

Mycorrhization helper bacteria: a case of specificity for altering ectomycorrhiza architecture but not ectomycorrhiza formation

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Abstract Mycorrhization helper bacteria (MHB), isolated from phylogenetically distinct ectomycorrhizal symbioses involving *Lactarius rufus*, *Laccaria bicolor* or *Suillus luteus*, were tested for fungus specificity to enhance *L. rufus*–*Pinus sylvestris* or *L. bicolor*–*P. sylvestris* mycorrhiza formation. As MHB isolated from the *L. rufus* and *S. luteus* mycorrhiza were originally characterised using a microcosm system, we assessed their ability to enhance mycorrhiza formation in a glasshouse system in order to determine the extent to which MHB are system-specific. *Paenibacillus* sp. EJP73, an MHB for *L. rufus* in the microcosm, significantly enhanced *L. bicolor* mycorrhiza formation in the glasshouse, demonstrating that the MHB effect of this bacterium is neither fungus-specific nor limited to the original experimental system. Although the five MHB strains studied were unable to significantly enhance *L. rufus* mycorrhiza formation, two of them did have a significant effect on dichotomous short root branching by *L. rufus*. The effect was specific to *Paenibacillus* sp. EJP73 and *Burkholderia* sp. EJP67, the two strains isolated from *L. rufus* mycorrhiza, and was not associated with auxin production. Altered mycorrhiza architecture rather than absolute number of mycorrhizal roots may be an important previously overlooked parameter for defining MHB effects.

Keywords Ectomycorrhizal fungi · *Laccaria bicolor* · *Lactarius rufus* · Mycorrhization helper bacteria · *Pinus sylvestris*

Introduction

A number of studies have demonstrated under laboratory, glasshouse or nursery conditions that strains of bacteria isolated from the ectomycorrhizosphere are able to enhance mycorrhiza formation by the plant–fungus symbiosis from which they were isolated (Garbaye and Bowen 1989; Duponnois and Garbaye 1991; Dunstan et al. 1998; Founoune et al. 2001, 2002; Poole et al. 2001). Those strains enhancing mycorrhiza formation have been termed mycorrhization helper bacteria (MHB) (Garbaye 1994). In all cases, enhanced mycorrhiza formation by MHB has been determined by counting the percentage of mycorrhizal short roots to total short roots. The impacts of MHB on more subtle aspects of mycorrhizal architecture, including the spatial arrangement and structure of roots, have received little attention. Several studies have indicated that some MHB can increase the formation of lateral roots (Poole et al. 2001; Schrey et al. 2005). However, effects on the extent of dichotomous branching of short roots have not been considered.

Mycorrhization helper bacteria have been isolated from a variety of ectomycorrhizal symbioses, although the nature of the systems, which have been used to identify MHB, vary considerably. For example, Duponnois and Garbaye 1991, Frey-Klett et al. 1997, 1999, and Brulé et al. 2001 have extensively studied *Pseudotsuga menziesii*–*Laccaria bicolor* bacteria interactions under non-sterile glasshouse conditions. These studies have characterised the MHB strains *Pseudomonas fluorescens* BBc6R8 and *Bacillus*

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subtilis MB3. By comparison, Poole et al. (2001) developed a gnotobiotic laboratory microcosm to test for MHB activity, which focussed on the *Pinus sylvestris*–*Lactarius rufus* symbiosis. Using this system a number of bacterial isolates were identified as MHB, including *Burkholderia* sp. EJP67 and *Paenibacillus* sp. EJP73. The microcosm was also used to identify *Bacillus* sp. EJP109 as a MHB for the *P. sylvestris*–*Suillus luteus* symbiosis (Bending et al. 2002). However, whether MHB activity is limited solely to the test system in which it was demonstrated has not been determined.

Although studies have been carried out since the late 1980s, still little is known about the mechanisms by which MHB produce the helper effect. An important question, which relates to understanding MHB mechanisms, is whether MHB–fungal interactions are specific. Duponnois et al. (1993) showed that MHB isolates taken from the *P. menziesii*–*Laccaria laccata* symbiosis had a positive effect on closely related ectomycorrhizal fungus *L. bicolor* but were unable to enhance mycorrhiza formation involving either *Hebeloma cylindrosporum* or *Paxillus involutus*. In fact, the bacteria had a significant negative effect on *H. cylindrosporum* mycorrhiza formation. These workers suggest that the apparent selectivity of MHB for specific ectomycorrhizal fungi would benefit the optimization of the efficiency of *L. laccata* inoculation in bare-root forest nurseries, the conventional approach involving soil fumigation with methyl bromide.

