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NITROGEN-FIXING (ACETYLENE REDUCING) BACTERIA ASSOCIATED WITH MYCORRHIZAE OF *EUCALYPTUS* IN TAIWAN

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ABSTRACT

Both *Eucalyptus camadulensis* and *E. urophylla* roots were heavily colonized by vesicular-arbuscular mycorrhizal (VAM) fungi. VAM spores, mostly *Glomus* sp. were abundant in soils of both *Eucalyptus* species. Bacterial cells were also present in roots of both species. Two unidentified nitrogen-fixing bacteria were isolated from surface-sterilized roots of *E. camadulensis* and *E. urophylla*. Nitrogen fixation measured by the acetylene reduction for nitrogenase, occurred in roots soils of both *Eucalyptus* species. Soils or excised roots incubated with 10% acetylene under 1% oxygen are able to reduce acetylene to ethylene, indicating the microaerophilic nitrogen fixation occurs in *Eucalyptus* rhizosphere. The nitrogenase activity (μ moles $\times 10^{-3}$ C_2H_4 formed/g/hr) for *E. camadulensis* roots with soils was 8.93 and 3.2 at Hualien and Tainan, respectively; the activity in *E. urophylla* roots with soils was 8.23 at Hualien and 3.93 at Tainan. The most probable number method was used to determine the populations of nitrogen-fixing bacteria in rhizosphere soils or surface-sterilized roots of both *Eucalyptus* species. The populations (cells/g dry soil) of nitrogen-fixing bacteria in rhizosphere soils were 1.3×10^3–2.5×10^5 for *E. camadulensis* from Tainan, 7.3×10^3–1.9×10^5 for *E. urophylla* from Tainan, 1.9×10^4–9.1×10^4 for *E. camadulensis* from Hualien and 2.4×10^3–8.1×10^4 for *E. urophylla*. The populations (cells/g fresh root) of nitrogen-fixing bacteria in surface-sterilized roots were 1.5×10^2–9.3×10^3 for *E. camadulensis* from Tainan, 5.5×10^1–1.5×10^4 for *E. urophylla* from Tainan, 1.3×10^1–1.3×10^3 for *E. camadulensis* from Hualien and 1.9×10^1–3.2×10^3 for *E. urophylla* from Hualien.

INTRODUCTION

Nitrogen is generally believed to be the most limiting nutrient in forests (Johnson *et al.*, 1982). In the absence of symbiotic N_2-fixing plants, known natural inputs are far below those required for good tree growth, and productivity is believed to be maintained by efficient cycling. Associative N_2 fixation (N_2 fixation in association with roots and mycorrhizae) was first suggested by Richards and Voigt (1964), and the phenomenon seems well established (Li and Hung 1987). However, its ecological significance is largely unknown.

In this study, we detect nitrogenase activity in the rhizospheres of *Eucalyptus camadulensis* and *E. urophylla* and to observe vesicular-arbuscular mycorrhizae on their roots and adjacent soils, as well as to isolate and observe the associative N_2-fixing bacteria in plant roots.

METHODS

Soil and root samples were collected from plantations of *E. camadulensis* and *E. urophylla* in Hualien and Tainan. Tree age in Hualien plantations was about one year old and was about three years old in Tainan plantations.

Nitrogenase activity was determined by placing 30 ml of roots and/ or soils in a 60 ml capacity serum bottle. The bottles were under microaerophilic conditions (1-2% O_2) and contained 10% acetylene of the total gas volume. After incubation at 30°C for 2-3 days, a 0.05 ml gaseous sample from each bottle was removed and analyzed for ethylene and acetylene with a gas chromatograph fitted with a 2 ml × 2.1 mm, 80-100 mesh, Porapack N column.

In order to isolate bacteria, roots were surface-sterilized with 0.5% NaClO for 1-2 min and were cut into small pieces (0.5-1 cm long). Five pieces of surface-sterilized roots were put in 30 ml Dobereiner's nitrogen-free liquid medium (Zuberer 1987) in a 60 ml capacity serum bottle. The method described above was used to test nitrogenase activity. The bottles with high nitrogenase activity were used to isolate bacteria. Bacteria were isolated and purified by streaking the cultures on Dobereiner's nitrogen-free agar medium. The method of Kormanik and McGraw (1982) was used to clear roots of *E. camadulensis* and *E. urophylla* and to stain vesicular-arbuscular mycorrhizae. Meanwhile, wet sieving and decanting method

(Daniels and Skipper 1982) was employed to collect VAM spores in soils.

Associative N_2-fixing bacteria populations in roots and soils were determined by most probable number method (Alexander 1982).

