RESEARCH ARTICLE

Ultramafic soils from New Caledonia structure *Pisolithus albus* in ecotype

Philippe Jourand[1], Marc Ducousso[1,2], Clarisse Loulergue-Majorel[1], Laure Hannibal[1], Sylvain Santoni[3], Yves Prin[4] & Michel Lebrun[4]

[1]Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UMII, Centre IRD, Nouvelle-Calédonie; [2]Institut Agronomique néo-Calédonien, Nouméa, Nouvelle-Calédonie; [3]INRA – Centre de Montpellier, UMR, Diversité et Adaptation des Plantes Cultivées, Montpellier, France; and [4]Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UMII, Montpellier, France

Correspondence: Philippe Jourand, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UMII, Centre IRD, BPA5, Promenade Roger Laroque, 98848 Nouméa cedex, Nouvelle-Calédonie. Tel.: +687 26 07 69; fax:+687 26 43 26; e-mail: philippe.jourand@ird.fr

Received 15 September 2009; revised 3 January 2010; accepted 10 January 2010.
Final version published online 22 February 2010.

DOI:10.1111/j.1574-6941.2010.00843.x

Editor: Philippe Lemanceau

Keywords
Pisolithus albus; serpentine soils; Ni tolerance; New Caledonia.

Abstract

Isolates of ectomycorrhizal *Pisolithus albus* were sampled from both ultramafic and volcano-sedimentary soils in New Caledonia, a tropical hotspot of biodiversity, to investigate the relationships between genetic diversity and edaphic constraint through tolerance to nickel (Ni). Carpophore description, spore morphology and phylogenetic analysis based on internal transcribed spacer (ITS) rDNA sequences confirmed that all isolates belong to *P. albus* and are closely related to other Australasian specimens. Using molecular tools, ITS-restriction fragment length polymorphism and amplified fragment length polymorphism markers, we showed the existence of two distinct genetic clusters within *P. albus*: ultramafic and volcano-sedimentary. Mycelia response to Ni toxicity supports such a population structure. *Pisolithus albus* from ultramafic soils included isolates with a high diversity of *in vitro* Ni tolerance, with both Ni-tolerant isolates (average Ni EC_{50} at 575 μM) and Ni-sensitive isolates (average Ni EC_{50} at 37 μM). In contrast, all isolates from volcano-sedimentary soils were found to be Ni sensitive (average Ni EC_{50} at 32 μM). We highlight that (1) *P. albus* population from ultramafic soils of New Caledonia are genetically structured in ecotype, and that (2) Ni tolerance among ultramafic isolates suggests an adaptive physiological response to Ni toxicity.

Introduction

Ultramafic soil structures are a weathered product of ultramafic bedrocks, and are characterized by low available concentrations of major plant nutrients (N, P, K), unbalanced Ca/Mg quotient and high levels of phytotoxic heavy metals, in particular nickel (Ni), chromium, manganese and cobalt (Brooks, 1987). Ni that may reach up to 10 g kg^{-1} in ultramafic soils as compared with the average 50 mg kg^{-1} in cultivated soils appears to be also the most bioavailable and phytotoxic element (Wenzel & Jockwer, 1999; Echevarria *et al.*, 2006). Further, other stress factors such as low content in organic matter and poor water retention are often associated with these environments (Brady *et al.*, 2005). Altogether, the ultramafic soil conditions strongly restrict plant and microbial growth and are known to select adapted populations to metal tolerance. Specific ecotypes were noticed either for plants (Proctor & Nagy, 1992; Proctor, 2003; Chiarucci & Baker, 2007), ectomycorrhizal fungi (Panaccione *et al.*, 2001; Moser *et al.*, 2005; Gonçalves *et al.*, 2009), arbuscular mycorrhizal fungi (Schechter & Bruns, 2008), or for microorganisms (Stoppel & Schlegel, 1995).

New Caledonia, a tropical archipelago located in the South Pacific Ocean, is a biodiversity hotspot where specific biological endemic ecosystems have evolved (Myers *et al.*, 2000). It is generally thought that the major reason for this high level of diversity is the presence of natural Ni-rich ultramafic soils that cover one-third of the main island (Latham, 1981). In these soils, a specific flora (Jaffré, 1992) as well as adapted fungi and microorganism populations have been reported (Amir & Pineau 1998a, b; Navarro *et al.*, 1999; Héry *et al.*, 2003; Chaintreuil *et al.*, 2007). Association

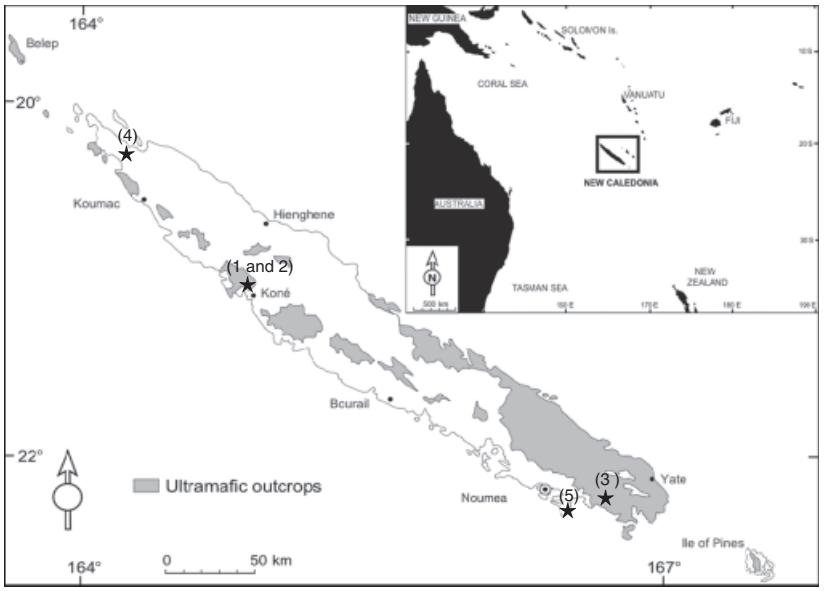

Fig. 1. General geographical map describing New Caledonian archipelago, location of ultramafic massifs (in gray) and sites where *Pisolithus* sp. isolates were collected (1, 2 and 3: ultramafic sites; 4 and 5: volcano-sedimentary sites).

of toxic metal-tolerant ectomycorrhizal fungi with plants is important for the establishment of sensitive plants in metal-polluted environment (Krznaric *et al.*, 2009). In this study, we focused on the fungi from the genus *Pisolithus* Alb. & Schwein. *Pisolithus* is a major ectomycorrhizal genus in the *Boletales*, distributed on a worldwide scale and forming ectomycorrhizal associations with a broad range of angiosperm and gymnosperm tree and shrub species (Marx, 1977; Martin *et al.*, 2002). *Pisolithus* is also regarded as an early colonizer that persists on sites subject to edaphic stresses (Anderson *et al.*, 1998). In New Caledonia, *Pisolithus* is very abundant and develops ectomycorrhizal symbiosis with many plants from various genera of the *Myrtaceae*, for example *Babingtonia*, *Melaleuca*, *Tristaniopsis* and one *Mimosaceae*, *Acacia spirorbis* (Perrier, 2005).

In this work, our objectives were: (1) to characterize at the molecular level *Pisolithus albus* from both ultramafic and volcano-sedimentary soils in New Caledonia by internal transcribed spacer (ITS) sequencing, (2) to assess *in vitro* the Ni-tolerance phenotype of isolates' mycelia from either soil type and (3) to determine by amplified fragment length polymorphism (AFLP) the genetic structure of those strains that have been phenotyped. Our ultimate goal was to investigate whether the *in vitro* Ni-tolerance phenotype relates to a specific genetic structure of the ultramafic population of *P. albus*.