Two of the MHB used in the study of Duponnois et al. (1993), *B. subtilis* MB3 and *P. fluorescens* BBc6, were subsequently used by Dunstan et al. (1998) in parallel with bacterial strains (*Bacillus* sp. Elf28 and a pseudomonad Elf29) isolated from *Laccaria fraterna* E710 sporocarps. All four bacterial strains were able to enhance *Eucalyptus diversicolor*–*L. fraterna* E710 mycorrhiza formation under glasshouse conditions. In contrast, mycorrhiza formation by *L. laccata* strain E766 was inhibited by *P. fluorescens* BBc6. There was no significant correlation between effects of bacteria on mycorrhizal formation for treatments with either *L. laccata* E766 or *L. fraterna* E710, indicating fungus-specific MHB effects for *E. diversicolor*–*Laccaria* spp. symbioses. In a more recent paper, however, *Pseudomonas monteilii* HR13 isolated from *Pisolithus alba* was found to stimulate mycorrhiza formation in symbioses of *Acacia holosericea* with *P. alba* and *Scleroderma* spp., and *P. monteilii* even enhanced root colonisation by the arbuscular mycorrhizal fungus *Glomus intraradices* (Duponnois and Plenchette 2003), suggesting that not all fungal–MHB interactions are specific. However, whether this is an isolated case of MHB non-specificity to enhance mycorrhizal formation or a more widespread phenomenon requires a comparative study of MHB from different ectomycorrhizal systems.

The existence of a glasshouse system used extensively to study *L. bicolor*–MHB interactions (Duponnois and Garbaye 1991; Frey-Klett et al. 1997, 1999; Brulé et al. 2001), as well as MHB, which was identified using a microcosm for two further phylogenetically distinct ectomycorrhizal fungi *L. rufus* and *S. luteus* (Poole et al. 2001; Bending et al. 2002), provides an ideal opportunity to study the system and fungus specificity of MHB. Therefore, the aims of this work were (1) to determine whether MHB activities are specific to the system in which it has been demonstrated, (2) to test the fungal specificity of MHB isolated from *L. bicolor* (MB3 and BBc6R8), *L. rufus* (EJP67 and EJP73) and *S. luteus* (EJP109) mycorrhiza, (3) to determine the effect of MHB on mycorrhiza formation by other spontaneous ectomycorrhizal fungi present in the glasshouse, and (4) to assess the effect of MHB on mycorrhizal architecture by assessing lateral root formation and the extent of dichotomous branching.

Materials and methods

Bacteria

Five bacterial strains were used. *P. fluorescens* BBc6R8 (a spontaneous rifampicin-resistant mutant of BBc6) and *B. subtilis* MB3 enhance mycorrhiza formation in the *P. menziesii*–*L. bicolor* symbiosis under glasshouse and nursery conditions (Duponnois and Garbaye 1991; Frey-Klett et al. 1997). *Paenibacillus* sp. EJP73 and *Burkholderia* sp. EJP67 (Poole et al. 2001), and *Bacillus* sp. EJP109 (Bending et al. 2002) stimulate mycorrhiza formation by *L. rufus* and *S. luteus*, respectively, on *P. sylvestris* in gnotobiotic microcosms. The bacterial strains were cultured on 1/10 strength tryptone soya agar (1/10 TSA).

