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In Vitro Nitrogen Fixation by Two Actinomycete Strains Isolated from *Casuarina* Nodules

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Acetylene reduction activity was demonstrated in pure cultures of two actinomycete strains isolated from nodules of *Casuarina equisetifolia*. This activity was comparable to that of free-living *Rhizobium* strains, but appeared to be less sensitive to pO_2 and more sensitive to the presence of combined nitrogen.

It is well established that some strains of

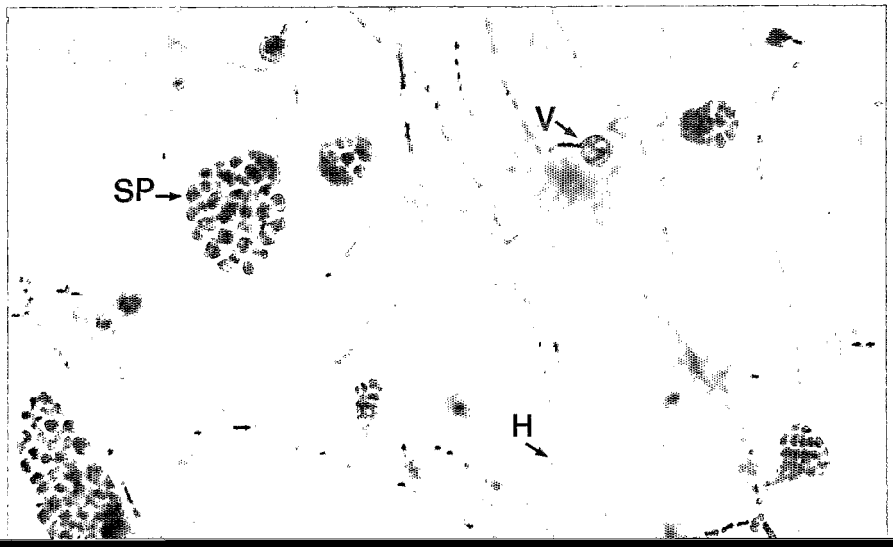
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TABLE 1. *Influence of different nitrogen sources on nitrogenase biosynthesis measured by the acetylene reduction method*[a]

Nitrogen source[b]	Specific acetylene reduction activity (nmol of C_2H_4 per h per mg of protein)	
	Strain D11	Strain G2
None (control)	34	14
Yeast extract (25 μg/ml)	42	20
Yeast extract (50 μg/ml)	30	12
Yeast extract (100 μg/ml)	17	12
L-Glutamine (0.25 mM)	32	16
L-Glutamine (0.54 mM)	3	1
L-Glutamine (1.1 mM)	3	1
KNO_3 (2 mM)	1	1
KNO_3 (5 mM)	0	0
KNO_3 (10 mM)	0	0
NH_4Cl (2 mM)	5	2
NH_4Cl (10 mM)	0	0
NH_4Cl (20 mM)	0	0

[a] Actinomycete strains were incubated in agitated vials filled with a gas mixture containing 10% O_2 and 90% argon at 30°C for 5 days. The specific acetylene reduction activity was calculated by using the slope of the C_2H_4 production curve.

[b] The medium contained malate (20 mM) and different amounts of combined nitrogen as indicated.

tion was ca. 10 to 30 nmol/h per mg of protein, which is comparable to values obtained for free-living *Rhizobium* strains which exhibited moderate acetylene reduction activities (2, 5). Since the incubation in the preliminary experiments reported here was carried out in agitated vials and the cell number was low, we assume that the concentration of dissolved O_2 in the culture was relatively high. Thus, it seems likely that our isolates fix nitrogen under more aerobic conditions than reported for free-living *Rhizobium* strains.

Nitrogenase synthesis by our isolates appeared to be more sensitive than *Rhizobium* strains to the presence of combined nitrogen, since *Rhizobium* strains are still able to form a significant amount of nitrogenase with an ammonium concentration of 40 mM (7).

Surprisingly, strains D11 and G2 did not initiate nodulation in the host plant. Failure to induce nodulation may indicate that these strains are noninfective in the pure culture condition as suggested by Knowlton et al. (7a) or that the requisite environmental conditions for nodulation were not achieved in our tests.

ADDENDUM IN PROOF

This note was in press when Tjepkema et al. (J. D. Tjepkema, W. Ormerod, and J. G. Torrey, Nature [London] **287**:633–635, 1980) reported that strains of *Frankia* isolated from *Comptonia peregrina* and *Alnus rubra* cultured in defined nutrient media could reduce acetylene.

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