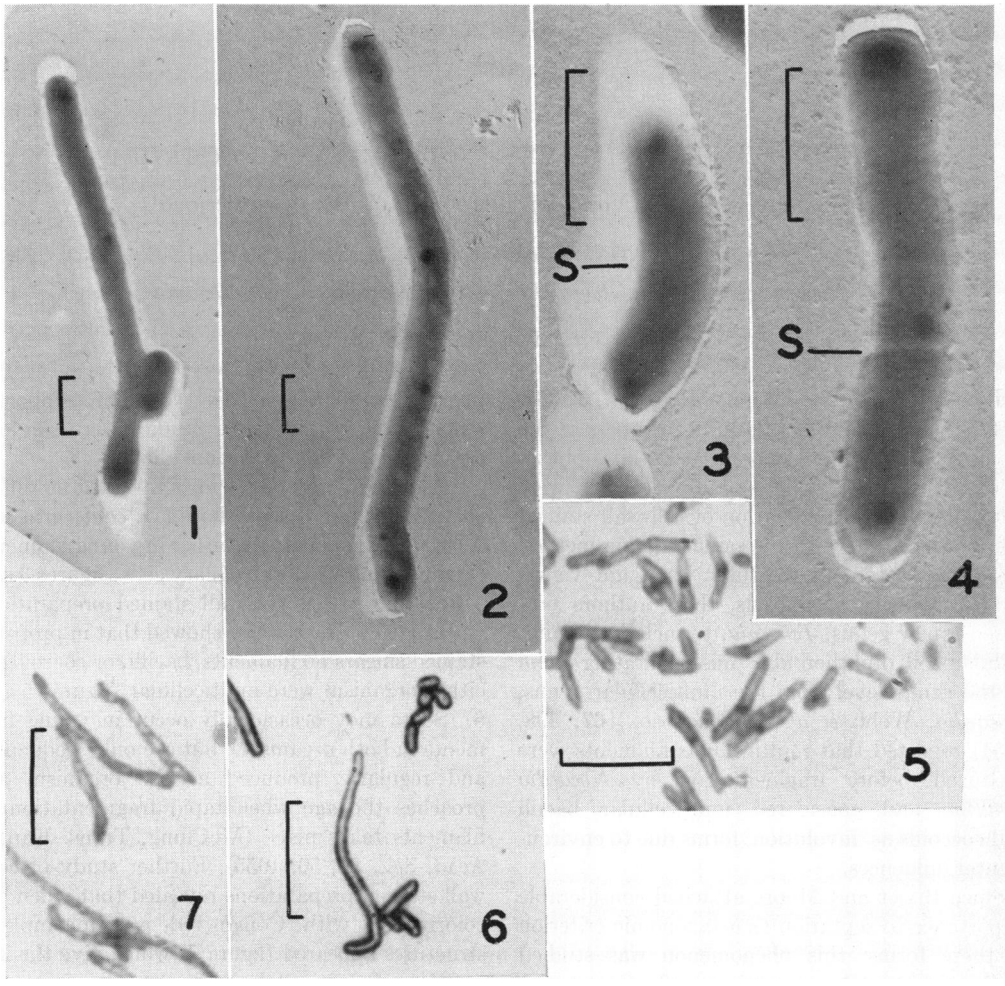

Figure 1–7. All photographs are of 18-hr nutrient agar cultures

Figure 1. Electron micrograph of *Jensenia canicruria*, platinum-palladium shadowed. Scale marker, 1 μ.

Figure 2. Electron micrograph of *Nocardia rubra*, platinum-palladium shadowed. Scale marker 1 μ.

Figure 3. Electron micrograph of *J. canicruria*, bacillary form showing septum (*S*), platinum-palladium shadowed. Scale marker, 1 μ.

Figure 4. Electron micrograph of *N. rubra*, bacillary form showing septum (*S*), platinum-palladium shadowed. Scale marker, 1 μ.

Figure 5. Cell wall stained smear of *J. canicruria*. Scale marker, 5 μ.

Figure 6. Cell wall stained smear of *N. rubra*. Scale marker, 5 μ.

Figure 7. Cell wall stained smear of *N. rubra*, incompletely decolorized with congo red. Scale marker, 5 μ.