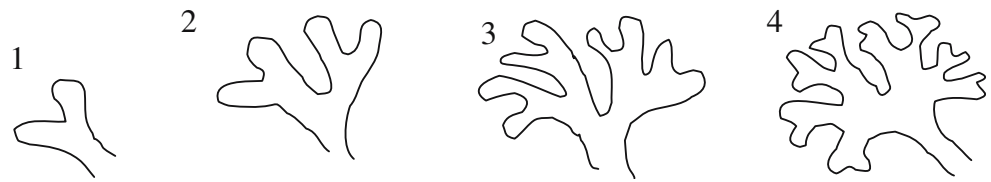
Fungi

The two ectomycorrhizal basidiomycetes, *L. bicolor* S238N (Di Battista et al. 1996) and *L. rufus* EO3 (Poole et al. 2001), were cultured on Pachlewski agar medium (Pachlewski and Pachlewski 1974). Solid inoculum was prepared in 1.6-l glass jars containing 1.3 l vermiculite–peat mixture (4:1, v:v) moistened with 650 ml liquid Pachlewski medium, inoculated with plugs from an agar culture and incubated for 6 weeks at 25°C (Duponnois and Garbaye 1991).

Glasshouse experiments

P. sylvestris L. seedlings were grown in black polythene trays containing 20 wells, each well 4 cm in diameter with a volume of 80 ml. Each well was filled with peat–

Fig. 1 Dichotomous short root branching patterns of *Lactarius rufus* mycorrhiza



vermiculite (1:1, v:v), which had been previously mixed with solid fungal inoculum at a ratio of 1:40 for *L. bicolor* or 1:10 (v:v) and 1:40 for *L. rufus*. Four seeds (obtained from the Forestry Commission, Alice Holt, Surrey, UK) were sown per well. Bacteria were introduced into each well by pipetting 3 ml of bacterial inoculum evenly over the peat-vermiculite surface. The bacterial inoculum was prepared by growing isolates on 1/10 strength TSA for 24 h, or 48 h in the case of slow-growing EJP67. Bacteria from the plates (approx. 30–40 plates per treatment) were each re-suspended in 20 ml $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 M) and bulked together. They were centrifuged (10,000 rpm, 10 min, 4°C) washed in $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, centrifuged twice (10,000 rpm, 5 min, 4°C), and re-suspended in $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The bacterial inoculum densities were then adjusted to 8.0×10^6 (MB3), 7.9×10^6 (EJP109), 6.1×10^7 (EJP73), 3.3×10^8 (EJP67), and 10^8 (BBc6R8) cfu ml⁻¹, respectively, with sterile distilled water. Polythene trays were inoculated with 4 ml culture per well, whereas control wells received the same volume of sterile distilled water. The experiment was divided into three blocks. Each block consisted of six microbial treatments: fungus alone, fungus plus each of the four bacterial strains, and non-inoculated control. Three trays were used per treatment, one tray randomly arranged within each of the three blocks. Seedlings were watered daily with a mist system and nutrient solution applied as described previously after 13 weeks (Frey-Klett et al. 1997). Wells were thinned to a single seedling as required after 4 weeks.

Plant harvest and mycorrhiza scoring

Three months after sowing, six plants were systematically sampled per block for each treatment. Each plant shoot was cut and shoot height measured. The washed root system was cut into sections, approximately 1–2 cm in length, and the mycorrhizal index (number of mycorrhizal short roots divided by the total number of short roots) was determined by randomly examining 100 short roots per seedling using a stereomicroscope. The short roots were scored as (1) inoculum mycorrhiza (formed by inoculated fungi *L. bicolor* or *L. rufus*), (2) mycorrhiza of a *Tomentella* ‘black-hairy’ morphotype resident in the glasshouse, (3) ‘other’ mycorrhiza (including *Thelephora* morphotypes), or (4) non-mycorrhizal, depending on short root morphology, colour and texture. After analysis, shoot and root

samples were dried and weighed. In the case of the *L. rufus* 1:10 fungal dose, the degree of root branching was also analysed. *L. rufus* mycorrhiza short roots were scored as either first, second, third or fourth order mycorrhiza (Fig. 1).

In vitro production of indole-3-acetic acid by bacterial strains

The method described in Frey-Klett et al. (2005) was used to determine whether the MHB strains could produce indole-3-acetic acid (IAA). Briefly, bacterial isolates were spotted with sterile toothpicks on 1/10 TSA containing 5 mM tryptophan. Plates were immediately overlaid with a nitrocellulose membrane and incubated at 28°C for 72 h. After incubation, membranes were removed from the plates and dipped in 2% Salkowski reagent (0.5 M FeCl_3 in 35% perchloric acid) for 1 h. Formation of a red halo on the membrane, in the area immediately surrounding the bacterial spots, indicated a bacterial strain producing IAA.

