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Detection of Nitrogen-fixing ability in an Epiphytic Orchid *Vanda testacea* (Linde) Reichb. F.

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Light microscopic observations of fresh sections of leaf and root of *Vanda testacea* (Linde) Reichb. F. showed cocco-bacillary shaped bacteria in parenchymatous cells of the cortex and the pith. These were isolated on nitrogen-free Ashby's mannitol agar and were characterized as *Azotobacter*. Cells of the aerial root, stem and leaf contained approximately 10^3 bacterial cells/mg of the fresh tissue. Bacterial isolates as also plant parts exhibited acetylene reduction ability which ranged between 16–261 nmol of C_2H_4/hr/mg of protein (bacteria) and 7–10 nmol of C_2H_4/hr/g of plant fresh weight. Bacterial isolates also produced indolyl 3-acetic acid (IAA).

Key Words: Epiphytic orchid, Acetylene reduction, *Azotobacter*, **Intracellular**

Introduction

As orchid flowers exhibit a great range of variation in colour, size and shape, they have been highly appreciated as ornamental plants and have been exploited commercially by horticulturists. Some orchids, known as epiphytes, grow predominantly on the trunks of forest trees, with aerial root hanging in the air; however they derive no nourishment from the host tree (Mukherjee 1983, Pradhan 1985). A variety of media are available for the artificial cultivations of orchids (Mukherjee 1983). However, there is little information concerning nutritional requirements of epiphytic orchids under natural conditions. This is specially true for their nitrogen requirement. In view of this, the nitrogen-fixing ability of an epiphytic orchid *Vanda testacea* (Linde) Reichb. F. was examined which is the subject of this report.

Materials and Methods

Plant Material

Vanda testacea was collected from Dehradun region, preserved in plastic bags under cold condition along with moss and fern fibers and kept wet with water to provide necessary humidity to orchids. Plants were examined within 24–36 hrs after collection.

Microscopic and Bacteriological Study

During this study, every care was taken to exclude the external contamination. Cleaning with a natural detergent like soap-nut, surface disinfection with 75% ethanol of different plant parts and subsequent sectioning as well as isolation of bacteria on Ashby's mannitol agar were carried out as described previously (Sharma et al. 1985).

Characterization of Isolated Bacteria

Isolates were characterized in terms of their morphological, cultural and biochemical characters using conventional procedures (Harrigan & McCance 1966). Following standard characters, as given in Bergey's Manual of Determinative Bacteriology (Becking 1974), an attempt was made for identification of isolates. Pectinolytic (both trans-eliminase and hydrolytic type) activity was examined using the thiobarbituric acid (TBA) method

(Ayers et al. 1966). Detection and estimation of IAA in the culture filtrate was carried out using Salkowsky reagent (Libbert & Risch 1969).

Acetylene Reduction Assay

Fresh plant material after surface disinfection and bacterial cultures grown aerobically on nitrogen free Ashby's mannitol agar slope for 48 hrs were used for assay as described (Turner & Gibson 1980). Plant material as well as bacterial cultures were incubated for 2 hr at 28–30°C under the atmosphere of air plus acetylene (90+10%) prior to assay of ethylene.

Results and Discussion

Light microscopic observations of fresh sections of leaf and aerial root revealed abundance of coccobacillary form of bacteria, mainly confined to parenchymatous cells of the pith and the cortex (figure 1). They showed sluggish movement which facilitated their observation.

Three isolates, OR_1, OR_2, & OR_3 were isolated on Ashby's mannitol agar, from the homogenates of different plant parts after surface disinfection.

All isolates were gram negative, capsulated, non-sporulating, aerobic, motile, short-rods. Colonies were raised, round, medium size (1–2 mm) smooth with entire edge and mucoid in nature. These isolates were urease and catalase positive. But they could be differentiated on the basis of other characters described in table 1. On the basis of the characters examined, all three isolates could be classified under the genus *Azotobacter*. Quantitative analysis revealed that OR_1 made up for more than 50% of the total population of nitrogen fixers, irrespective of the plant part analysed. Average bacterial population ranged between 1 to 2×10^3 cells/mg of the fresh tissue (root, stem and leaf).

