Symbiosis, 8 (1990) 233–250
Balaban, Philadelphia/Rehovot

Interactions Between Monokaryotic and Dikaryotic Isolates of *Laccaria bicolor* on Roots of *Pinus banksiana*

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Received March 15, 1990; Accepted July 19, 1990

Abstract

Seedling roots of *Pinus banksiana* were coinoculated under aseptic conditions with pairs of isolates of the ectomycorrhizal fungus *Laccaria bicolor*. Fungal cultures reisolated from second-order lateral roots were identified using isozyme patterns in combination with other phenotypic characters. There were examples where both inoculants persisted on the same root system and even in a single mycorrhiza over the two week duration of our experiments. Furthermore, one monokaryon (mating type A_1B_1) could persist on roots in the presence of a compatible dikaryon (mating type $A_3B_3 \times A_4B_4$). In this case, newly formed dikaryons as results of dikaryon-monokaryon matings were also found on roots. They were consistently constituted of the A_1B_1 and A_4B_4 nuclei (according to isozyme analysis) and the cytoplasm of the monokaryon (according to restriction fragment analysis of the mitochondrial DNA). This study raised questions concerning the contribution of vegetative propagation of monokaryons to the population genetics of ectomycorrhizal fungi.

Keywords: monokaryons, dikaryons, mating, ectomycorrhiza, *Laccaria bicolor*

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Abbreviations: LAP = leucine aminopeptidase; MMN = modified Melin-Norkran; RFLP = restriction fragment length polymorphism; SSC = sodium citrate; sodium chloride; SDS = sodium dodecyl sulfate

1. Introduction

Laccaria bicolor (Maire) Orton is an heterothallic basidiomycete with a bifactorial tetrapolar incompatibility system (Fries and Mueller, 1984; Kropp and Fortin, 1988; Doudrick and Anderson, 1989). Like many other species of the Basidiomycetes (Malloch et al., 1980), it forms ectomycorrhizal associations with roots of many trees and shrubs. The symbiotic organs known as ectomycorrhizae enhance nutrient uptake in the plant partner while the fungal partner benefits from carbon sources produced by the plant (Harley and Smith, 1983).

Several studies have shown that the ectomycorrhizal aggressiveness of different isolates of the same fungal species can vary greatly (Mason, 1975; Molina, 1979; Marx, 1981), even among sib-monokaryotic isolates (Kropp et al., 1987; Lamhamedi et al., 1990). Susceptibility to ectomycorrhizal colonization has also been reported to vary with host genotypes (Marx and Bryan, 1971; Dixon et al., 1987; Tonkin et al., 1989). Genetic interactions may therefore determine the dominant fungal colonizer of a given root system under specific conditions. Although the presence of different genera of ectomycorrhizal fungi on a single root system has been demonstrated (Zak and Marx, 1964; McAfee and Fortin, 1986; Gibson and Deacon, 1988), to our knowledge there has been only one report which mentions the occurrence of two conspecific isolates on a root system (Malajczuk et al., 1990). These latter authors had distinguished two isolates of *Pisolithus tinctorius* (Pers.) Coker and Couch by the intensity of their yellow pigmentation. The general lack of reliable markers for identifying conspecific isolates is the major reason for the absence of ecological information at the intraspecific level.

In past studies, Gardes, Kropp, Fortin and Lalonde (Abstr. Annu. Meet. Mycol. Soc. Am., 1987, *MSA Newsletter* **38(1)**: 25) and Gardes et al. (1990a, 1990b) have developed molecular markers to recognize monokaryotic and dikaryotic isolates of *L. bicolor*. In the present study, such markers are used to evaluate whether different isolates of *L. bicolor* could coexist on seedling roots of *Pinus banksiana* Lamb. In particular, we examined the persistence of monokaryons on root systems in the presence of other isolates because monokaryons are able to form ectomycorrhizal structures (Debaud et al., 1988; Wong et al., 1989) and because the dikaryotic form is usually considered to be the stable state of basidiomycetes.

2. Materials and Methods

Organisms

Fungal cultures

The monokaryotic and dikaryotic isolates of *L. bicolor* used in this study (Table 1) were maintained at 26°C as static liquid cultures. Our MMN culture medium was identical to the modified Melin-Norkrans medium of Marx (1969) except that glucose was substituted for sucrose and a ferric citrate solution for ferric chloride. Subculturing was performed every month by fragmenting liquid cultures in a Waring blender for 10 s and transferring 5 ml of the resultant hyphal slurry to 50 ml of fresh MMN medium in 125 ml Erlenmeyer flasks. Two-week old cultures were harvested for isozyme analysis and DNA extraction.