Statistical analysis

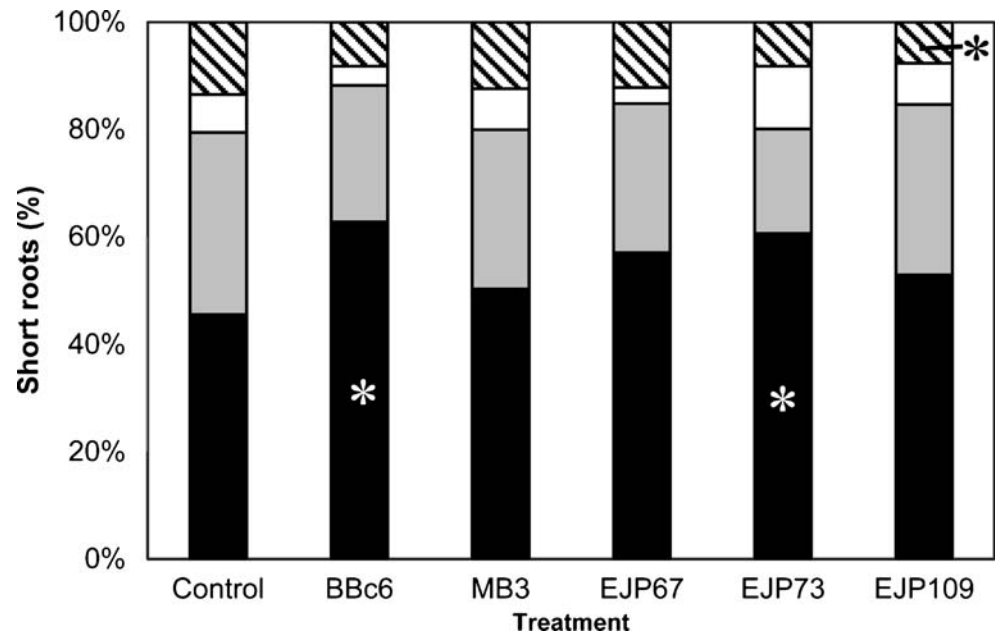
L. rufus and *L. bicolor* mycorrhiza formation and plant data were analysed separately by a two-way (block, bacterial treatment) analysis of variance (ANOVA). Analysis of the mycorrhiza architecture data was carried out using ANOVA and canonical variate analysis (CVA). Significance of treatment effects were determined at the probability level of 0.05 using Genstat 7.1 (VSN International).

Results

Mycorrhizal status of the seedlings

In the *L. bicolor* experiment (1:40 fungal dose), *L. bicolor* mycorrhiza formation in the control treatment was 45.5% (Fig. 2). Bacterial treatments BBc6R8 and EJP73 significantly ($P < 0.05$) increased mycorrhiza formation by *L. bicolor*. The mycorrhizal indices for these bacteria were 62.7 and 60.6%, respectively. The other MHB, EJP67, EJP109 and MB3 had no significant effect on mycorrhiza formation by *L. bicolor*.

Fig. 2 Effect of bacterial treatment on the ectomycorrhiza formation in root systems of *Pinus sylvestris* by *Laccaria bicolor* 3 months after inoculation. Short roots indicated as: colonised by *L. bicolor* (black), non-mycorrhizal (grey), *Tomentella* morphotype (blank), or other (hatching). Asterisks indicate values significantly different (l.s.d. 5%) from the control. Analyses are based on six replicates in each of three blocks