Materials and methods

Site description and soil characterization

A map location of New Caledonia archipelago including studied sites is presented in Fig. 1. The ultramafic sites (ferralsol) were the Koniambo Massif (sites 1 and 2, Fig. 1) located in the Northern Province of New Caledonia (20°59′S, 164°49′E) and Mont Dore (site 3, Fig. 1) located in the Southern province (22°15′21″S; 164°36′42″E). Jaffré (1974) and Perrier *et al.* (2006a, b) have already reported a full description, including climate, geology, geomorphology and vegetation structure of these ultramafic sites. Nonserpentine sites (sites 4 and 5, Fig. 1) were also selected for isolation of *Pisolithus*. The volcano-sedimentary sites are situated in the extreme north of New Caledonia, near Poum (site 4, Fig. 1; 20°12′58–59″S, 164°04′40–43″E) and Ouen Toro Hill (site 5, Fig. 1; 22°18′29–33″S and 166°27′09–15″E), both ferric acrisol soils (Latham, 1981; Gillepsie & Jaffré, 2003) and vegetation consisting of savannah with trees or sclerophyll forest (Morat *et al.*, 1981). Soil sampling and mineral analyses were performed at Laboratoire de Chimie (IRD, Noumea, New Caledonia) as described by Perrier *et al.* (2006a, b).

Fungal isolates and culture conditions

Sporocarps of ectomycorrhizal fungi identified as *P. albus* were collected. The collection number, geographical localization and putative plant host are reported in Table 1a. Sporocarps mineral Ni concentration analysis was performed on an aseptic piece of the *pileus trama* dry tissue at Laboratoire de Chimie (IRD) as described in Perrier *et al.* (2006b). Stock cultures from each sporocarp were obtained by transferring aseptically a piece of the *pileus trama* to a solid modified Melin–Norkrans (MMN) medium (Marx, 1969) containing: KH_2PO_4 ($0.5\,g\,L^{-1}$), $(NH_4)_2HPO_4$ ($0.25\,g\,L^{-1}$), $CaCl_2$ ($0.05\,g\,L^{-1}$), NaCl ($0.025\,g\,L^{-1}$), $MgSO_4 \cdot 7H_2O$ ($0.15\,g\,L^{-1}$), thiamine hydrochloride ($100\,\mu g\,L^{-1}$), $FeCl_3 \cdot 6H_2O$ ($0.03\,g\,L^{-1}$), glucose ($10\,g\,L^{-1}$), malt extract ($3\,g\,L^{-1}$) and agar ($14\,g\,L^{-1}$). The pH

was adjusted to 5.6 with 1 M HCl and the medium was autoclaved for 20 min at 120 °C. All fungal strains were maintained as subcultures at 24 °C on the same medium.

ITS sequencing and phylogenetic analysis

Total genomic fungal DNA was extracted and purified from mycelial cultures using the DNAeasy Plant Qiagen Kit (Qiagen, Courtabœuf, France). An approximately 650-base pair (bp) fragment of nuclear ribosomal ITS rDNA containing the 5.8S region was amplified with specific primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990). PCR reactions were prepared in a total volume of 25 μL containing aliquots of 1 μL of genomic DNA at 50 ng μL^{-1}, 1 μM of each primer, 1.5 U of *Taq* DNA polymerase (Promega, Charbonières, France), 1 × Promega *Taq* polymerase buffer, 2 mM $MgCl_2$ and 200 μM dNTP. Amplification was performed with a DNA thermocycler Mastercycler® Eppendorf system (Eppendorf, Hamburg, Germany) programmed as follows: one cycle for 5 min at 95 °C followed by 35 cycles at 95 °C for 30 s, 55 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 7 min. Mock-DNA controls were included to test for the presence of DNA contamination in the reagents. PCR products were separated by electrophoresis in 1% w/v agarose gel in 1 × TAE with ethidium bromide at 10 μg mL^{-1} in the running buffer as described previously by Moyersoen *et al.*, 2003. Amplified ITS products were purified using the Qiagen Gel extraction Kit (Qiagen). DNA sequencing was performed by Macrogen, using ABI chemistry and an ABI3730 capillary electrophoresis-based genetic analyzer (Macrogen, Seoul, Korea). All ITS sequences were then deposited in the EMBL nucleotide database for assignment of accession numbers (Table 1a). ITS sequences (including ITS1, 5.8S rRNA gene and ITS2) from this study were then aligned together with *Pisolithus* reference ITS sequences (Table 1b), and *Suillus luteus* ITS sequence was included as an outgroup. The alignment was produced using MUSCLE 3.6 (Edgar, 2004) and corrected manually using GENEDOC (Nicholas *et al.*, 1997). Molecular phylogeny of ITS sequences was built under the maximum parsimony (MP) criterion (to treat gaps as informative) using PAUP4 (Swofford, 2000). MP parameters were as follows: initial tree by random addition (1000 replicates), gaps treated as fifth base, character state optimization by accelerated transformation,

Table 1a. *Pisolithus* isolates included in the study along with details of their collection number, putative host plant, site collection reference, geographical origin (locality, GPS coordinates, map localization) and EMBL accession code for ITS sequences

Isolate	Putative host plants	Site number	Map location, locality and GPS coordinates	ITS accession code	Reference
MD07-112	*As*	1	Main road, Koniambo massif, Kone, (21°00′10″S; 164°47′00″E)	AM947078	This study
MD07-113	*As*	1	Main road, Koniambo massif, Kone (21°00′07″S; 164°46′51″E)	AM947079	This study
MD07-114	*As*	1	Main road, Koniambo massif, Kone (21°00′06″S; 164°46′46″E)	AM947080	This study
MD07-116	*As*	1	Main road, Koniambo massif, Kone (21°00′09″S; 164°46′28″E)	AM947081	This study
MD07-117	*As*	1	Main road, Koniambo massif, Kone (21°00′22″S; 164°46′08″E)	AM947082	This study
MD07-118	*As*	1	Main road, Koniambo massif, Kone (21°00′28″S; 164°45′56″E)	AM947083	This study
MD07-165	*As*, *B*, *Mq*	2	Pindjen waterfall road, Kone (20°02′30″S; 164°46′09″E)	AM947107	This study
MD07-166	*As*, *B*, *Mq*	2	Pindjen waterfall road, Kone (20°02′30″S; 164°46′09″E)	AM947108	This study
MD07-167	*As*, *B*, *Mq*	2	Pindjen waterfall road, Kone (20°02′30″S; 164°46′09″E)	AM947109	This study
MD07-168	*As*, *B*, *Mq*	2	Pindjen waterfall road, Kone (20°02′30″S; 164°46′09″E)	AM947110	This study
MD07-169	*As*, *B*, *Mq*	2	Pindjen waterfall road, Kone (20°02′30″S; 164°46′09″E)	AM947111	This study
MD07-214	*As*, *B*	3	Mont-Dore, Plum road (22°15′21″S; 166°36′16″E)	AM947112	This study
MD07-215	*As*, *B*	3	Mont-Dore, Plum road (22°15′21″S; 166°36′16″E)	AM947113	This study
MD07-217	*As*, *B*	3	Mont-Dore, Plum road (22°15′18″S; 166°36′42″E)	AM947114	This study
MD07-218	*As*, *B*	3	Mont-Dore, Plum road (22°15′18″S; 166°36′42″E)	AM947115	This study
MD07-219	*As*, *B*	3	Mont-Dore, Plum road (22°15′18″S; 166°36′42″E)	AM947116	This study
MD07-220	*As*, *B*	3	Mont-Dore, Plum road (22°15′18″S; 166°36′42″E)	AM947117	This study
MD06-378	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	AM947123	This study
MD06-379	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	FN390950	This study
MD06-380	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	FN390951	This study
MD06-381	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	AM947124	This study
MD06-382	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	AM947125	This study
MD06-383	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	AM947126	This study
MD06-384	*B*, *Mq*	4	Poum (20°13′02″S; 164°04′38″E)	AM947127	This study
MD07-227	*As*, *Mq*	5	Ouen-Toro, Nouméa (22°18′30″S; 166°27′13″E)	AM947118	This study
MD07-228	*As*, *Mq*	5	Ouen-Toro, Nouméa (22°18′30″S; 166°27′13″E)	AM947119	This study
MD07-229	*As*, *Mq*	5	Ouen-Toro, Nouméa (22°18′29″S; 166°27′13″E)	AM947120	This study
MD07-230	*As*, *Mq*	5	Ouen-Toro, Nouméa (22°18′29″S; 166°27′13″E)	FN390952	This study

As, *Acacia spirorbis* Labill.; *B*, *Babingtonia* sp.; *Mq*, *Melaleuca quinquenervia* (Cav.) S.T. Blake.