Plant seedlings

Seeds of *P. banksiana*, collected from a single tree (see bank no. 34.200-6730940.0, National Tree Seed Centre, Petawawa National Forestry Institute, Chalk River, Ont., Canada), were surface sterilized in 30% H_2O_2 and left for 2 weeks to germinate aseptically on 0.7% bacteriological agar (Quelab, Montréal, Qué, Canada) and 1% sucrose. The seedlings were transferred to the Petri dish system of Wong and Fortin (1989), which maintains aseptic conditions of the root system, during growth on a sugar-free mineral salt-agar medium buffered at pH 5.5 with 25 mM MES (2-[*N*-morpholino]ethanesulfonic acid; ICN Biochemicals, Cleveland, OH, USA). The plates were incubated in a growth chamber with a 16 hr photoperiod of 10.5 klx (200 $\mu E \cdot m^{-2} \cdot S^{-1}$ for 400–700 nm) and day:night temperatures of 23:18°C.

Phenotypic characters

General colony morphology, radial growth on MMN agar medium, and purple pigmentation were noted as characters for certain isolates of the fungus. The presence of clamp connections on hyphae (observed using light microscopy) was used as an indicator of the dikaryotic state. The number of nuclei per cell was occasionally verified by staining newly emerged hyphae with 0.5% fluorochrome DAPI (4′, 6-diamidino-2-phenylindole; Sigma Chemical Co, St. Louis, MO, USA) (Meixner and Bresinsky, 1988).

Table 1. List of *Laccaria bicolor* cultures used for fungal confrontation experiments

CRBR[a]	History or earlier reference	Type of isolate	Mating type and biological species[b]	Ectomycorrhizal colonization mantle[c]	Hartig net[d]
0101	CG-211 (Godbout and Fortin, 1985)	dikaryon	bs II[e]	thin	3
0599	$A_3B_3 \times A_4B_4$ (Kropp and Fortin, 1988)	dikaryon	$A_3B_3 \times A_4B_4$, bs I	few hyphae	2 (rare)
0345	ss10 (Kropp et al., 1987)	monokaryon	A_1B_1, bs I	few hyphae	1 (rare)
0345*	spontaneous dedikaryotization of ss4×ss10 (Kropp et al., 1987)	monokaryon	A_1B_1, bs I	few hyphae	1 (rare)
0351	A_3B_4 (Kropp and Fortin, 1988)	monokaryon	A_3B_4, bs I	rare hyphae	0
0568	$A'_1B'_1$ (Kropp and Fortin, 1988)	monokaryon	$A'_1B'_1$, bs II[e]	few hyphae	1 (rare)
0572	$A'_2B'_2$ (Kropp and Fortin, 1988)	monokaryon	$A'_2B'_2$, bs II[e]	few hyphae	2

[a] strain number in the collection of Centre de Recherche en Biologie Forestière (CRBF)

[b] A, B, A′, B′ are mating type factors; bs I and bs II are intersterile biological species (see Kropp and Fortin, 1988)

[c] thin = thin mantle; few/rare hyphae = amount on root surface

[d] the number refers to the maximum number of root cell layers observed to be penetrated by the Hartig net; rare = Hartig net formation was rarely observed

[e] sib-monokaryons 0568 and 0572 are members of a progeny from 0101

Molecular markers

Isozyme analysis

Mycelium was harvested by filtration, washed, and then ground in liquid nitrogen with a mortar and pestle. The mycelial powder was resuspended in 0.16 M Tris-HCl (pH 6.8), centrifuged at 12000 g for 15 min at 4°C and the supernatant stored at −70°C. Discontinuous nondenaturing polyacrylamide gel electrophoresis (Davis, 1964) was carried out using a stacking gel of 3% acrylamide (pH 6.7), a running gel of 7.5% acrylamide (pH 8.9) and an electrode buffer of 0.04 M Tris-glycine (pH 8.3). After electrophoresis at constant current of 25 mA, leucine aminopeptidase (LAP; E.C. 3.4.11.1) activity was detected in the gel according to the procedure of Cheliak and Pitel (1984). Diagrammatic representation of the LAP patterns of each fungal isolate studied is shown on Fig. 1.