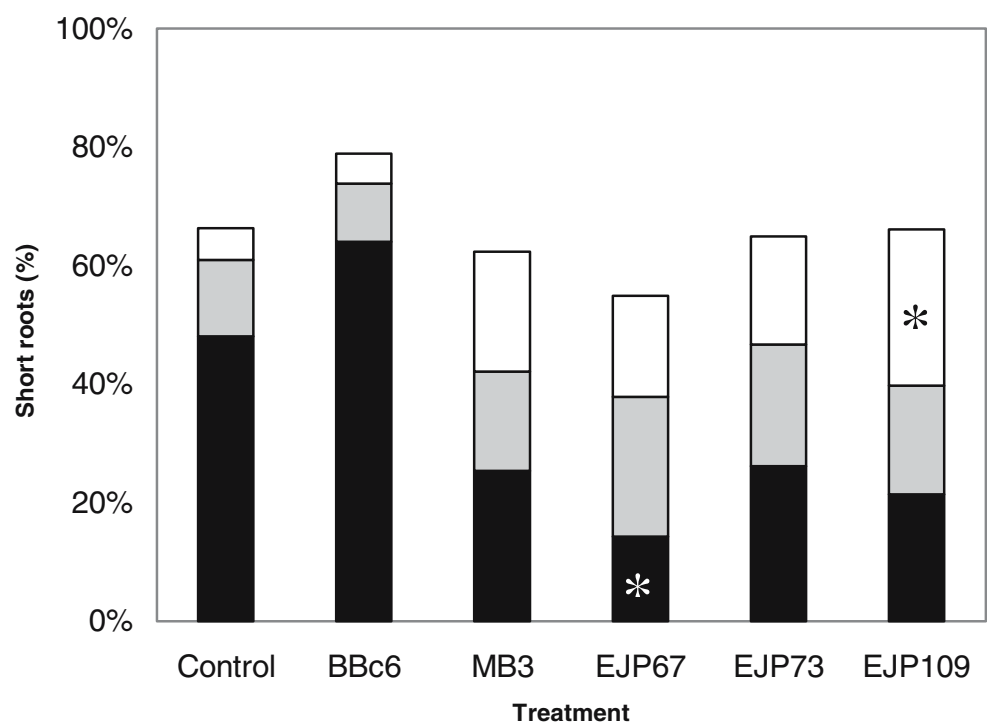


For *L. rufus* at the same 1:40 fungal dose, all bacterial treatments had no significant effect on *L. rufus* mycorrhiza formation except EJP67, which significantly decreased mycorrhiza formation by *L. rufus* to 14.3% compared to 48.1% in the control treatment (Fig. 3). At the 1:10 fungal dose (data not shown), none of the MHB had a significant effect on *L. rufus* mycorrhiza formation, which was 56.5% in the control treatment (48.1% at the 1:40 fungal dose).

Aside from determining the effect of MHB on mycorrhiza formation by the inoculated fungus (*L. rufus* and *L.*

bicolor), we also assessed the effect of MHB on the number of ectomycorrhiza formed by non-inoculated fungi originating from the glasshouse environment. These were grouped as either *Tomentella* morphotype or other. EJP109 was the only MHB to have significant effects ($P < 0.05$) on these two types of ectomycorrhiza. In the *L. bicolor* treatment (Fig. 2), EJP109 significantly ($P < 0.05$) decreased the percentage of other mycorrhiza from 13.5% in the control to 7.7%. In contrast in the *L. rufus* treatment (Fig. 3), EJP109 significantly ($P < 0.05$) increased *Tomen-*

Fig. 3 Effect of bacterial treatment on ectomycorrhiza formation in root systems of *P. sylvestris* by *Laccaria rufus* 3 months after inoculation. Short roots indicated as: colonised by *L. rufus* (black), non-mycorrhizal (grey), *Tomentella* morphotype (blank), or other (hatching). Asterisks indicate values significantly different (l.s.d. 5%) from the control. Analyses are based on six replicates in each of three blocks



tella mycorrhiza from 5.3% in the control to 26.3%. During scoring of mycorrhizal roots, it became apparent that root architecture, particularly the extent of dichotomous branching, appeared altered in *L. rufus* treatments inoculated with EJP67 and EJP73 relative to the other treatments, although quantification of effects was not possible.

Effect of MHB on dichotomous branching of pine short roots

Mycorrhiza formation by *L. rufus* and *L. bicolor* was increased in all treatments at the 1:10 dose, and the bacterial treatments had no significant effect on the percentage of mycorrhiza formation by inoculated or other fungi. However, since earlier analysis of the 1:40 dose treatments had indicated that EJP73 and EJP67 had changed mycorrhiza structure, all treatments at the 1:10 dose were further analysed for effects on dichotomous branching.