Table 1b. *Pisolithus* and *Suillus* DNA ITS sequences used as reference for the ITS molecular phylogeny study with information concerning their code use in the cladogramme, putative host plant, geographical origin, ITS GenBank database accession number and bibliographical reference

Isolate	*Pisolithus* sp.	Plant host	Locality	ITS GenBank	Reference
H4937	*P. albus*	*Eucalyptus tereticornis*	Fanning River, Qld, Australia	AF374670	Martin *et al*. (2002)
REB2078	*P. albus*	*Kunzea ericoides*	Tauhara, New Zealand	AY318746	Moyersoen *et al*. (2003)
CA02	*P. albus*	*Eucalyptus* sp.	Canberra, ACT, Australia	AF270782	Martin *et al*. (2002)
Gemas	*P. albus*	*Acacia mangium*	Gemas, Malaysia	AF374638	Martin *et al*. (2002)
COI24	*P. albus*	*Acacia holosericea*	Sinthiou Malème, Senegal	AF374622	Martin *et al*. (2002)
Pasoh01all1	*P. aurantioscabrosus*	*Shorea macroptera*	Paosh, Malaysia	AF415226	Martin *et al*. (2002)
KS871	*P. marmoratus*	*Eucalyptus calophylla*	Denmark, WA, Australia	AF374719	Martin *et al*. (2002)
MU98/22	*P. marmoratus*	*Eucalyptus globulus*	Albany, WA, Australia	AF374660	Martin *et al*. (2002)
441	*P. microcarpus*	*Eucalyptus citriodora*	São Paulo, Brazil	U62666	Martin *et al*. (2002)
UFSC132	*P. microcarpus*	*Eucalyptus dunni*	Tres Barras, SC, Brazil	AF374704	Martin *et al*. (2002)
F22	*P. tinctorius*	*Pinus pinaster*	Mimizan, France	AF374707	Martin *et al*. (2002)
MARX270	*P. tinctorius*	*Pinus elliotii*	Georgia	AF374632	Martin *et al*. (2002)
K915	*Pisolithus* sp.1	*Afzelia quanzensis*	Arabuko, Kenya	AF228653	Diez *et al*. (2001)
5105	*Pisolithus* sp.1	*Afzelia quanzensis*	Arabuko, Kenya	AF003915	Martin *et al*. (2002)
Cr04	*Pisolithus* sp.3	*Cistus ladanifer*	Abenojar, Spain	AF228643	Diez *et al*. (2001)
Pt03	*Pisolithus* sp.4	*Quercus ilex*	Valencia, Spain	AF228648	Diez *et al*. (2001)
MH728	*Pisolithus* sp.5	*Eucalyptus* sp.	Yanxi, China	AF374679	Martin *et al*. (2002)
MSN	*Pisolithus* sp.5	*Pinus kesiya*	ChangMai, Thailand	AF374625	Martin *et al*. (2002)
MU98101	*Pisolithus* sp.8	*Eucalyptus camaldulensis*	Eneabba, WA, Australia	AF374661	Martin *et al*. (2002)
MH56	*Pisolithus* sp.8	*Eucalyptus* sp.	Kalbarri, WA, Australia	AF374708	Martin *et al*. (2002)
CSH4461	*Pisolithus* sp.10	*Acacia* sp.	Townsville, Qld, Australia	AF374624	Martin *et al*. (2002)
MU986	*Pisolithus* sp.10	*Eucalyptus globulus*	Scott R, WA, Australia	AF374646	Martin *et al*. (2002)
S193	*Suillus luteus*		UK	AJ272416	Ruiz-Diez *et al*. (2006)

branch swapping option by tree-bisection-reconnection, consensus tree obtained by the 50% MajRule method. A bootstrap analysis was performed under the MP criterion with 1000 bootstrap replicates by fast stepwise addition (using PAUP4).

ITS restriction fragment length polymorphism (RFLP) profiles

Four restriction enzymes, i.e. MboI, HinfI, AluI and TaqI, were selected to characterize ITS diversity within *Pisolithus* as reported previously (Gomes *et al*., 1999; Anderson *et al*., 2001; Moyersoen *et al*., 2003). Digestions were performed for 6 h at 37 °C on 1 μg of the amplified ITS as indicated by the provider (Promega). Restriction fragments were separated by electrophoresis in a 4% NuSieve® agarose gel (VWR, Fontenay, France) and stained with ethidium bromide before visualization under UV light. The 100 pair of bases (pb) DNA ladder was used as a marker (Promega).

AFLP profiles

The AFLP analysis was performed using the AFLP® Core Reagent Kit (Invitrogen, Cergy Pontoise, France) following the AFLP® Analysis System Manual with some modifications. A small quantity of genomic DNA (10–50 ng) was double digested with EcoRI (Pharmacia Biotech, Sweden) and MseI (New England Biolabs, Canada) and the resulting fragments were ligated to adaptors specific for the EcoRI and MseI restriction sites. Preselective amplification was carried out with EcoRI+A and MseI+C primers on an Eppendorf MasterCycler PCR machine under the following conditions: 2 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 60 s at 56 °C and 60 s at 72 °C. An aliquot of the preamplification product was tested for a visible smear on a 0.8% agarose gel. The PCR products were then diluted 10-fold with water and used as the template for selective amplifications using both EcoRI+3 and MseI+3 primers. EcoRI+3 primers were 5′-end labeled with fluorescent dyes 6-FAM, HEX or NED. The list and characteristics of the AFLP primer combinations for selective amplification are presented in Supporting Information, Table S2. Amplified products were detected using an ABI 3130xl Genetic Analyser (Applied Biosystems, Courtabœuf, France). For each genotype, samples were prepared by mixing 1 μL each of three diluted (10-fold with water) PCR products obtained with different fluorescent dyes with 0.15 μL GenSize 500HD Rox and 16.85 μL water. Reading of the profiles was performed using the GENEMAPPER V3.7 software (Applied Biosystems). The presence/absence (scored as 1 or 0, respectively) of polymorphic fragments within 34–527 bp was determined for each AFLP profile corresponding to the nine-primer combination used for each sample and transformed into a binary matrix. Then a genetic distance matrix was constructed using Nei's distance coefficient (Nei & Li, 1979). A phenogram was constructed with the unweighted pair group method with the arithmetic

mean (UPGMA) algorithm in the PHYLIP software package, and the robustness of the phenogram topology was assessed by bootstrap analysis (Felsenstein, 1993). In addition, a principal component analysis (PCA) was conducted using the binary matrix of all individuals as the input matrix and performed using the XLSTAT computer software (Addinsoft, 2005). The scatter plot was generated by the first two principal components.