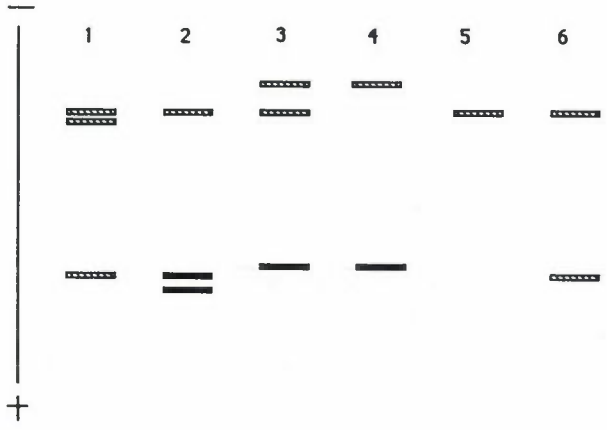

Figure 1. Diagrammatic representation of the LAP (leucine aminopeptidase) pattern for each of the different *Laccaria bicolor* isolates studied. Lanes (1) dikaryon 0101; (2) dikaryon 0599 (constituent nuclei: $A_3B_3 \times A_4B_4$) and monokaryotic constituent of 0599 with mating type A_3B_3; (3) Newly formed dikaryon from the compatible combination 0345*/0599; (4) monokaryon 0345 and monokaryon 0345*; (5) monokaryon 0351; (6) monokaryon 0568 and monokaryotic constituent of 0599 with mating type A_4B_4.

Restriction fragment analysis

A mitochondrial DNA probe for restriction fragment length polymorphism (RFLP) analysis was prepared from DNA of *L. bicolor* CRBF 1043. Total cellular DNA was isolated from freeze-dried mycelium according to the procedure of Murray and Thompson (1980). Mitochondrial DNA was separated

from nuclear DNA and purified by ultracentrifugation in a cesium chloride-bisbenzimide (Hoescht 33258, Sigma Chemical Co.) gradient according to the method of Hudspeth et al. (1980), and then digested with the restriction enzyme *Bam*HI (Pharmacia, Uppsala, Sweden) according to the conditions recommended by the manufacturer. *Bam*HI total digest was shotgun-cloned into the plasmid vector pBR 328 (Soberon et al., 1980). A clone containing a mitochondrial insert of 6.6 kb in size (Gardes et al., 1990b) was chosen for use as a mitochondrial marker.

For restriction fragment analysis, total DNA was extracted according to Lee et al. (1988), digested with *Bgl*II (Pharmacia) and subjected to electrophoresis on 0.8% agarose gel in TBE (89 mM Tris-HCl, 89 mM boric acid, 2.0 mM EDTA, pH 8.0) at 20-50 V for 10–15 hr (for maxigels, 30 cm length), using lambda-*Hin*dIII digest as size markers. Capillarity transfer of DNA fragments from gels to nylon membranes (Genescreen Plus, NEN Research Products, Boston, MA, USA), was done according to the manufacturer's recommendations.

Plasmid probe DNAs were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; ICN Biomedicals) using a nick translation kit from Bethesda Research Laboratories (Gaithersburg, MD, USA). On the average, specific radioactivity of 1×10^8 cpm/mg DNA was obtained. Ten ng of bacteriophage lambda DNA were usually added to 500 ng of plasmid DNA during nick translation in order to visualize the lambda marker.

Prehybridization was done at 65°C for 2 hr in 6×SSC, 5×Denhardt's solution (Maniatis et al., 1982) and 0.5% SDS (sodium dodecyl sulfate). Hybridization was done at 65°C for 16–24 hr in 6×SSC, 5×Denhardt's solution, 0.5% SDS, 10% Dextran sulfate, 150 mg/ml of sheared salmon sperm DNA and 10–20 ng/ml probe DNA. Just before use for hybridization, salmon and probe DNAs were brought to 1 ml with double distilled water and denatured by boiling for 10 min. Blots were washed twice at room temperature for 5 min in 2×SSC, twice at 65°C for 30 min in 2×SSC–1% SDS, and finally twice at room temperature for 10 min in 0.1×SSC. Autoradiography was performed using Cronex Lightening-Plus intensifying screens and Kodak X-Omat AR films at −70°C for 4–10 hr.

Fungal confrontation experiments

On plants

Each single fungal inoculum was prepared by transferring a one week-old liquid culture into a 50 ml, sterile tube, centrifuging and resuspending the mycelial pellet in 10 ml of sterile distilled water. The mycelium was then

homogenized by repeated passages (10–15 times) through a 10 ml syringe equipped with an 18 G 1/2 needle. Each mixed inoculum was prepared by pairwise mixing of single inocula on a volume basis and passing the mixture through a syringe.