The extent of dichotomous branching in the *L. rufus* treatment at the 1:10 fungal dose was analysed using CVA. CVA of the four *L. rufus* mycorrhiza architecture types showed that EJP67 and EJP73 were significantly different from the control and other MHB treatments, but similar to each other (Fig. 4a). By removing one of the mycorrhiza architectures in turn and carrying out CVA, we found that the pattern was preserved when either first or fourth order mycorrhiza were removed (Fig. 4b,e). Removing either second or third order mycorrhiza resulted in a loss of this pattern (Fig. 4c,d) indicating that second and third order mycorrhiza were together responsible for the observed difference.

Effect of MHB and ectomycorrhizal fungi on growth and development of *P. sylvestris*

The addition of *L. bicolor* significantly changed shoot height ($P<0.05$), which increased from an average of 30.9 mm in the control to 36.1 mm. The bacterial treatments had no additional effect to *L. bicolor* on shoot height. Inoculation with *L. bicolor* alone or in combination with the bacteria had no significant effect on shoot, root or whole plant dry weight, or root to shoot ratio relative to uninoculated plants.

Shoot height significantly increased from an average of 30.9 to 34.20 mm after inoculation with *L. rufus* ($P<0.05$). However, the bacterial treatments had no additional effect on plant height to that of *L. rufus*. Inoculation with *L. rufus* significantly reduced ($P<0.05$) shoot dry weight, from an average of 71.2 to 64.3 mg. When *L. rufus* was inoculated with the bacterial strains MB3, EJP67 and EJP73, the inhibition of shoot growth was the same as when the fungus was inoculated alone. However, when BBc6R8 and EJP109 were inoculated, *L. rufus* had no effect on shoot growth

