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Isolation of *Azospirillum* from the Rhizosphere of Jojoba [*Simmondsia chinensis* (Link) Schneider] and Guayule (*Parthenium argentatum*)

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Nitrogenase activity was associated with the roots of Jojoba and Guayule. Pure cultures of *Azospirillum* were isolated from the rhizosphere and rhizoplane of both these plants and also from inside the roots of Jojoba. While the N_2-ase activity of these cultures did not significantly vary, the isolates from inside the roots showed markedly less activity. All these cultures exhibited more activity with glucose as sole carbon source as compared to malate. The rhizosphere of both these plants maintained higher populations of fungi, bacteria and actinomycetes than the non-rhizosphere.

Key Words: *Azospirillum*, Rhizosphere *Simmondsia chinensis Parthenium argentatum*

Introduction

Since the isolation of nitrogen-fixing bacterium, *Azospirillum* sp. from the roots and rhizosphere of tropical grasses, (Dobereiner & Day 1976) many other plants were later found to have this organism associated with their roots, (Lakshmi-Kumari et al. 1976 and Sylvester-Bradley 1976). The rhizosphere association of this bacterium depends primarily on the root exudation pattern of each plant species (Dommergues et al. 1973). However, no information is available on the association of this organism with the roots or the rhizosphere of two economic plants, namely Jojoba (*Simmondsia chinensis*) —an oil-yielding plant, and Guayule (*Parthenium argentatum*) which yields rubber from the whole plant. These species are native to Mexican desert and have been introduced into India for their economic exploitation. The present paper reports the isolation of *Azospirillum* sp. from the rhizosphere of Jojoba and Guayule and their characterisation. Besides, the rhizosphere effect of these plants on the population of fungi, actinomycetes and bacteria was also studied.

Materials and Methods

The nitrogenase activity of the roots of two-years-old field grown plants of Jojoba and Guayule was assayed at four different intervals during 1980-81. The soil free roots of one g each were kept in five 250 ml Erlenmeyer flasks which were later closed with a rubber cork having a serum cap in the centre. Ten per cent of the air inside the flasks was replaced with acetylene and these were incubated at 30±1°C for 24 hr. The ethylene produced after the incubation was estimated by gas chromatography as described elsewhere (Venkateswarlu & Rao 1982). In the September, 81 sampling, the rhizosphere populations of fungi, bacteria and actinomycetes were estimated by dilution plate technique employing Martin's rose bengal agar, Thronton's agar and Ken-Knights agar media respectively. The most probable numbers (MPN) of *Azospirillum* in the rhizosphere and rhizoplane were estimated as per the method described by Okon et al. (1977).

Pure cultures of *Azospirillum* from the rhizosphere and rhizoplane were obtained by repeated sub-culturing from the respective positive MPN tubes into the N-free semi-solid malate medium and ultimately plating on potato-sucrose-malate agar for single cell colonies. Isolates from within the roots were obtained by crushing small surface sterilized (0.1% $HgCl_2$ for 2 mts) root bits in a pestle and mortar under aseptic conditions and the suspension thus obtained was used for inoculating the culture tubes. The N_2-ase activity of these cultures was assayed in the semi-solid malate medium as described earlier (Venkateswarlu & Rao 1982). The activity with glucose (0.5%) as sole carbon source was tested in the same medium by replacing malic acid. The N_2-ase activity of the cultures was expressed as n moles of C_2H_4 produced/tube/hr.

Results and Discussion

The roots of both Jojoba and Guayule had exhibited N_2-ase activity irrespective of the sampling season (table 1). In case of Jojoba the activity was higher during September which corresponds to the onset of it's growth period, while the activity remained more or less constant in case of Guayule throughout the year. However, in both the cases the seasonal variation in the activity was not statistically significant. Further Jojoba roots had shown significantly higher N_2-ase activity compared to Guayule in all the four samplings. The most probable numbers (MPN) of *Azospirillum* estimated from the rhizosphere of Jojoba and Guayule were 69.7×10^2 and $38.9 \times 10^2 g^{-1}$ oven dry soil respectively which means that the population was nearly two times higher in the rhizosphere of the former. The rhizoplane of Jojoba also yielded much higher numbers as compared to Guayule. This may partly explain the higher N_2-ase activity observed with the roots of Jojoba. However, this bacterium was completely absent in the non-rhizosphere soil which indicates that it is primarily a rhizosphere inhabitant (Dobereiner & De-polli 1980). While *Azospirillum* could also be isolated from inside the roots (Surface sterilized and crushed) of Jojoba this was not so with Guayule indicating that it's association was more intimate with the roots of Jojoba.