Six-to-seven weeks after the start of seed germination, seedlings were inoculated by depositing a drop of a single or a mixed fungal inoculum near the apex of a first-order lateral root. Two weeks after inoculation, fungi were reisolated from second-order lateral roots near the site of inoculation, by transferring root segments to MMN agar medium. The reisolates were grown on MMN agar medium for 1–2 weeks and plugs from the periphery of the colonies were then transferred to MMN liquid medium for another week before subculturing as described previously.

Control treatments (without plants)

There were 2 types of control treatment for each combination of fungal isolates tested. Control treatment A was performed in parallel with the experiments with plants. A drop of the original mixed inoculum was placed on MMN agar medium for 2 weeks and plugs from the periphery of the resultant fungal colony were taken for reisolation. Control treatment B consisted of pairwise confrontations of MMN agar medium grown cultures by placing plugs of two isolates side-by-side on fresh MMN agar medium.

Ectomycorrhizal colonization

Roots inoculated with single isolates were sampled for evaluation of ectomycorrhizal colonization. Second-order laterals near inoculation sites were fixed in glutaraldehyde, dehydrated in a graded ethanol series and embedded in Spurr's resin as previously described (Wong and Fortin, 1989). Transverse root sections (1 μm thick) were cut using an ultramicrotome with glass knives, stained with 0.05% toluidine blue O in 1% sodium tetraborate and examined using light microscopy. The results shown on Table 1 are assessments made after examining 3–9 root segments.

3. Results

Seven *L. bicolor* cultures were used in this study (Table 1), 2 dikaryons and 5 monokaryons. Five combinations of isolates were tested as inocula in coinoculation experiments, in order to illustrate certain interactions between monokaryons and dikaryons (Table 2). Two independent experiments with a total of 4–6 seedlings were performed with each combination of isolates,

Table 2. Results of fungal confrontation experiments on root systems

Confrontation type[a]		Inoculum	Ratio	No. of	Co-inoculation of plant[b]				Control treatment A for mixed inoculant[c]			
pairing	compatibility	isolates	(v/v)	expt.	no.	roots	ID[d]	method[e]	no.	plugs	ID	method[e]
mon-mon	compatible	0568:0572	1:1	2	5	12	0568 x 0572	clamps	3	3	0568 x 0572	clamps
	intersterile	0568:0345	1:1	2	6	8	0568	LAP, no clamp	3	7	0568	LAP, no clamp
di-mon	compatible	0599:0345*	1:1	2	3	10	0345*	LAP, no clamp	3	7	0345*	LAP, no clamp
					2	6	NFD	LAP, clamps				
					1	4	0345* (2)	LAP, no clamp				
							NFD (2)	LAP, clamps				
			1:5	1	1	3	0345*	LAP, no clamp	2	2	0345*	LAP, no clamp
					1	2	NFD	LAP, clamps				
	insterile	0101:0345*	1:1	2	4	12	0101 (11)	LAP, clamps	3	7	0101	LAP, clamps
							0345* (1)[f]	LAP, no clamp				
			1:5	1	2	4	0101	LAP, clamps	2	2	0345*[g]	LAP, no clamp
	hemicomp.	0599:0351	1:1	2	5	12	0599 (11)	LAP, clamps	4	5	0351	LAP, no clamp
							mix (1)[h]	see text				
			5:1	1	2	5	0599	LAP, clamps	2	3	0351[i]	LAP, no clamp

[a] monokaryon-monokaryon or dikaryon-monokaryon pairings, sexual compatibility of nuclei

[b] number of plants tested, number of reisolates from root segments, identification of reisolates, method of identification

[c] number of colonies grown on MMN agar medium, number of reisolates from plugs taken from the periphery of these colonies, identification of reisolates, method of identification

[d] a number in parenthesis is used when necessary to indicate the number of reisolates having the corresponding identification, NFD = newly formed dikaryon

[e] LAP patterns were identical for isolates 0345 and 0345*

[f] this monokaryotic reisolate had been recovered along with two dikaryotic 0101 reisolates from a single seedling

[g] colony contained sectors of isolate 0101, as identified by LAP and presence of clamps

[h] mixture of 0351 and 0599 (see text)

[i] colony contained sectors of isolate 0599, as identified by LAP and presence of clamps

mixed at a ratio of 1:1. An additional experiment was performed using an 1:5 mixture of certain isolates. For all experiments, parallel inoculations of plants with single fungal inocula had been performed and the inoculants successfully reisolated from corresponding root segments.