Table 1 *Characteristics of three bacterial isolates from orchid* Vanda testacea

Character	Bacterial isolates		
	OR_1	OR_2	OR_3
Pigmentation	White	Light brown	White
Opacity of colony	Trans-parent	Opaque	Semi trans-parent
Slime production	Less	Moderate	High
Starch utilization	+	+	+
Mannitol utilization	+	+	+
Rhamnose utilization	–	+	+
Protease activity	+	+	–
Pectinolytic activity	+	–	–
IAA production	+	+	+

IAA – Indolyl 3-Acetic Acid
+ Positive reaction; – Negative reaction

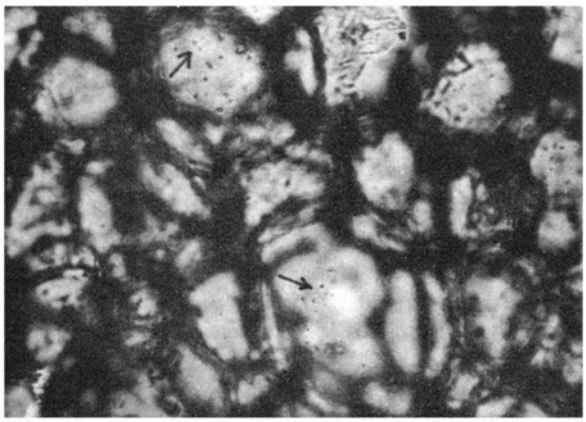

Figure 1 Light micrograph (×750) of a transverse section of aerial root of orchid showing the presence of coccobacillary shaped bacteria in the cells of the cortex region

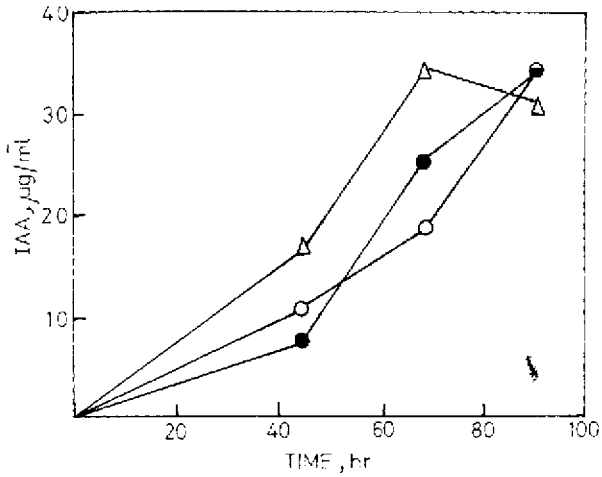

Figure 2 Time course of the IAA production from tryptophan by isolates OR_1 0-0-0, OR_2 ●-●-● and OR_3 Δ-Δ-Δ

The acetylene reduction capacity in leaf, stem, and aerial roots ranged between 7–10 nmol of C_2H_4/hr/g of fresh weight. Nitrogenase activity of isolates OR_1, OR_2, & OR_3 under aerobic condition in nitrogen-free medium was found to be 261, 66 & 16 nm/hr/mg of protein respectively. These observations indicate that nitrogen fixing bacteria do contribute to the nitrogenous requirement of an epiphytic orchid.

In a recent study on *in vitro* culture of *Vanda* hybrid by Mathews and Rao (1985), IAA has been shown to favour proliferation and differentiation of protocorm units. The production of IAA by rhizobia (Kefford et al. 1960), mycorrhizae (Slankis 1973), *Azospirillum brasilense* (Reynders & Vlassak 1979) and *Azotobacter paspali* (Barea & Brown 1974) is implicated in influencing the host plant metabolism. Detection of the ability of IAA production by all three isolates from *Vanda testacea* (figure 2) suggests that this might play a role in growth and differentiation of orchid plant under natural conditions.

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