There were no marked differences in the nitrogenase activity between the cultures

Table 1 *N_2-ase activity and MPN of Azospirillum associated with the roots of Jojoba and Guayule*

Plant	Nitrogenase activity (n moles of C_2H_4 day^{-1} g^{-1} fresh roots)				Population of *Azospirillum*	
	Dec, 80	Mar, 81	June, 81	Sept, 81	Rhizosphere*	Rhizoplane**
Jojoba	30.03	22.62	25.70	32.00	69.7×10^2	93.4×10^2
Guayule	19.78	17.20	16.70	18.60	38.9×10^2	10.2×10^2

*MPN g^{-1} ovendry soil **MPN g^{-1} fresh roots

Table 2 *N_2-ase activity of* Azospirillum *isolates from Jojoba and Guayule*

Isolate No.	Source	N_2-ase activity (n mole C_2H_4/hr) the medium containing Malic acid	Glucose
G_1	Rhizosphere of Guayule	107.03	132.52
G_2	,,	116.68	172.29
G_3	Rhizoplane of Guayule	143.28	194.66
G_4	,,	123.46	202.00
J_1	Rhizosphere of Jojoba	121.57	233.53
J_2	,,	142.87	195.40
J_3	Rhizoplane of Jojoba	129.50	242.78
J_4	,,	149.50	214.39
J_5	Inside the roots of Jojoba	48.61	128.07
J_6	,,	56.51	135.41

C.D. at 5% level for isolates: N.S., Carbon source: 28.9, interaction 41.7

isolated from Jojoba and Guayule. Nor the source of isolation, i.e., rhizosphere or rhizoplane had any effect on the activity. However isolates from within the roots of Jojoba showed significantly less activity as compared to those from the rhizosphere and rhizoplane which might be due to the greater dependence of these isolates on the host supplied nutrients by virtue of their close association and consequently unable to multiply fully in culture medium. All the isolates irrespective of their origin had shown higher N_2-ase activity in medium containing glucose as sole carbon source as compared to malate. Utilization of glucose resulted in acid production and fall in *p*H. Based on this and other cell morphological characters suggested by Tarrand et al. (1978) these isolates were identified as *A. lipoferum.*

Both Jojoba and Guayule had exerted a typical rhizosphere effect on all microorganisms (table 3). The populations of total bacteria, fungi and actinomycetes were significantly higher in the rhizosphere than the non-rhizosphere. Further the rhizosphere effect was more distinct in case of Jojoba. The R/S ratio for all these groups of organisms was much higher in Jojoba than in Guayule, which indicated that the root exudates in Jojoba might be rich in nutrients which stimulate the multiplication of soil microflora.

These preliminary results indicate the occurrence of N_2-fixing *Azospirillum* in the roots/rhizosphere and it's possible role in the N-economy of these economic plants which grow mostly in the desert soils.

Table 3 *Rhizosphere microflora of Jojoba aud Guayule*

Organism	Population of microorganisms g^{-1} oven dry soil: Non-rhizosphere	Rhizosphere: Jojoba	Guayule
Fungi	11.28×10^3	76.76×10^3 (6.7)	59.22×10^3 (5.31)
Actinomycetes	74.15×10^4	356.1×10^4 (4.8)	121.1×10^4 (1.61)
Bacteria	10.26×10^5	202.02×10^5 (19.7)	49.67×10^5 (4.81)

Figures in parenthesis indicate the rhizosphere effect (R/S ratio)

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