Cell Morphology and Flagellation of Nitrogen-Fixing Spirilla

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ABSTRACT. Twenty isolates of N₂-fixing spirilla were isolated from the rhizosphere of maize and sugar cane grown in Egyptian and Belgian soils. Electron microscopy distinguished two morphological groups. The first includes short and thick curved rods with an unipolar flagellum while cells of the second group are much longer with the typical appearance of spiral cells and most probably possess a bipolar tuft of flagella.

Spirillum lipoferum Beijerinck is a very common soil and root inhabitant occurring mainly in the tropics (Becking 1963; Dobereiner and Day 1976; Dobereiner et al. 1976). The bacterium was found to be among the major organisms responsible for nitrogenase activity on roots of field-grown tropical grain and forage grasses (von Bulow and Dobereiner 1975). Several isolates were obtained and studied for their different nutritional requirements and other physiological characteristics (Sampaio et al. 1976; Okon et al. 1976a, b). Neyra et al. (1977) were able to distinguish three major groups of isolates on the basis of nitrate reduction. Little information is available concerning cell morphology and flagellation of the organism. We now report on such characteristics of the type culture S. lipoferum ATCC 29145, as well as of representative strains occurring in the rhizosphere of maize and sugar cane planted in Egyptian and Belgian clay soils.

MATERIALS AND METHODS

Isolation of strains. Maize and sugar cane were planted in Egyptian and Belgian clay soils under subtropical conditions provided in a greenhouse to study nitrogenase activity in the rhizosphere. Segments from different parts of active roots were partially surface-sterilized by shaking for 2 min in absolute ethanol and washed twice in sterilized distilled water. They were transferred to 10 ml of 0.5 % malate semi-solid medium (Day and Dobereiner 1976) in 50-ml test-tubes and incubated for 2—5 d at 30 °C. The resulting enrichment cultures were examined for the presence of characteristic white sub-surface pellicles of spirilla, nitrogenase activity and for active motility. Purification of selected strains was achieved by 3—5 successive transfers to semisolid malate medium followed by isolation of single colonies developed on malate agar supplemented with 0.5 % yeast extract and on potato agar as well as nutrient agar plates respectively (Dobereiner et al. 1976).
Assay of nitrogenase activity. Root enrichments and pure cultures grown in semi-solid medium for 2–5 d at 30 °C were subjected to C2H2 reduction assay. The cotton plugs of the tubes were replaced by tight serum stoppers and 10 % of the gas phase was replaced by acetylene. Samples (1 ml) of the gas phase were taken at 1-h intervals for ethylene determination with a Hewlett-Packard gas chromatograph using a Poropak R column (Vlassak and Jain 1976).

Microscopy. Purified strains as well as a type culture of S. lipoferum (ATCC 29145) were examined microscopically for cell morphology, type of motility and flagellation (Leifson’s stain), and for the presence of lipid bodies (Sudan Black stain). Formvar-coated grids prepared from young (1–3 d) and aged (> 7 d) cells grown in semi-solid malate medium were negatively stained with phosphotungstic acid (2 %, W/V, pH 6.8) according to Hegazi (1975). Electron micrographs were taken with a JEOL U electron microscope.

RESULTS AND DISCUSSION

Malate enrichments prepared from surface-sterilized roots of various stages of maize and sugar cane growth were positive to the acetylene reduction assay, and showed the presence of the characteristic white sub-surface pellicle of the N2-fixing spirilla. The malate medium compared to other N-deficient media, e.g. Ashby’s (Hegazi and Niemela 1976) and Becking’s (Becking 1959) media, furnished with glucose as carbon source has provided better growth of the organism and yielded highest nitrogenase activity. Lactate was utilized efficiently with all strains. This is explained by Okon et al. (1976) who found that tricarboxylic-acid cycle intermediates as well as lactate and pyruvate supported active O2 uptake on both cell suspensions and cell-free extracts. The efficiency of N2-fixation reported for tested strains as determined by semi-micro Kjeldahl analysis was in the range of 13.6—27.6 mg N assimilated per g malate, which is close to the estimates of 8—24 mg N per g carbon substrate reported by Okon et al. (1976).

Phase-contrast and electron microscopy revealed two types of cell morphology irrespective of plant and soil origin. Plate 1A—D represents the first morphological group where the cells are oval or like thick, slightly curved, rods (2.0—3.0 × 1.5 to 3.0 μm). They occur mainly in pairs and sometimes single, and contain several refractive lipid bodies easily stained with Sudan Black. They move very fast in various directions with a mode of motility probably similar to that of Azomonas insignis except that the cells of the N2-fixing spirilla show rather mobile cytoplasm while moving or standing. Electron micrographs (Plate 2) confirmed the earlier suggestions of Becking (1963) and Krieg (1976) that they are unipolar flagellated. The type culture of Dobereiner (ATCC 29145) fits in this group; in some pictures the attachment site of the flagellum to the cell surface is resolved (Plate 3). The second group of isolates include those having the typical appearance of spirilla. The cells are much longer and thinner (3.5—5.0 × 1.0—1.5 μm) with more than one turn per cell (Plate 1E, F). Cells occur mainly single and sometimes in pairs and packed with lipid bodies (Plate 4). They move forward and backward, spinning along the axis. Electron microscopy suggests that these cells possess bipolar tufts of flagella. Members of Aquaspirillum which is suggested to include the N2-fixing spirilla are reported to possess bipolar tufts of flagella (Krieg 1976). The placement of our isolates in any of the three physiological groups of Neyra et al. (1977) must await further investigations.