Interaction between monokaryotic isolates

A compatible combination

All 12 reisolates from roots coinoculated with the sib-monokaryons 0568 and 0572 had clamp connections (Table 2), indicating dikaryotization. Dikaryotic hyphae were also observed at the periphery of colonies grown on MMN agar medium from drops of the original mixed inoculum (control treatment A). These 2 monokaryons also crossed readily during pairwise confrontations on MMN agar medium (control treatment B), with dikaryotization observed within 2 weeks at the confrontation zone between their colonies.

An interstile combination

Isolates 0345 and 0568 belonged to two different biological species (i.e. reproductively isolated groups) but had similar ectomycorrhizal abilities (Table 1). All eight reisolates from coinoculated roots showed a LAP pattern identical to that of isolate 0568. Seven reisolates from control treatment A were also similarly identified as isolated 0568 (Table 2). Furthermore, the growth rate of isolate 0568 on MMN agar medium was visibly greater than that of isolate 0345. These observations suggest that in this combination, isolate 0568 outgrew isolate 0345 on the plant and in control treatment A.

Interaction between dikaryotic and monokaryotic isolates

A compatible combination

The monokaryon 0345* and the dikaryon 0599 had apparently similar abilities to colonize roots (Table 1). Among the total of 25 reisolates from all experiments, two kinds of LAP pattern were observed (Fig. 2). Fifteen reisolates had a LAP pattern identical to that of monokaryon 0345* whereas ten others had a LAP pattern distinct from that of either inoculant (Table 2). The latter group of reisolates also differed from the former by having clamp connections. The presence of two cathodal LAP bands (Figs. 2 and 3) in the latter group suggested that mating between monokaryon 0345* and dikaryon 0599 had occurred. On one seedling, monokaryon 0345* and the newly formed dikaryon were recovered from different root segments.

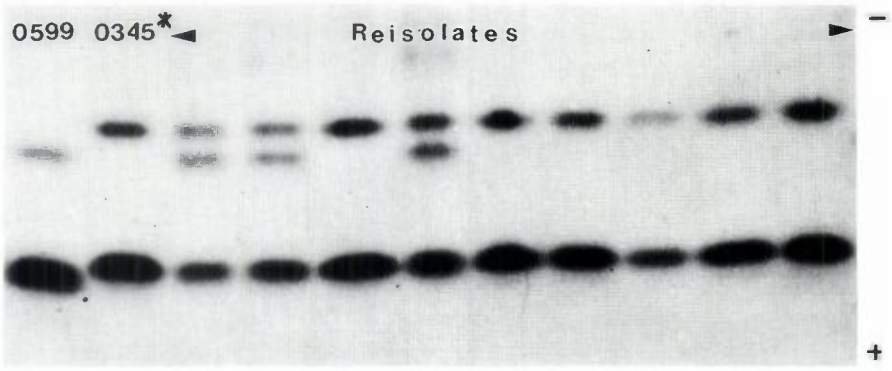

Figure 2. LAP pattern of *Laccaria bicolor* monokaryon 0345*, dikaryon 0599 and 9 reisolates from roots of 6 *Pinus banksiana* plantlets coinoculated with them. The two strains belong to the same biological species and have sexually compatible nuclei.

LAP pattern comparisons of the sib-monokaryotic constituents of dikaryon 0599 (mating type A_3B_3 and A_4B_4; Kropp and Fortin, 1988) suggested that the newly formed dikaryons contained the nucleus with mating type A_4B_4 and that of monokaryon 0345* (Fig. 3). This result was confirmed

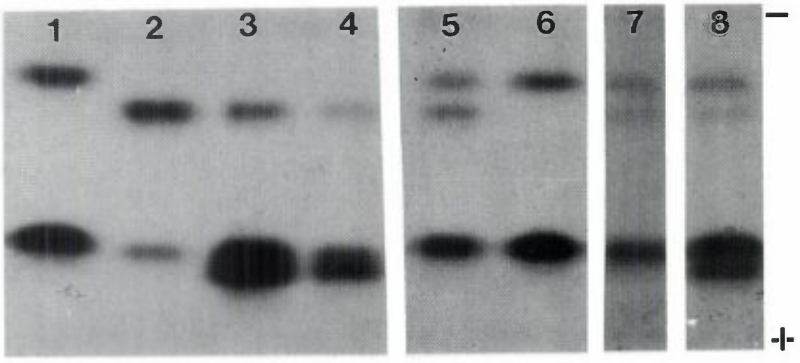

Figure 3. LAP pattern of *Laccaria bicolor* isolates indicating that the newly formed dikaryon, from interactions between monokaryon 0345* and dikaryon 0599 (constituent nuclei: $A_3B_3 \times A_4B_4$), contained the nucleus of 0345* and that of A_4B_4: Lanes (1 & 6) monokaryon oft; (2) monokaryon with mating type A_4B_4; (3) dikaryon 0599; (4) monokaryon with mating type A_3B_3; (5) newly formed dikaryon; (7) protein sample from a mycelial mixture of 0345* and A_4B_4; (8) protein sample extracted from a mycelial mixture of 0345* and A_3B_3.