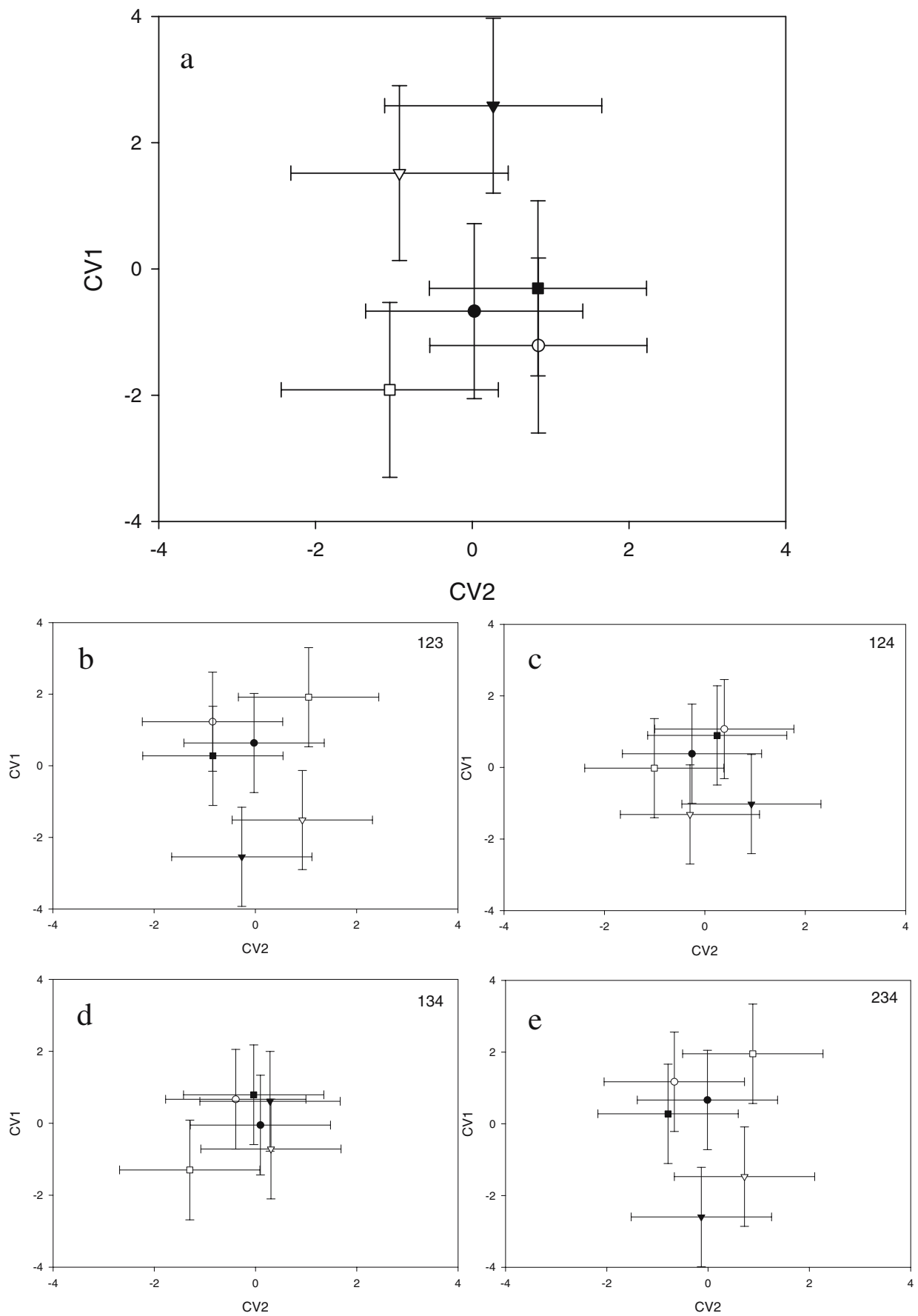
relative to the uninoculated control. Inoculation with *L. rufus* significantly reduced ($P<0.05$) root growth from an average of 80.9 to 61.6 mg. When inoculated with BBc6R8, MB3 and EJP67, the inhibition of root growth caused by *L. rufus* was the same as when the fungus was inoculated alone. However, inoculation of EJP73 and EJP109 significantly increased ($P<0.05$) root dry weight relative to inoculation of the fungus alone, with average root weights of 71.2 and 72.5 mg recorded, respectively. Total plant dry weight was reduced from an average of 152.1 to 125.9 mg after inoculation with *L. rufus*. The bacterial treatments inhibited plant dry weight to the same extent as the fungus alone, with the exception of EJP109 in which the whole plant dry weight was significantly higher (139.5 mg) than when the fungus was inoculated alone. Compared to the uninoculated control, *L. rufus* significantly reduced ($P<0.05$) the root/shoot ratio from 1.16 to 0.96. When *L. rufus* was inoculated with BBc6R8 and MB3, there was no additional effect to the fungus alone. However, the root/shoot ratios in treatments receiving EJP67, EJP73 and EJP109 were 1.14, 1.11 and 1.09, respectively, which were not significantly different to the uninoculated control, but were significantly higher ($P<0.05$) than when the fungus was inoculated alone.

In vitro production of indole-3-acetic acid by bacterial strains

Strains EJP109, BBc6R8 and MB3 were found to produce IAA in vitro (formation of a red halo around bacterial spot), but EJP73 and EJP67 did not produce IAA (no halo formed).

Discussion

Bacterial strains previously isolated from *L. bicolor* (BBc6R8 and MB3) were originally characterised as MHB under glasshouse and nursery conditions, whereas those isolated from *L. rufus* and *S. luteus* symbioses (EJP67, EJP73 and EJP109) were originally characterised as MHB using a microcosm system. In our experiment, both bacterial strains BBc6R8 and EJP73 significantly enhanced *L. bicolor* mycorrhiza formation. This shows that EJP73 is an MHB in symbioses involving *L. bicolor* under glasshouse conditions and, as demonstrated previously, for *L. rufus* using gnotobiotic microcosms (Poole et al. 2001). The fact that the MHB strain MB3 had no significant effect on *L. bicolor* mycorrhiza formation in this particular experiment is perhaps due to bacterial dose effect. The strain MB3 has been previously shown to inhibit fungal colonisation at high bacterial inoculum doses (Duponnois 1992).