Ni-tolerance assays

The response of fungal isolates to Ni was determined on pure mycelial cultures on medium at a pH of 5.6. To avoid metal precipitation in the assays (Hartley *et al.*, 1997), Ni was added to the medium after autoclaving to the desired final concentration from 1 M stock solutions of $NiCl_2$ (or $NiSO_4$) sterilized by filtration through 0.45-μm membrane filters (Millipore). In the initial Ni-tolerance screening of all isolates, plugs (8 mm) were cut from the actively growing edge of a 10-day-old mycelium culture and placed on solid MMN medium amended with $NiCl_2$ (or $NiSO_4$) at varying concentrations (10, 100 μM, 1 and 10 mM). The control was medium without Ni added. For each isolate, three independent experiments were carried out with five replicates for each concentration of Ni tested. In the second assay, for each Ni-tolerant isolate, plugs (8 mm) of a 10-day-old mycelium culture were transferred to MMN medium plates covered with cellophane and amended with Ni to final concentrations ranging from 0 to 2 mM (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1 and 2 mM). For Ni-sensitive isolates, MMN medium was amended with Ni to final concentrations ranging from 0 to 100 μM (0, 10, 25, 50, 75 and 100 μM). After 2 weeks of growth, mycelia were frozen at $-80\,^{\circ}C$, and subsequently freeze-dried before weighing. The dry weight increment during the 2-week test period was determined and consequently, the biomass was expressed as the mycelial dry weight in micrograms. A tolerance index (TI) was calculated for each isolate as the percentage of biomass retained on the metal-enriched media compared with growth on the control medium. The Ni effective concentration inhibiting growth by 50% (EC_{50}) was determined as reported by Colpaert *et al.* (2004). Mean values of EC_{50} were analyzed using a one-way ANOVA and compared with the Newman–Keuls multiple range test ($P \leq 0.05$) using the XLSTAT computer software (Addinsoft, 2005).

Results

Soil characteristics, description of fungal isolates and the putative host plant

Full geochemical soil analyses are reported in Table S1. Soils from sites 1, 2 and 3 showed geochemical characteristics of ultramafic soils (ferralsol) with low content of major plant nutriments (N, P and K elements), unbalanced Ca/Mg ratio and high levels of the toxic metals Mn, Cr, Co and Ni. Total concentrations of Ni ranged from 2.8 ± 0.5 to $3.5 \pm 0.7\,mg\,g^{-1}$ of soil, while extractable diethylene triamine pentaacetic acid (DTPA)-Ni concentrations ranged from 19 ± 3 to $25 \pm 2\,\mu g\,g^{-1}$ of soil. In contrast, volcano-sedimentary soils (ferric acrisol) contained low N and P, but higher K, lower unbalanced Ca/Mg ratio and very low content of toxic metals. Total Ni concentrations ranged from 0.08 ± 0.02 to $0.14 \pm 0.02\,mg\,g^{-1}$ of soil, while extractable DTPA-Ni concentrations were 83–190 times lower and ranged from 0.1 ± 0.05 to $0.3 \pm 0.05\,\mu g\,g^{-1}$ of soil.

The fruiting bodies used for fungal isolation displayed the morphological and anatomical characteristics of *P. albus* (Cooke & Massee) Priest (1998) (Fig. 2). In particular, the color of the spore prints, the size of globose spores ranging from 8 to 10 μm in diameter and the ornamentation with erect spines are similar to the type specimen deposited at the Royal Botanic Gardens (Kew, UK). *Pisolithus alba* collected in both types of soils were associated with major host plants belonging to *Myrtaceae* such as *Babingtonia* Lindl. and *Melaleuca quinquenervia* (Cav.) S.T. Blake or the *Mimosaceae*, *A. spirorbis* Labill (Table 1a).

Phylogenetic analysis of ITS sequence

Phylogenetic diversity of *P. albus* isolates was first analyzed by studying the ITS sequence (ITS1/5.8S rRNA gene/ITS2) of each isolate, a commonly used phylogenetic marker for fungal species. Amplification of the ITS region resulted in a single product of 650 pb for all 28 isolates. All ITS sequences analyzed were deposited in EMBL DNA databank and are referenced under an accession number (Table 1a). The alignment (available on request) of all ITS sequences was 890 pb length, including the 28 taxa, with 127 characters as constant, 167 variable characters as parsimony uninformative and 625 characters as parsimony informative. A molecular phylogeny was produced using the MP criterion, allowing the treatment of gaps as informative, to take into account the insertions/deletions (indels). A total of $3.9e^{+10}$ rearrangements were attempted and 206 510 trees were obtained, the best tree score being tree length = 3733. The 50% MajRule consensus synthetic tree obtained from the MP trees is presented in Fig. 3 including ITS sequences of *P. albus* from the sampling sites in New Caledonia. The molecular phylogeny based on the comparison of ITS sequences grouped our isolates together with other strains named as *P. albus*, indicating that these strains are very closely related. In addition, *P. albus* from New Caledonia grouped with *P. albus* isolated from Australia, New Zealand, Malaysia and Senegal.

Genetic diversity of *P. albus* based on ITS-RFLP

To further investigate the molecular diversity of the New Caledonian *P. albus*, the amplified fragments of the ITS

Fig. 2. *Pisolithus albus* from New Caledonia. (a) *P. albus* MD07-117 from the Koniambo massif; (b) *P. albus* MD07-228 from the Ouen-Toro, Noumea; (c) cross-section of *P. albus* MD07-166 from Pindjen waterfall and (d) globose spores (8.77–9.62 μm) of *P. albus* MD06-379 from Poum, erected spines (1.2 μm) are clearly visible. The white double bar represents 2 cm at the focus of the fungus; the black single bar represents 20 μm.

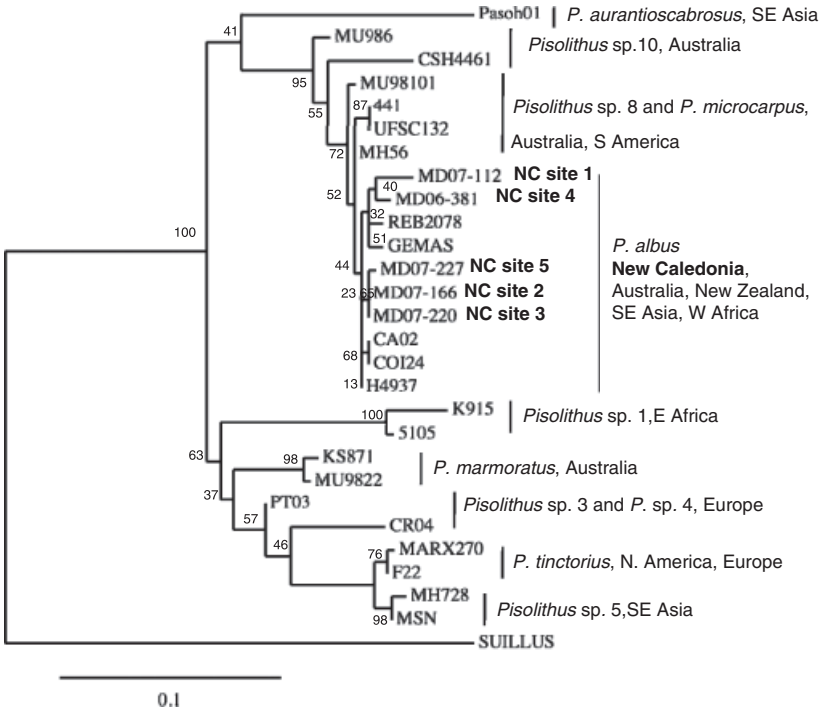

Fig. 3. Phylogenetic synthetic relationships among representative *Pisolithus* sp. from New Caledonia collection sites and worldwide reference isolates. The phylogeny is based on the analysis of the rDNA ITS1, 5.8S and ITS2 sequences. The tree shown is a 50% majority rule consensus of the most parsimonious trees (tree length = 3733) obtained with PAUP4 (see Materials and methods). Values indicated at tree nodes are percentage values of 1000 bootstrap replicates under the MP criterion using fast stepwise addition (only values > 50% are shown). The tree was rooted with *Suillus luteus* ITS sequences. Significant bootstrap frequencies are indicated. S America, South America; SE Asia, South East Asia; W Africa, West Africa.

region (650 pb) were digested by four different restriction enzymes. Pattern sequences of the ITS region obtained from 28 isolates grouped into distinct profiles (profile A and profile B) are presented in Fig. 4a. Profile A groups the 17 isolates from ultramafic soils while profile B groups the 11 isolates from volcano-sedimentary soils. Digestions with MboI and TaqI ITS supported major differences between profiles (Table 2).

