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Distribution of *Gymnostoma* spp. microsymbiotic *Frankia* strains in New Caledonia is related to soil type and to host-plant species

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Abstract

The diversity of the *Frankia* strains that are naturally in symbiosis with plants belonging to the *Gymnostoma* genus in New Caledonia was investigated. A direct molecular characterization of DNA extracted from nodules was performed, followed by characterization by restriction fragment length polymorphism (RFLP) of the ribosomal *rrs–rrl* (16S–23S) intergenic spacer (IGS) polymerase chain reaction (PCR)-amplified region. Seventeen different patterns were identified among the 358 microsymbiotic strains studied in the eight species of host plant present in New Caledonia. This genotypical approach permitted us to show that a large diversity existed among the patterns and that these did not exhibit a strict specificity to any host-plant species comparable with that previously found in the *Casuarina* and *Allocasuarina* symbioses in Australia. Despite this lack of specificity, a correspondence analysis nevertheless showed that the distribution of these patterns was related to soil type and to host-plant species. Furthermore, several *Frankia* strains were exclusively associated with the ultramafic soils.

Keywords: diversity, *Frankia*, *Gymnostoma*, New Caledonia, nickel, ultramafic soil

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Introduction

The family Casuarinaceae is composed of the four genera of plants *Allocasuarina*, *Casuarina*, *Ceuthostoma* and *Gymnostoma*, of which *Gymnostoma* is considered the most

with the actinomycete *Frankia*, permitting the plants to develop on nitrogen-poor soils.

In spite of its small surface area (19 000 km^2), New Caledonia has an abundance of endemic Casuarinaceae spe-

Various *Gymnostoma* species have been used successfully

were *Elaeagnus*-infective but not *Casuarina*-infective.

Table 1 Geographical, soil types and host-plant species origins of samples used in this study

Region	Localization	No. of sites sampled	Soil type*	*Gymnostoma* species†
South	Far south	11	O–U	D–G–I–L–P–W
	Tontouta	2	B–O	C–G–I–P
Centre-east	Canala/Thio	7	A–O	N–P–W
	Kouaoua	5	B–O	C–G–I
	Tiwaka	1	A	N
Centre-west	Me Maoya	2	B–O	P–G
	Boulinda Kopeto	4	B–O–U	C–G–P–W
	Koniambo	1	B	C
Northwest	Kaala	1	B	C
	Tiebaghi	4	A–B–U	C–N–W
	Poum	1	B	C
Northeast	Tao	2	A	N–W
	Mandjelia	1	A	W

*A, acidic alluvium; B, brown hypermagnesian inceptisol; O, oxisol; U, ultramafic alluvium.
†C, *G. chamaecyparis*; D, *G. deplancheanum*; G, *G. glaucescens*; I, *G. intermedium*; L, *G. leucodon*; N, *G. nodiflorum*; P, *G. poissonianum*; W, *G. webbianum*.

from the sampling stands belonged to four classes, based on parent rock nature, soil origin, evolution degree, structure and chemical composition (Latham *et al.* 1978). Soil from acidic rocks was represented by alluvial soils (A), essentially with an acidic pH and a low nickel concentration (0.016%; Jaffré *et al.* 1994a). Although poor in phosphorus, calcium and potassium, and abnormally rich in nickel (from 0.2 to 0.87%; Jaffré *et al.* 1994a), soil from ultramafic rocks appeared to be more diversified than soil from acidic rocks. According to their degree of evolution, three soils were identified: brown hypermagnesian inceptisols (B), oxisols (O) and ultramafic alluvial soils (U).

DNA extraction

DNA from nonisolated strains present in nodules was extracted and purified according to a modification of the protocol described initially by Simonet *et al.* (1984) and

performed using primers FGPS989e (5′-GGGGTCCTTAGGGGCT-3′) (Bosco *et al.* 1992) and FGPL73′ (5′-ATCGGCTCGAGGTGCCAAGGGTC-3′) (Navarro *et al.* 1992) targeting the ribosomal 16S–23S intergenic spacer (IGS) with the standard conditions described by Simonet *et al.* (1991) including the use of the *Taq* DNA polymerase from Gibco BRL and a PTC-100TM programmable thermal controller (MJ Research). To verify the efficiency of the amplification, 1/10 of the amplification reaction was analysed by electrophoresis on a 2% (w/v) agarose gel.

