This medium, dispensed in 100-ml amounts in 250-ml Erlenmeyer flasks, was inoculated with an agar block containing L colonies which had been growing 5 days following 15 passages on a staphyloccocal L form maintenance agar (Marston, J. Infectious Diseases, 108, 75-84, 1961). Slight turbidity and a granular sediment developed in the liquid medium in 4 days at 37 C and serial transfer of the sediment resulted in more abundant growth in a shorter time.

The sediment was composed of minute, morphologically indistinct elements and some large bodies; no cocci were observed. Many of the large bodies appeared to contain particles which showed Brownian movement. That the granular sediment contained L forms was demonstrated by transferring 0.1 ml to brain heart infusion agar plates with final concentrations of 5% NaCl, 10% horse serum and 1,000 units/ml penicillin G. This transfer resulted in appearance of typical L colonies. Fig. 1 shows the granular elements in liquid media and an L colony derived from them. The colonial morphology was, in all respects, identical to the L colonies used as the initial inoculum.

No reversion of the L forms to cocci was observed following continued cultivation or repeated serial transfer to media free of penicillin and with a lower salt concentration.

Serological and biochemical studies of staphyloccocal L forms cultivated in this manner are rendered more tenable and should contribute to the expanding knowledge of them.

MORPHOLOGY OF NITROSOMONAS EUROPAEA AND CLASSIFICATION OF THE NITRIFYING BACTERIA

M. S. ENGEL

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received for publication December 16, 1960

Interest in the biochemical transformations of the nitrifying bacteria has focused attention on the chemoautotroph Nitrosomonas europaea. Descriptions of the morphology of the organism are hampered by difficulties that prevent the application of the usual bacteriological growth techniques to this organism. Until recently, N. europaea was grown in calcium or magnesium carbonate-containing media for periods varying from weeks to months. The cells grown under such conditions were often described as nonmotile (Mecklejohn, J. Gen. Microbiol., 21, 185, 1950), and are listed in Bergey's Manual of Determinative Bacteriology (7th ed., The Williams and Wilkins Company, Baltimore, 1967) as nonmotile or with a single polar flagellum, rarely having one at either end. Lewis and Pramer (J. Bacteriol., 76, 524, 1958) published a picture of N. europaea that shows no flagellation.

The organism used in these investigations was kindly supplied by R. L. Starkey and was grown as described by Engel and Alexander (J. Bacteriol., 76, 217, 1958). When cultures of N. europaea were grown for biochemical investigations, the cell levels attained were high enough to prepare electron micrographs. The cells came from cultures having a generation time of approximately 11 hr and growing to a final titer of 2 X 10^8 viable cells. Cells taken from a late log phase culture were motile and had two subterminal flagella (Fig. 1a). The cells were ellipsoidal rods measuring approximately 0.8 by 1.2 μ. Another picture, also showing two flagella, appears elsewhere (Engel, Ph.D. thesis, Cornell University, 1959).

The difficulties in showing flagella in an electron micrograph were as follows: Centrifugation caused rupture and produced a flagella-free population (Fig. 1b). This rupturing could also explain the absence of flagella in the pictures of Lewis and Pramer (personal communication) and Hoffman and Lees (Biochem. J., 53, v., 1953). When the population reached a titer of 2 X 10^9 cells, the nitrite-nitrogen level in the medium was more than 1 mg/ml. This nitrite level causes corrosion of the copper grids on which the bac-
bacteria are mounted for electron microscopy. To circumvent corrosion, the grids were dried under vacuum immediately after the culture was placed on them.

The conclusion is that *N. europaea* has two flagella, and that lack of flagellation or motility in other cultures may be ascribed to experimental artifact.

Now let us examine the criteria for the differentiation of *N. europaea* and *N. monocella*. *N. monocella* is described in *Bergey's Manual* as ovoid with yellowish brown, irregular colonies on silica gel, and *N. europaea* as a rod, forming small, sharply defined brown colonies on solid media. Fig. 1 and pictures by Lewis and Pramer (1958) show that *N. europaea* is an oval cell, not a rod. Kingma-Boltjes (Arch. Mikrobiol., 6, 79, 1935) investigated swarming in liquid medium as a reflection of motility. He also described alteration of colony appearance of a pure culture on solid media. The shape of the colonies thus seems to depend on the medium, not the cell. Cell size seems to be the only remaining difference between the two species according to *Bergey's Manual*. The criteria of size and shape are not valid for the differentiation of the species because of the dependence on age of culture, and spherical cells of *N. monocella* are described as coming from a young culture.

The criteria for the existence of other genera of nitrifiers are also open to question. In 1935, Kingma-Boltjes showed that he could produce a nitrosocystis-like culture when growing Nitrosomonas in MgCO₃-containing media. His pictures show "zooglea" and "capsule" formation, these being the characteristics separating Nitrosomonas from Nitrosocystis. Persistent attempts by many people to isolate nitrifiers other than Nitrosomonas, and the fact that these other cultures do not now exist make it questionable whether the listing of the genera Nitrosococcus, Nitrospira, Nitrosocystis, or Nitrosoglea should be continued in *Bergey's Manual*. The great difficulties associated with obtaining and maintaining pure cultures of Nitrosomonas have been reviewed by Lewis and Pramer (1958). Only recently have pure cultures become available, so that the existence of other nitrifiers is doubtful, especially since the morphological descriptions differentiating them can be accounted for on the basis of cultural conditions (Hofman and Lees, 1952; Kingma-Boltjes, 1935; and Meiklejohn, Nature, 168, 56, 1951) and impure culture (Grace, Nature, 168, 117, 1951; Imseenecky, Nature, 157, 877, 1946).

Fig. 1a. Electron micrograph of *Nitrosomonas europaea*. A sample from a log phase culture was placed on a collodion-coated copper grid and immediately dried. Shadow casting at a 4:1 angle was carried out using uranium nitrate. Scale marker, 1 μm.

Fig. 1b. Cells of *N. europaea* that were centrifuged and washed before fixing on the copper grid. Scale marker, 1 μm.