Table 2. ITS-fragment sizes of *Pisolithus albus* ITS-RFLP types following digestion with MboI, HinfI, AluI, TaqI

ITS-RFLP type		ITS-RFLP fragment size (pb)			
Soil origin	ITS size (pb)	MboI	HinfI	AluI	TaqI
Ultramafic	650	73, 107, 205, 239, **334**	274, 382	68, 80, 421	50, **66**, **106**, 123
Nonultramafic	650	73, 107, 205, 239	274, 382	68, 80, 421	50, **80**, 123, **228**

Differences are highlighted in bold.

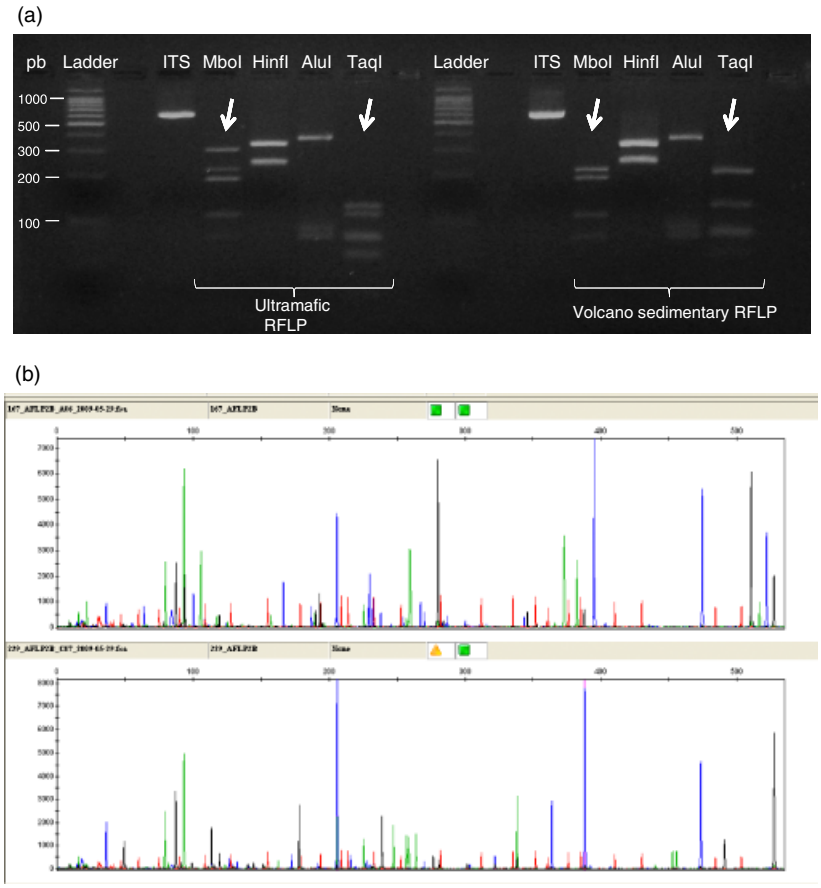

Fig. 4. (a) Representative patterns of ITS RFLP profiles of *Pisolithus albus* isolates from both ultramafic and volcano-sedimentary soils compared with both undigested amplified ITS and 100-pb DNA ladder (Promega). Arrows highlight major differences between profiles. (b) Chromatograms from AFLP analyses of representative *P. albus* isolates (MD07-167 and MD07-229) illustrating the high level of AFLPs observed in this species. The blue, green and black peaks represent fragments generated by selective amplification with respectively the 6-FAM-EcoRI+ACT/MseI+CAA, HEX-EcoRI+AAG/MseI+CAA and NED-EcoRI+ACG/MseI+CAG primer combinations. The red peaks represent the size standard.

AFLP polymorphism and genetic relatedness of *P. albus* isolates

Electrophoregrams generated for the nine selective primer pairs EcoRI-ANN and MseI-CNN, each pair tested on all genomic DNA from isolates, exhibited clear and easily identifiable peaks, corresponding to 882 different DNA fragments of which the identified size ranged from 34 to 527 DNA pair of bases. Figure 4b shows two chromatograms from AFLP analysis of representative *P. albus* isolates (ultramafic isolate MD07-167 and volcano-sedimentary isolate MD07-229), and illustrates the high level of AFLP polymorphisms observed in this species. Both genetic similarity and distance matrices are reported in Tables S3a, b. Nei's genetic distance coefficient varied between 0.169 and 0.900. The bootstrap consensus UPGMA tree obtained for 882 AFLP-scored fragments on the 27 *P. albus* isolates indicates the clustering of isolates from ultramafic soils vs. isolates from volcano-sedimentary soils (Fig. 5a). To further explore the relationship between genetic AFLP polymorphism and soil origin of isolates, a PCA was conducted. The two principal components accounted for 40% of the total variation (Fig. 5b).

Ni tolerance of *P. albus* mycelia

Ni concentration of *P. albus* collected in New Caledonia was analyzed before *in vitro* culture. The Ni concentration varied