PCR product restriction analysis

Restriction endonuclease digestions were carried out by using 15 μL of a positive control PCR reaction for each enzyme. The endonucleases, *Msp*I, *Hae*III and *Cfo*I, found to be the most resolutive in a preliminary study (data

Table 2 *Frankia* pattern (%) distribution in the *Gymnostoma* species

Plant species*	Soil types†	Pattern																Absolute no. of samples
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
C	B	0	2.1	0	86.3	4.2	0	2.1	0	0	4.2	0	0	1.1	0	0	0	0	95
D	O	0	7.1	17.1	35.7	31.4	7.1	0	0	0	1.4	0	0	0	0	0	0	0	70

Table 4 *Frankia* pattern (%) distribution in the species *Gymnostoma webbianum* as a function of soil type

	Pattern																	
Soil types*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Absolute no. of samples
A	0	40.0	0	0	33.3	0	0	26.7	0	0	0	0	0	0	0	0	0	15
U	4.5	27.3	4.5	31.8	31.8	0	0	0	0	0	0	0	0	0	0	0	0	22
Absolute no. of samples	1	12	1	7	12	0	0	4	0	0	0	0	0	0	0	0	0	37

*A, acidic alluvium; U, ultramafic alluvium.

The multiple correspondence analysis of the *Frankia* pattern distribution as a function of the host-plant species and of the soil type is shown in Fig. 2. The first horizontal axis, which explains 10.3% of the total inertia (average correlation ratio for the first axis 0.90), opposes

Gondwana supercontinent and is characterized by an important plant endemicity (Jaffré *et al.* 1994a) and a large proportion of soils overlying ultramafic substrata. These soils are rich in nickel and manganese and thus constitute an original environment to study microbial eco-