by mixing colonies of 0345* with A_3B_3 or A_4B_4 for simultaneous protein extraction. The anodal band of monokaryon A_4B_4 migrated only slightly more than

that of 0345* and only one anodal band was observed when the two strains were mixed as observed in the newly formed dikaryon (Fig. 3). Two anodal bands were observed for 0599 and A_3B_3 (one being identical in migration to that of A_4B_4) and two anodal bands were observed when colonies of 0345* and A_3B_3 were mixed (Fig. 3). RFLP of the mitochondrial DNA from four of the newly formed dikaryons (reisolated from three different seedlings) indicated that they had the 3.5 kb fragment characteristic of isolate 0345* and not the 2.4 kb fragment characteristic of isolate 0599. This latter result suggested that the cytoplasmic constitution of the newly formed dikaryons was identical to that of isolate 0345* (Fig. 4) and confirmed the invasion of the homokaryon by the dikaryon.

All nine reisolates from control treatment A were identified as monokaryon 0345* (Table 2). Isolate 0345* grew visibly faster on MMN agar medium than isolate 0599.

In control treatment B, purple dikaryotic sectors developed at the interface of white colonies grown from plugs of 0345* and 0599 after 1–2 weeks. Mycelia from two of these sectors were cultured and found to have a LAP pattern identical to that of the newly formed dikaryon reisolated from seedling roots.

In this combination, it appears that the monokaryon 0345* was more successful than the dikaryon 0599. However, selective invasion of the monokaryon 0345* by one of the two nuclear types from dikaryon 0599 was often observed and the newly formed dikaryons could be found on roots.

An intersterile combination

The dikaryon 0101 colonized roots better than the monokaryon 0345* (Table 1). A total of 15 reisolates had clamp connections and a LAP pattern typical of isolate 0101 (Fig. 5). One reisolate lacked clamp connections and had a LAP pattern typical of isolate 0345*. This monokaryotic reisolate had been recovered along with two dikaryotic reisolates from a seedling inoculated with an 1:1 mixed inoculum.

For the 1:1 mixed inoculum of 0101:0345*, the three colonies obtained from control treatment A were identified as isolate 0101. For the 1:5 mixed inoculum, the two resulting colonies were similar to isolate 0345* (identical LAP pattern, no clamp connection)but they contained dikaryotic sectors. LAP analysis of one reisolate from one of the dikaryotic sectors confirmed that it was isolate 0101. Colonies of isolate 0101 grew visibly faster on MMN agar medium than those of 0345*.

In this combination, dikaryon 0101 colonized root better and grew faster. It was generally more successful than monokaryon 0345* on roots and in control

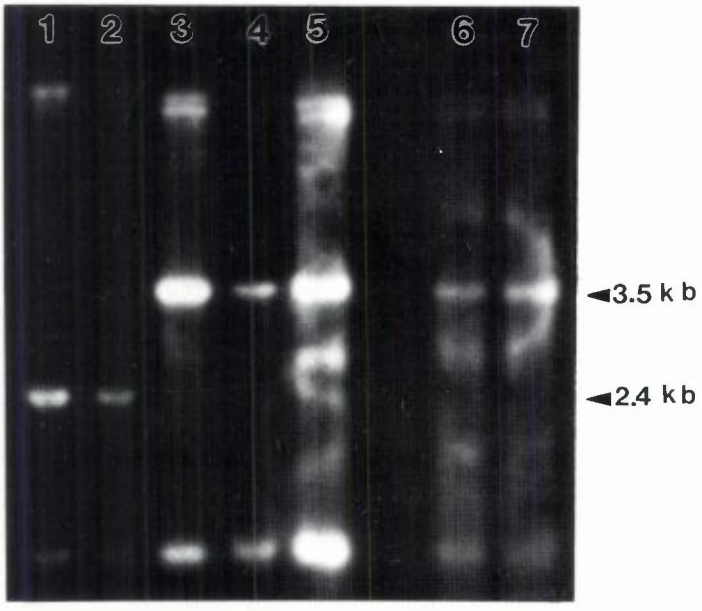

Figure 4. DNA fragment patterns of *Laccaria bicolor* isolates indicating that the newly formed dikaryon, from interactions between monokaryon 0345* and dikaryon 0599, contained the mitochondrial type of 0345*. Autoradiogram of total DNA digested with *Bgl*II and probed with the cloned mitochondrial DNA fragment pLbm6.6. Lanes (1 & 2) 0599, two independent tracks; (3) 0345*; (4 to 7) 4 reisolates from 3 *Pinus banksiana* plantlets coinoculated with monokaryon 0345* and dikaryon 0599. The two strains belong to the same biological species and have sexually compatible nuclei. The characteristic fragment for 0345* is 3.5 kb in size and that for 0599 is 2.4 kb.