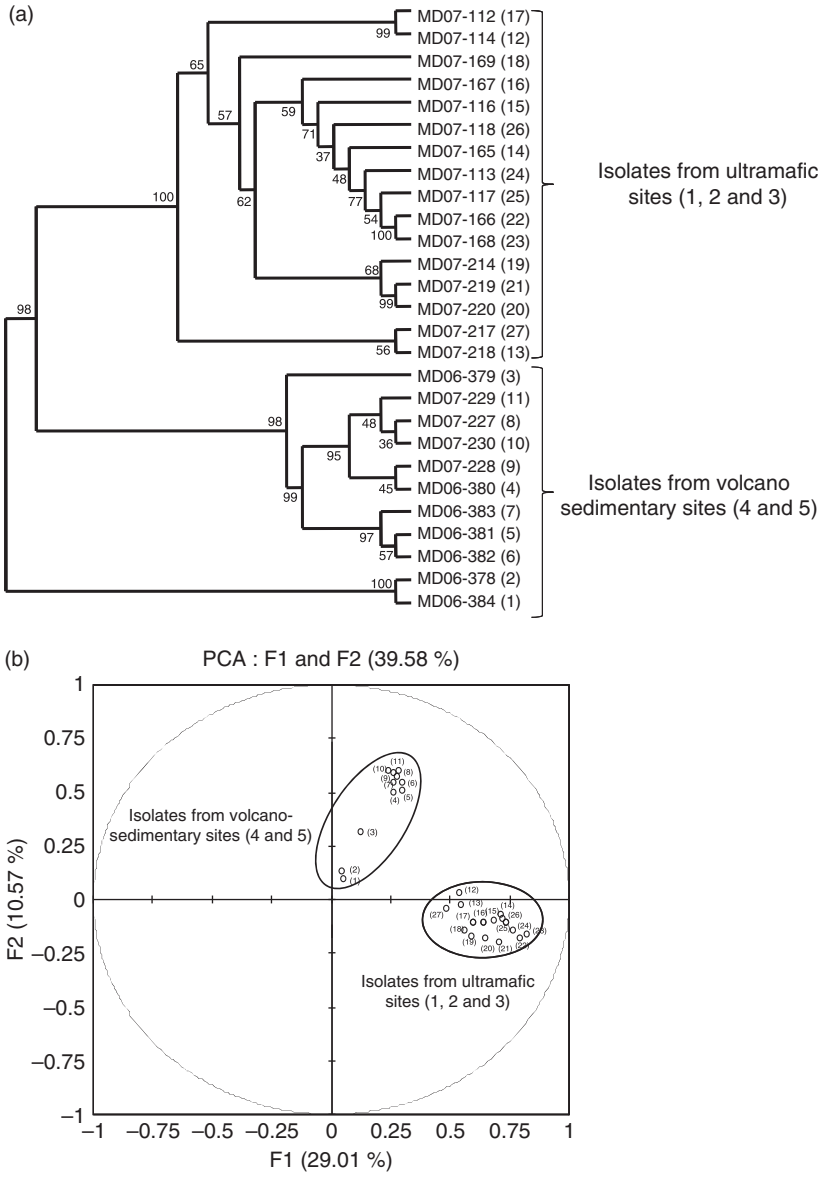

Fig. 5. Genetic relationship within *Pisolithus albus* isolates from New Caledonia according to AFLP analysis. (a) Bootstrap consensus UPGMA tree obtained for 882 AFLP scored fragments obtained with the nine selective primers pairs on the 27 *P. albus* isolates (100 replicates). (b) Plot representing the PCA of the AFLP genotypes and their clustering into ecotypes. The first two principal components (PC1 and PC2) account for 39.58% of the total variation observed. Numbers in parentheses refer to isolates in both dendogram and PCA plot.

between 5.3 ± 0.35 and $6.15 \pm 0.25\,\mu g\,g^{-1}$ (average Ni = $5.7\,\mu g\,g^{-1}$) in *P. albus* from ultramafic soils. In contrast, tissue of carpophores of isolates collected from volcano-sedimentary soils contained 2.5 times less Ni (average Ni = $2.35\,\mu g\,g^{-1}$).

In vitro growing mycelia from collected strains were screened for their tolerance to increasing Ni concentrations. The full results of the Ni tolerance screening are presented in Table S4. Among isolates from ultramafic soils, nine were able to tolerate Ni (either as $NiCl_2$ or $NiSO_4$) at concentrations ranging from 100–1 mM, while eight isolates could not tolerate Ni at concentrations higher than 75 μM. In contrast, isolates from volcano-sedimentary soils were all unable to tolerate Ni at concentrations higher than 75 μM. No particular effect on the fungal Ni tolerance was observed, irrespective of the counter-anion used (Cl^- or SO_4^-).

Ni tolerance (or sensitivity) was determined in terms of the Ni TI (= % of the initial biomass) and Ni concentration that inhibited growth by 50% (Ni EC_{50}) (Table 3). Concentrations of Ni higher than 0.6 mM affected the biomass yield of the nine Ni-tolerant isolates collected from ultramafic soils (average TI = 39%). In addition, Ni-tolerant isolates presented a high average Ni EC_{50} ($575 \pm 30\,\mu M$). In contrast, for the Ni-sensitive isolates from either ultramafic (eight isolates) or volcano-sedimentary (11 isolates) soils, low Ni concentrations (50 μM) were sufficient to affect the

Table 3. *Pisolithus albus* isolates Ni-tolerance screening, means of carpophore tissue Ni content, means of Ni-tolerance indices (TI, %) and effective concentrations of Ni inhibiting biomass yield by 50% (Ni EC_{50})

Ni-tolerance screening	Carpophore Ni content in $\mu g\ g^{-1}$ dry weight tissue	Ni-TI determination							Ni EC_{50} (μM)
Ni-tolerant ($Ni \geq 100\ \mu M$) isolates		Initial biomass (μg) at Ni = 0	Ni concentration tested (mM) 0.1	0.2	0.4	0.6	0.8	1.0	2.0	
Soil origin			TI (% of initial biomass)							
Ultramafic soils ($n = 9$)	5.3 ± 0.35a	41.2 ± 1.6	95 ± 3	90 ± 4	81 ± 8	39 ± 10	10 ± 5	5 ± 1	0	575 ± 32a
Volcano sedimentary soils ($n = 0$)	ND	ND	ND	ND	ND	ND	ND	ND		ND
Ni-sensitive ($Ni \leq 10$ mM) isolates		Initial biomass (μg) at Ni = 0	Ni concentration tested (μM) 10	25	50	75	100			
Soil origin			TI (% of initial biomass)							
Ultramafic soils ($n = 8$)	6.15 ± 0.25a	52.0 ± 2.4	104 ± 5	65 ± 6	25 ± 8	4 ± 2	0			37 ± 6b
Volcano sedimentary soils ($n = 11$)	2.35 ± 0.15b	82.4 ± 6.4	103 ± 10	69 ± 9	22 ± 8	2 ± 2	0			32 ± 8b

All data are means ($\pm$ SD) of five replicates per isolate tested.
Ni carpophore content and Ni EC_{50}: data followed by same letter are nonsignificantly different ($P \leq 0.05$).
ND, not determined.

biomass production in a similar proportion (average TI = 25%). These latter isolates had a mean Ni EC_{50} value of only $34.5 \pm 7\ \mu M$ Ni, which was 17-fold lower than that of the Ni-tolerant isolates.

Discussion

Phylogeny of *P. albus* from New Caledonia

This set of results is the first report on the ectomycorrhizal *P. albus* populations from New Caledonia, an archipelago in tropical south Pacific that is considered to be a hotspot of biodiversity (Myers *et al.*, 2000). Both anatomy and morphology of *P. albus* collected from either ultramafic or volcano-sedimentary New Caledonian soils correspond to the *P. albus* specimen voucher reported originally from Australia (Saccardo's Syll. fung. XI: 167; XII: 1013). Molecular identity and phylogeny based on rDNA-ITS sequencing confirmed this result and indicated that New Caledonian *P. albus* groups with Australian, New Zealand, Malaysian and Senegal specimens. In addition, the ecology of *P. albus* isolated from New Caledonia revealed an association with *Myrtaceae* (e.g. *Babingtonia*, *Melaleuca*, *Tristaniopsis*) and the endemic New Caledonian *Mimosaceae*, *A. spirorbis*. Altogether, these data are in agreement with the phylogeography of the ectomycorrhizal *Pisolithus* inferred from rDNA-ITS sequences, which suggests that (1) evolutionary lineages within *Pisolithus* are related to the biogeographical origin of the plant hosts (Martin *et al.*, 2002) and (2) a long-distance dispersal of ectomycorrhizal fungi from Australia might explain the introduction of *Pisolithus* species in the South Pacific zone (Moyersoen *et al.*, 2003).

