

A FREE-LIVING RHIZOBIUM SP. ABLE TO GROW ON  $N_2$  AS SOLE NITROGEN SOURCE

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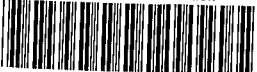
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Some strains of Rhizobium, mainly slow growing, are known to fix N<sub>2</sub> when cultivated in free-living conditions. However, such strains are unable to grow at the expense of N<sub>2</sub> as sole N source and combined nitrogen (e.g. glutamate) must be added to the medium to support growth. We report here for the first time the case of a tropical, fast-growing strain of Rhizobium characterized by its ability to grow on N<sub>2</sub>. This strain (ORS571) was originally isolated from stem-nodules of Sesbania rostrata (L., J.).

Strain ORS 571 was cultured in batch under a constant flow (1 l/min) of an O<sub>2</sub>/Ar (3%-97%) or O<sub>2</sub>/N<sub>2</sub> (3%-97%) mixture with internal agitation (400 rpm). Two liquid media were used: (1) LN containing per 1000 ml: Na-lactate, 10 g; Na-glutamate or Ammonium-sulfate, 1 g; K<sub>2</sub>HPO<sub>4</sub>, 1.67 g; KH<sub>2</sub>PO<sub>4</sub>, 0.87 g; NaCl, 0.05 g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1 g; CaCl<sub>2</sub>, 40 mg; FeCl<sub>3</sub>, 4 mg; trace elements; biotin, 2 mg; nicotinic acid, 2 mg; pantothenic acid, 2 mg. pH 6.8. (2) LO, a N-free medium differing from LN in that Na-glutamate or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> were omitted and vitamins content increased tenfold. The absence of contaminants in the cultures and their nodulation ability were systematically checked.

Strain ORS 571 assimilated ammonium when the required vitamins were provided in the medium. In LN medium, with ammonium sulfate as source, the generation time of strain ORS 571 grown under air or under the O<sub>2</sub>/Ar mixture was 3 h at 30 or 37°.

When strain ORS 571 was inoculated under the O<sub>2</sub>/N<sub>2</sub> mixture in LO medium, nitrogenase activity was detected after 2 h, and optical densities increased from 0.15 to 2 with a generation time of 7 h at 37°. Nitrogenase activity was maximum (1200 nmoles C<sub>2</sub>H<sub>4</sub>/mg protein/h) for an optical density of 0.4. Under O<sub>2</sub>/Ar, maximum O.D. was 0.3.

The ability of strain ORS 571 to grow on N<sub>2</sub> as sole N source could be explained by the fact that it readily assimilated ammonium whether produced by nitrogenase or added to the growth medium. This unique property allows the isolation and characterization of nif mutants of Rhizobium, using standard bacterial genetic.

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