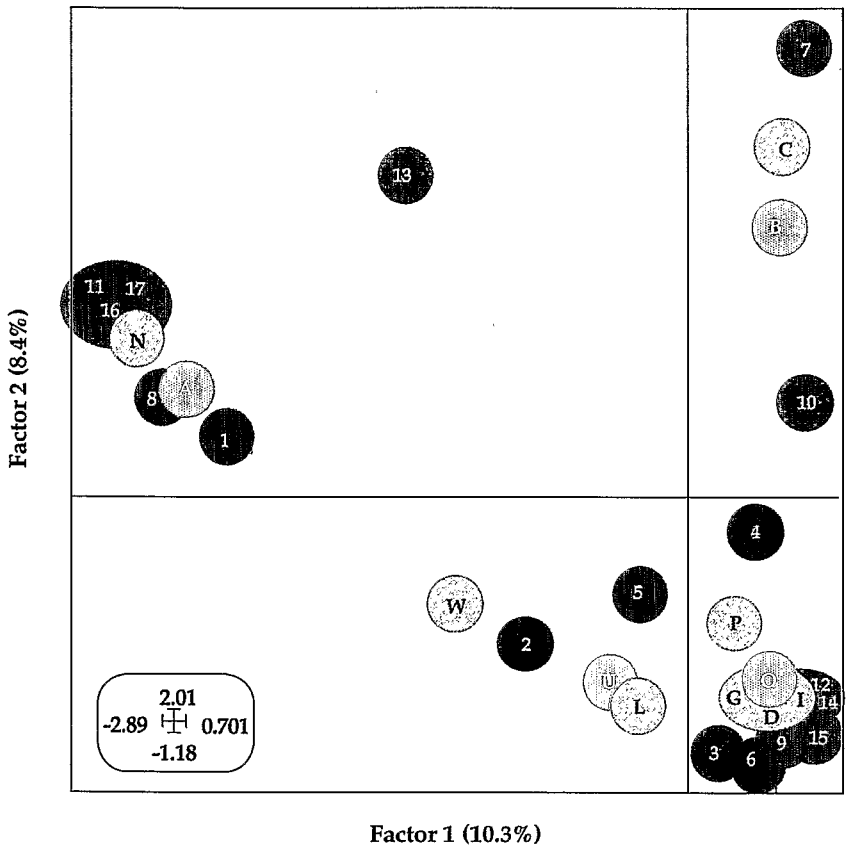

Fig. 2 Multiple correspondence analysis of *Frankia* patterns, soil type and host-plant species. The soil types are in red (A, acidic; B, brown hypermagnesian ultramafic; O, oxysol; U, ultramafic alluvium), the *Gymnostoma* host plants are in green (D, *G. deplancheanum*; C, *G. chamaecyparis*; G, *G. glaucescens*; I, *G. intermedium*; L, *G. leucodon*; N, *G. nodiflorum*; P, *G. poissonianum*; W, *G. webbianum*) and the 17 patterns are in blue (dominants in dark blue). The range of the first two factors is given in the lower left.

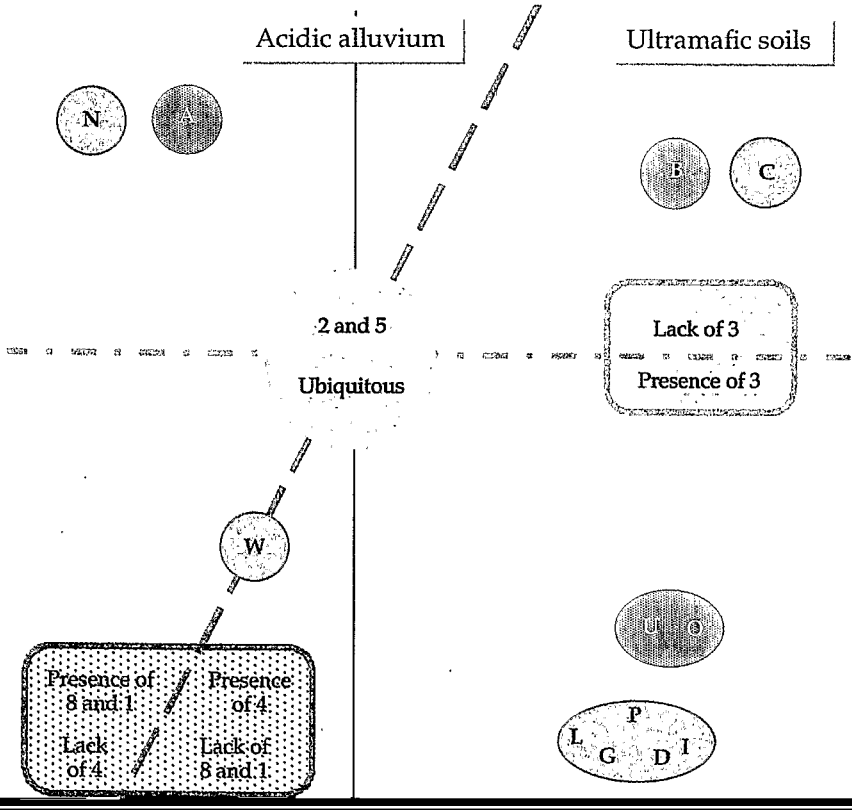

Fig. 3 Schematic representation of the multiple correspondence analysis results. For legend, see Fig. 2.

between *Gymnostoma* and *Casuarina/Allocasuarina* (Maggia & Bousquet 1994).

The study of the genetic diversity of *Gymnostoma* microsymbionts in New Caledonia clearly indicates that a large diversity exists, as the 358 DNAs tested were classified into 17 different patterns. The present factorial analysis shows some associations between *Frankia* patterns, *Gymnostoma* host-plant species and soil types. The major differentiating

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