Types of soil genetically structure populations of *P. albus*

Among ecological constraints that might structure plant, fungi and microorganism populations, unfavorable mineral composition of soils has often been evoked as evolution pressure factors. In ultramafic soils, it has recently been hypothesized that this constraint was responsible for the structure of plant communities and the development of endemic and adapted species (Kazakou *et al.*, 2008). In fungi, recent results of analysis of the fungal genetic divergence of *Cenococcum geophilum* collected from ultramafic soils were hypothesized to reflect adaptation to soil factors (Panaccione *et al.*, 2001). However, more recently, Gonçalves *et al.* (2007) using AFLP analysis could not distinguish between ultramafic and nonultramafic isolates of *C. geophilum*. Here, for the first time, we report on a specific distribution within New Caledonian *P. albus*. Two groups of genotypes based on the ITS-RFLP analysis and confirmed by AFLP correspond to different ecotypes (ultramafic vs. volcano-sedimentary). Our results strongly support the hypothesis of a correlation between a phylogenetic link and ecological adaptation due to the particular mineral constraints of the ultramafic soils of New Caledonia. This is in agreement with work showing that ultramafic constraints structure ectomycorrhizal communities (Urban *et al.*, 2008),

as well as arbuscular mycorrhizal fungal assemblages (Schechter & Bruns, 2008).

Pisolithus albus tolerance to Ni

Ni tolerance and accumulation in tissues has often been used as a classic tool to study plant and fungal potential ecological adaptation to ultramafic constraints (Kazakou *et al.*, 2008), even to characterize microfauna (Boyd *et al.*, 2006). Within *Pisolithus* spp., previous studies have noted that some isolates were able to tolerate high concentrations of Ni. Isolates of *Pisolithus tinctorius*, with Ni EC_{50} ranging from 126 to 170 μM, have been reported (McCreight & Schroeder, 1982; Tam, 1995). Aggangan *et al.* (1998) also described one isolate of *P. tinctorius* from ultramafic soils able to grow on Ni from 20 to 200 μM. More recently, Blaudez *et al.* (2000) and Ray *et al.* (2005) reported isolates of *P. tinctorius* that are able to grow on medium with Ni concentrations ranging from 17 to 350 μM. The mycelia from *P. albus* isolates from New Caledonian ultramafic soils displayed both *in vitro* Ni-sensitive and Ni-tolerant phenotypes. The Ni-tolerant isolates presented a noteworthy tolerance to Ni with an average Ni EC_{50} two to three times higher than the Ni EC_{50} already reported for other *Pisolithus* and mentioned above.

To explain the high variability in Ni-tolerance observations, we might first hypothesize high real fluctuations of Ni content in ultramafic soils while soils sampling and Ni analysis inform more about a global average of either the total Ni content (53 mmol kg^{-1}) or extractable Ni-DTPA (374 μmol kg^{-1}). Recently, Echevarria *et al.* (2006) demonstrated that extractable Ni-DTPA correlated well with the plant bioavailable Ni fraction, which is presumably what the fungus is also exposed to. Perrier *et al.* (2006a) reported that DTPA-Ni concentrations in New Caledonian ultramafic soils are in the range of 17–980 μmol kg^{-1}. In our study, *P. albus* isolates were obtained from soil with DTPA-Ni average concentrations at 374 μmol kg^{-1} in ultramafic soils. We can assume that the average Ni-DTPA concentration does not reflect real fluctuations of bioavailable Ni in ultramafic soils. According to the range of Ni-DTPA concentrations in ultramafic soils reported by Perrier *et al.* (2006a, b), it is not surprising to find isolates of *Pisolithus* with high variations in Ni tolerance from the same ultramafic site. On the other hand, with an average concentration of Ni-DTPA at 3.4 μmol kg^{-1} in volcano-sedimentary soils, *P. albus* isolates were all Ni sensitive. Similar variations in metal-tolerant fungal populations in correlation to metal–soil content have already been reported. For instance, in Suilloid fungi, populations displayed Zn tolerance relative to Zn concentrations in polluted soils, suggesting an evolutionary adaptation of fungi to the soil environment (Colpaert *et al.*, 2004). More recently, evidence of adaptation to Ni was provided in isolates of *C. geophilum* from ultramafic soils (Gonçalves *et al.*, 2009). In our study, no clear relationship between the phenotypic physiological response to Ni and the population genetic differentiation could be established: Ni-tolerant isolates from ultramafic soils did not cluster in a homogeneous group. It is tempting to speculate that the capacity of some *P. albus* isolated from ultramafic soils to tolerate high Ni concentrations reflects the expression of an adaptative response to high concentrations of bioavailable Ni in soils as suggested for other fungi in response to high heavy metal levels (Hartley *et al.*, 1997; Colpaert *et al.*, 2004; Gonçalves *et al.*, 2009). However, if ultramafic constraints structure New Caledonian population of *P. albus* into ecotypes, Ni tolerance alone might not be a sufficient feature to explain such results. Thus, the ultramafic constraints should be grasped as a whole, even if each factor (N, P, K contents, Ca/Mg imbalance, heavy metal presence) is studied separately, as suggested by Kazakou *et al.* (2008).

References

Addinsoft (2005) *XLSTAT software version 7.5.2*. Addinsoft. http://www.xlstat.com.

Aggangan N, Dell B & Malajczuk N (1998) Effects of chromium and nickel on growth of the ectomycorrhizal fungus *Pisolithus* and formation of ectomycorrhizas on *Eucalyptus urophylla* S.T. Blake. *Geoderma* **84**: 15–27.

Amir H & Pineau R (1998a) Effects of metals on the germination and growth of fungal isolates from New Caledonian ultramafic soils. *Soil Biol Biochem* **30**: 2043–2205.

Amir H & Pineau R (1998b) Influence of plants and cropping on microbiological characteristics of some New Caledonian Ultramafic soils. *Aust J Soil Res* **36**: 457–470.

Anderson IC, Chambers SM & Cairney JWG (1998) Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. *Mycol Res* **102**: 295–300.

Anderson IC, Chambers SM & Cairney JWG (2001) ITS-RFLP and ITS sequence diversity in *Pisolithus* from Australia sclerophyll forests. *Mycol Res* **105**: 1304–1312.

Blaudez D, Jacob C, Turnau K, Colpaert JV, Ahonen-Jonnarth U, Finlay R, Botton B & Chalot M (2000) Differential responses of ectomycorrhizal fungi to heavy metals *in vitro*. *Mycol Res* **104**: 1366–1371.

Boyd RS, Wall MA & Jaffré T (2006) Nickel levels in arthropods associated with Ni hyperaccumulator plants from an ultramafic site in New Caledonia. *Insect Sci* **13**: 271–277.

Brady KU, Kruckeberg AR & Bradshaw HD Jr (2005) Evolutionary ecology of plant adaptation to serpentine soils. *Annu Rev Ecol Evol S* **36**: 243–266.

Brooks RR (1987) *Serpentine and its Vegetation: A Multidisciplinary Approach*. Dioscorides Press, Portland, OR.

Chaintreuil C, Rigault F, Moulin L, Jaffré T, Fardoux J, Giraud E, Dreyfus B & Bailly X (2007) Nickel resistance determinants in *Bradyrhizobium* strains from nodules of the endemic New Caledonia legume *Serianthes calycina*. *Appl Environ Microb* **73**: 8018–8022.

Chiarucci A & Baker AJM (2007) Advances in the ecology of serpentine soils. *Plant Soil* **293**: 1–217.

Colpaert JV, Muller LAH, Lambaerts M, Adriaensen K & Vangronsveld J (2004) Evolutionary adaptation to Zn toxicity in populations of *Suilloid* fungi. *New Phytol* **162**: 549–560.

Diez J, Anta B, Manjon JL & Honburia M (2001) Genetic variability of *Pisolithus* isolates associated with native hosts and exotic *Eucalyptus* in the western Mediterranean region. *New Phytol* **149**: 577–587.

Echevarria G, Massoura S, Sterckeman T, Becquer T, Schwartz C & Morel JL (2006) Assessment and control of the bioavailability of Ni in soils. *Environ Toxicol Chem* **25**: 643–651.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**: 1792–1797.