RESULTS AND DISCUSSION

Both *E. camadulensis* and *E. urophylla* roots were heavily colonized by VAM fungi based on the observation of clearing and staining roots under light microscope. VAM spores, mostly *Glomus* sp. were abundant in soils of both *Eucalyptus* species. Some mycelia with clamp connections were also found on and in roots which indicated that exdomycorrhizae or ectendomycorrhizae might lived with *Eucalyptus*. But neither Hartig net nor mycelial mantle was observed.

Bacterial cells were also present in roots of both tree species under observation of scanning electron microscope. Some bacterial cells were also observed near by mycelia of VAM in the roots. Two unidentified nitrogen-fixing bacteria were purified from surface-sterilized roots of *E. camadulensis* and *E. urophylla*. However, inoculation of the nitrogen-fixing bacteria to host plants has not been tested yet.

Nitrogenase activity in roots of two *Eucalyptus* species from both locations was the highest except that nitrogenase activity in roots of *E. urophylla* from Tainan was absent in one of three samples. So its nitrogenase activity was omitted (Table 1). Nitrogenase activity in roots *E. camadulensis* from both locations was more than 100 μ moles $\times 10^{-3}$ C_2H_4 formed/g/hr. The second one was roots plus soils ranging from 3.20 to 8.93 (μ moles $\times 10^{-3}$ C_2H_4 formed/g/hr). Nitrogen activity in soils was the lowest ranging from 0.30 to 4.27 (μ moles $\times 10^{-3}$ C_2H_4 formed/g/hr).

Table 1. Nitrogenase activity (μ moles $\times 10^{-3}$ C_2H_4 formed/g/hr) of two *Eucalyptus* species roots and adjacent soils.

Plant species	Source	Treatment	μ moles $\times 10^{-3}$ C_2H_4 formed[a]
E. urophylla	Hualien	Roots	62.36
		Soils	4.27
		Roots & Soils	8.23
	Tainan	Roots	—
		Soils	2.31
		Roots & Soils	3.93
E. camadulensis	Hualien	Roots	130.06
		Soils	3.50
		Roots & Soils	8.93
	Tainan	Roots	125.23
		Soils	0.30
		Roots & Soils	3.20

[a] Averages of 3 replicates under microarophilic conditions.

The most probable number method (Alexander 1982) was used to determine the populations of nitrogen-fixing bacteria in rhizosphere soils or surface-sterilized roots of both *Eucalyptus* species. The populations (cells/g dry soil) of nitrogen-fixing bacteria in rhizosphere soils were $1.3\times10^3-2.5\times10^5$ for *E. camadulensis* from Tainan, $7.3\times10^3-1.9\times10^5$ for *E. urophylla* from Tainan, $1.9\times10^4-9.1\times10^4$ for *E. camadulensis* from Hualien and $2.4\times10^3-8.1\times10^4$ for *E. urophylla* (Table 2). The populations (cells/g fresh root) of nitrogen-fixing bacteria in surface-sterilized roots were $1.5\times10^2-9.3\times10^3$ for *E. camadulensis* from Tainan,$5.5\times10^1-1.5\times10^4$ for *E. urophylla* from Tainan, $1.3\times10^1-1.3\times10^3$ for *E. camadulensis* from Hualien and $1.9\times10^1-3.2\times10^3$ for *E. urophylla* from Hualien. The results show that associative N_2-fixing bacteria populations in soils were relatively higher than those in roots. It is probably because pre-crops are sugar canes and corns which were known in association with associative N_2-fixing bacteria.

Table 2. The most probable number (cells/g dry soil or fresh root) of nitrogen-fixing bacteria in adjacent soils and surface-sterilized roots of two *Eucalyptus* species.

Plant species	Source	Treatment	No. cell per g dry soil or fresh root[a]
E. urophylla	Hualien	Soils	2.4×10^{3}—8.1×10^{4}
		Roots	1.9×10^{1}—8.1×10^{3}
	Tainan	Soils	7.3×10^{3}—8.1×10^{4}
		Roots	5.5×10^{1}—8.1×10^{4}
E. camadulensis	Hualien	Soils	1.3×10^{4}—8.1×10^{4}
		Roots	1.3×10^{1}—8.1×10^{3}
	Tainan	Soils	1.3×10^{3}—8.1×10^{4}
		Roots	1.5×10^{2}—8.1×10^{3}

[a] Five replicates for each treatment.

This study shows that nitrogen-fixing bacteria was associated with vesicular-arbuscular mycorrhizae of two *Eucalyptus* species in Taiwan. In addition, nitrogen-fixing bacteria isolated from roots of both *E. camadulensis* and *E. urophylla* appears not to have been previously described. However, influences of nitrogen-fixing bacteria and VAM fungi on the host plants have not been elucidated yet.

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