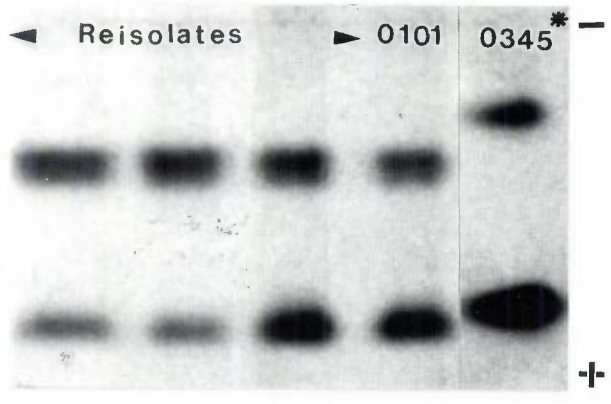

Figure 5. LAP pattern of *Laccaria bicolor* dikaryon 0101, monokaryon 0345*, and 3 isolates from roots of 2 *Pinus banksiana* plantlets coinoculated with them. The 2 strains belong to different biological species.

treatment A. With the 1:5 mixed inoculum, although the higher biomass of monokaryon 0345* permitted its dominance in control treatment A, it was still the dikaryon 0101 which was found on roots.

An hemicompatible combination

The dikaryon 0599 appeared to be able to colonize roots better than the monokaryon 0351 (Table 1). Of a total of 17 reisolates, 16 were identified as isolate 0599 by their clamp connections, LAP pattern (Fig. 6; Table 2) and compact mycelium. However, one of two reisolates from a seedling inoculated

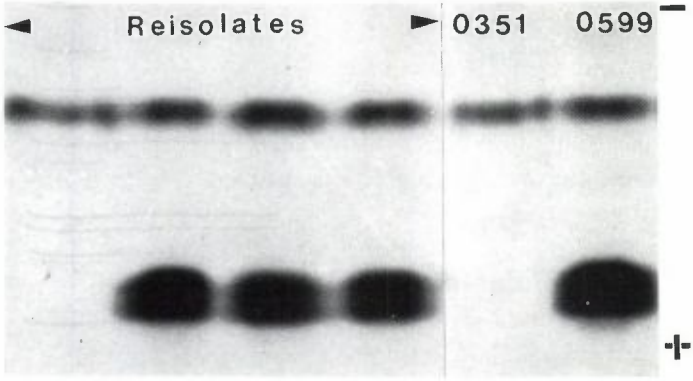

Figure 6. LAP pattern of *Laccaria bicolor* dikaryon 0599, monokaryon 0351, and 4 reisolates from roots of 2 *Pinus banksiana* plantlets coinoculated with them. The 2 strains belong to the same biological species and have sexually hemicompatible nuclei.

with an 1:1 mixture yielded a colony with dispersed mycelium at the periphery. Three independent plugs from this colony were transferred on to fresh MMN agar medium and subsequent growth produced sectors, some with and others without clamp connections. LAP analysis of one of the dikaryotic sectors confirmed that it was isolate 0599, while one of the monokaryotic sectors was confirmed to be isolate 0351. These results suggested that both isolates 0599 and 0351 were present on the same root segment.

Control treatment A for the 1:1 mixed inoculum yielded colonies recognized as isolate 0351. For the 5:1 mixed inoculum of 0599:0351, the two resulting colonies were similar to isolate 0351 (identical LAP pattern, no clamp connections) but they contained dikaryotic sections, one of which was identified as isolate 0599 by its LAP pattern. Isolate 0351 grew visibly faster on MMN agar medium than isolate 0599.

In this combination, the dikaryon 0599 grew slower than the monokaryon 0351. However, it was the better root colonizer and in coinoculation experiments on roots, it was more successful.