Felsenstein J (1993) *PHYLIP (Phylogeny Inference Package) Version 3.5c*. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Gillepsie TW & Jaffré T (2003) Tropical dry forests in New Caledonia. *Biodivers Conserv* **12**: 1687–1697.

Gomes EA, Barros EG, Kasuya MCM & Araújo EF (1999) Molecular characterization of *Pisolithus* spp. isolates by rDNA PCR-RFLP. *Mycorrhiza* **8**: 197–202.

Gonçalves SC, Portugal A, Gonçalves MT, Vieira R, Martins-Louçao MA & Freitas H (2007) Genetic diversity and differential *in vitro* responses to Ni in *Cenococcum geophilum* isolates from serpentine soils in Portugal. *Mycorrhiza* **17**: 677–686.

Gonçalves SC, Martins-Louçao MA & Freitas H (2009) Evidence of adaptative tolerance to nickel in isolates of *Cenococcum geophilum* from serpentine soils. *Mycorrhiza* **19**: 221–230.

Hartley J, Cairney JWG & Meharg AA (1997) Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment? *Plant Soil* **189**: 303–319.

Héry M, Nazaret S, Jaffré T, Normand P & Navarro E (2003) Adaptation to nickel spiking of bacterial communities in New Caledonian soils. *Environ Microbiol* **5**: 3–12.

Jaffré T (1974) La végétation d'un massif de roches ultrabasiques de Nouvelle-Calédonie: le Koniambo. *Candollea* **29**: 427–456.

Jaffré T (1992) Floristic and ecological diversity of the vegetation on ultramafic rocks in New Caledonia. *The Vegetation of Ultramafic Soils* (Baker AJM, Proctor J & Reeves RD, eds), pp. 101–107. Intercept Ltd, Andover, UK.

Kazakou E, Dimitrakopoulos PG, Reeves RD, Baker AJM & Troumbis AY (2008) Hypotheses, mechanisms, and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. *Biol Rev* **83**: 495–508.

Krznaric E, Verbruggenc N, Wevers JHL, Carleerb R, Vangronsveld J & Colpaert JV (2009) Cd-tolerant *Suillus luteus*: a fungal insurance for pines exposed to Cd. *Environ Pollut* **157**: 1581–1588.

Latham M (1981) *Atlas de la Nouvelle-Calédonie*. ORSTOM, Paris, France.

Martin F, Diez J, Dell B & Delaruelle C (2002) Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from ribosomal DNA ITS sequence. *New Phytol* **153**: 345–357.

Marx DH (1969) The influence of ectotropic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. *Phytopathology* **59**: 153–163.

Marx DH (1977) Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. *Can J Microbiol* **23**: 217–223.

McCreight JD & Schroeder DB (1982) Inhibition of growth of nine ectomycorrhizal fungi by cadmium, lead and nickel *in vitro*. *Environ Exp Bot* **22**: 1–7.

Morat P, Jaffré T, Veillon JM & MacKee HS (1981) Végétation. *Atlas de la Nouvelle-Calédonie et Dépendances*. IRD, Paris, France.

Moser AM, Petersen CA, D'Allura JA & Southworth D (2005) Comparison of ectomycorrhizas of *Quercus garryana* (*Fagaceae*) on serpentine and non-serpentine soils in southwestern Oregon. *Am J Bot* **92**: 224–230.

Moyersoen B, Beever RE & Martin F (2003) Genetic diversity of *Pisolithus* in New-Zealand indicates multiple long-distance dispersal from Australia. *New Phytol* **160**: 569–579.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GABDA & Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Navarro E, Jaffre T, Gauthier D, Gourbiere F, Rinaudo G, Simonet P & Normand P (1999) Distribution of *Gymnostoma* spp. microsymbiotic *Frankia* strains in New Caledonia is related to soil type and to host-plant species. *Mol Ecol* **8**: 1781–1788.

Nei M & Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *P Natl Acad Sci USA* **76**: 5269–5273.

Nicholas KB, Nicholas HB & Deerfield DW (1997) GeneDoc: analysis and visualization of genetic variation. *Embnew News* **4**: 14.

Panaccione DG, Sheets NL, Miller SP & Cumming JR (2001) Diversity of *Cenococcum geophilum* isolates from serpentine and non-serpentine soils. *Mycologia* **93**: 645–652.

Perrier N (2005) Bio-géodiversité fonctionelle des sols latéritiques miniers: application à la restauration écologique (Massif du Koniambo, Nouvelle-Calédonie). PhD Thesis, University of New Caledonia, New Caledonia.

Perrier N, Ambrosi JP, Colin F & Gilkes RJ (2006a) Biogeochemistry of a regolith: the New Caledonian Koniambo ultramafic massif. *J Geochem Explor* **88**: 54–58.

Perrier N, Amir H & Colin F (2006b) Occurrence of mycorrhizal symbioses in the metal-rich lateritic soils of the Koniambo Massif, New Caledonia. *Mycorrhiza* **16**: 449–458.

Priest MJ (1998) *Pisolithus albus* (Cooke & Massee). *Fungi of Southern Australia* (Bougher NL & Syme K, eds), pp. 122. University of Western Australia Press, Perth, WA, Australia.

Proctor J (2003) Vegetation and soil and plant chemistry on ultramafic rocks in the tropical far east. *Perspect Plant Ecol* **6**: 125–146.
Proctor J & Nagy L (1992) Ultramafic rocks and their vegetation: an overview. *The Vegetation of Ultramafic (Serpentine) Soils* (Baker AJM, Proctor J & Reeves RD, eds), pp. 469–494. Intercept, Andover, UK.
Ray P, Tiwari R, Reddy GU & Adholeya A (2005) Detecting the heavy metal tolerance level in ectomycorrhizal fungi *in vitro*. *World J Microb Biot* **21**: 309–315.
Ruiz-Diez B, Rincon AM, de Felipe MR & Fernandez-Pascual M (2006) Molecular characterization and evaluation of mycorrhizal capacity of *Suillus* isolates from Central Spain for the selection of fungal inoculants. *Mycorrhiza* **16**: 465–474.
Schechter SP & Bruns TD (2008) Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. *Mol Ecol* **17**: 3198–3210.
Stoppel RD & Schlegel HG (1995) Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. *Appl Environ Microb* **61**: 2276–2285.
Swofford DL (2000) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer, Sunderland, MA.
Tam PCF (1995) Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. *Mycorrhiza* **5**: 181–187.
Urban A, Puschenreiter M, Strauss J & Gorfer M (2008) Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil. *Mycorrhiza* **18**: 339–354.
Wenzel WW & Jockwer F (1999) Accumulation of heavy metals in plants grown on mineralised soils of the Austrian Alps. *Environ Pollut* **104**: 145–155.
White TJ, Bruns TD, Lee SB & Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* (Innis MA, Gefland DH, Sninsky JJ & White TJ, eds), pp. 315–322. Academic Press, London, UK.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Geochemical characteristics of rhizospheric soils site: major element content.
Table S2. List and characteristics of the nine AFLP primer combinations for selective amplification.
Table S3a. Similarity matrix calculated with Nei's coefficient based on 882 scored AFLP fragments obtained with the nine selective primers pairs EcoRI–ANN and MseI–CNN.
Table S3b. Distance matrix calculated with Nei's coefficient based on 882 scored AFLP fragments obtained with the nine selective primers pairs EcoRI–ANN and MseI–CNN.
Table S4. *Pisolithus* isolates Ni-tolerance screening by measuring capacity to grow on MNM medium at different concentrations of either $NiCl_2$ or $NiSO_4$.
Table S5. AFLP number loci scored for each DNA of *P. albus* isolates tested.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.