4. Discussion

In agreement with previous reports (Kropp et al., 1987; Debaud et al., 1988; Kropp and Fortin, 1988; Wong et al., 1989; Lamhamedi et al., 1990), we found that monokaryons of an ectomycorrhizal fungus can colonize roots under artificial conditions. The monokaryon in each of three dikaryon-monokaryon combinations of *L. bicolor* had been reisolated from roots after coinoculation. In one case, the monokaryon 0345* was reisolated from a root segment even though the dikaryotic coinoculant 0101 was a better root colonizer and grew faster on the reisolation medium. To our knowledge, the persistence of monokaryotic hyphae on roots in the presence of conspecific hyphae has not been previously reported in ectomycorrhizal fungi. Studies over longer periods of time and in more naturals systems are required in order to understand the reasons for the apparent absence of monokaryons in ectomycorrhizae collected from the field. There may generally be an abundance of sexually compatible nuclei, which permits frequent and rapid dikaryotization of monokaryotic hyphae growing from spores which arrive by aerial dispersal. Other possibilities include that monokaryons are excluded in nature by competitive interactions with other microorganisms.

Rapid dikaryotization of two sib-monokaryotic isolates (0568 and 0572) was observed on roots and on agar medium without roots. Furthermore, the mating system in Basidiomycetes is remarkably flexible in that monokaryons are also capable of mating with dikaryons (Buller, 1958). Thus, newly formed dikaryons were recovered from roots coinoculated with monokaryon 0345* and dikaryon 0599. Even though both nuclear types in the dikaryon 0599 had the potential to mate with monokaryon 0345*, only one of them (mating type of A_4B_4) was found in the newly formed dikaryons. The phenomenon of nuclear selection during dikaryon-monokaryon matings in Basidiomycetes has often been reported (Raper, 1966; Beeching et al., 1989) with numerous mechanisms hypothesized, one of the most popular being a selection for the most dissimilar genome (Coates and Rayner, 1985). Mating studies using dikaryon 0599 and other monokaryons could be used to evaluate this hypothesis in *L. bicolor*.

Only one mitochondrial type, that of the monokaryon, was found in the newly formed dikaryons from the mating of dikaryon 0599 and monokaryon 0345*. In other Homobasidiomycetes such as *Agaricus bitorquis* (Hintz et al., 1988) and *Coprinus cinereus* (Baptista-Ferreira et al., 1983; May and

Taylor, 1988), only one mitochondrial restriction fragment length phenotype was also observed in newly formed dikaryons and in these cases, nuclear migration had occurred without mitochondrial migration. The mechanism responsible for uniparental inheritance of mitochondria in *L. bicolor* may be revealed by detailed studies of mitochondrial inheritance and nuclear migration. Although we have only observed dikaryon formation at confrontation zones, Doudrick and Anderson (1989) observed clamp connections at the periphery of monokaryotic colonies during dikaryon-monokaryon confrontations. This difference in observations concerning nuclear migration in *L. bicolor* may be a result of differences in the media used as well as in the genetic constitution of the strains.

The results of the control treatment performed along with our coinoculation experiments indicate that the reisolation procedure generally favours the recovery of isolates which grow faster on MMN agar medium. However, the isolate recovered from the control treatment was not always the one reisolated from roots, the latter generally appears to be the better root colonizer (e.g. 0101 from a 1:5 mixture of isolates 0101:0345*, 0599 from a 1:1 mixture of 0351:0599). The newly formed dikaryotic genotype, reisolated from roots coinoculated with isolates 0345* and 0599, may also be a relatively good root colonizer. It appears that genetic determinants can favour root association, and thus possibly survival, among individuals of a fungal population. *In situ* localization of hyphae, using antibodies or oligonucleotide probes, is a technique which may be useful for revealing the mechanisms controlling fungal interactions on roots.

Ectomycorrhizal fungi may successfully propagate and disperse in nature as spores and as vegetative mycelium. Whether or not the mycelium in mycorrhizae can be monokaryotic in nature remains to be determined. Nevertheless, monokaryon-dikaryon matings probably occur in nature and the study of interactions between monokaryotic and dikaryotic hyphae on roots can therefore contribute to a better understanding of the population genetics of ectomycorrhizal basidiomycetes. Our study shows that the use of molecular markers, specific to the nuclear and the mitochondrial genomes of fungi, can provide information concerning genomic composition and exchange during mycelial interactions. Furthermore, the development of more sensitive molecular techniques to identify fungal genomes directly from root and soil samples would permit us to avoid the fungal reisolation step and thus facilitate future laboratory and field studies (Gardes et al., 1990c).

Acknowledgements

We thank Bruno Marcotte for technical assistance and Harry Kope for reviewing this manuscript. This work was supported by the Natural Sciences and Engineering Research Council (Canada) through a scholarship to K. Wong and a grant to A. Fortin.

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