◀ **Fig. 4** Canonical multivariate analysis showing effect of MHB treatment on *Lactarius rufus* mycorrhiza dichotomous short root branching patterns. **a** Analysis of patterns 1–4, **b–e** patterns analysed indicated in top right of figure. CV1 and CV2 accounted for **a** 79.9 and 18.8%, **b** 80.5 and 19.3%, **c** 68.2 and 31.3%, **d** 86.2 and 9.6%, and **e** 85.1 and 13.9% variability, respectively. Bars represent 95% confidence limits [shaded circle BBc6R8, white circle EJP109, shaded inverted triangle EJP67, white inverted triangle EJP73, shaded square MB3, white square control (fungus alone)]

In the *L. rufus* experiment, the strain EJP67 had a significant negative effect on *L. rufus* mycorrhiza formation. As the strain EJP67 was characterised as an MHB for the *P. sylvestris*–*L. rufus* symbiosis using a laboratory microcosm bioassay, it suggests that EJP67 was unable to produce the helper effect under glasshouse conditions. However, it is known that this particular strain operates within a narrow dose range, at least in microcosms (Aspray et al. 2006), and may have proliferated in the glasshouse experiment to a concentration that was detrimental to the symbiosis. Strain EJP73, another MHB of the *P. sylvestris*–*L. rufus* symbiosis in microcosms, was also unable to produce the helper effect for this symbiosis under glasshouse conditions, whereas it produced a helper effect on the *P. sylvestris*–*L. bicolor* symbiosis. Therefore, the lack of helper effect on *L. rufus*, by strains EJP73 and EJP67, may be due to a difference in behaviour of the fungus and/or bacteria in the glasshouse system. It is also possible that the discrepancy results from different mechanisms being dominant depending on the culture system. For example, the effect of the bacteria on the survival and/or the growth of the fungus during its presymbiotic life cannot be tested in the microcosms where the fungus is inoculated in an already established root system. This is the first time that *L. rufus*–MHB interactions have been studied under glasshouse conditions; therefore, an optimal fungal dose may need to be determined to detect *L. rufus* helper effects.

As *L. rufus*–MHB interactions have not previously been studied in the glasshouse bioassay, we chose to study two fungal doses (1:40 and 1:10) to determine their effect on MHB interactions. At the 1:40 dose, we observed stimulated mycorrhiza formation by BBc6R8 over the control. In addition, we were able to detect a significant negative effect for EJP67 on mycorrhiza formation. In contrast, at the 1:10 dose, *L. rufus* efficiently colonised *P. sylvestris* and MHB induced no significant negative or positive effects. Using the lower fungal dose (1:40) and either BBc6R8 or EJP73, we were able to obtain a higher mycorrhizal index than using the higher fungal dose (1:10) and no bacterial inoculation. This result supports previous work where *L. bicolor* was inoculated in a nursery experiment at different doses. At the lower fungal dose, the addition of BBc6R8 produced a mycorrhizal index comparable with the one obtained in the control treatment with the higher fungal

dose (Frey-Klett et al. 1999). These authors state that this is beneficial for using MHB for controlled mycorrhization of forest planting stocks since bacterial cultures are easier and faster to produce than ectomycorrhizal fungi.

Several previous studies have shown that MHB have fungus specificity (Duponnois et al. 1993; Dunstan et al. 1998). In our experiment, the strain EJP73 was found to significantly enhance *L. bicolor* mycorrhiza formation, illustrating the lack of fungal specificity of this strain. The lack of specificity observed supports previous work where *P. monteilii* HR13 enhanced mycorrhiza formation for *Pisolithus* and *Scleroderma* strains (Duponnois and Plenchette 2003). Interestingly though, EJP73 is a *Paenibacillus* sp. showing that Gram-positive and Gram-negative MHB can lack fungus specificity. In our work, enhanced mycorrhiza formation was also observed when EJP109, isolated from *S. luteus* mycorrhiza, was inoculated in the *L. rufus* experiment. However, EJP109 enhanced mycorrhiza formation by a *Tomentella* morphotype, an environmental strain present in the glasshouse, and not *L. rufus* itself. Duponnois et al. (1993) recorded a negative effect of MHB *Pseudomonas* sp. SBc5 and *P. fluorescens* BBc6 on a ‘contaminant’ white fungus in nursery experiments. Our results indicate that in a non-sterile environment containing complex ectomycorrhizal communities, the presence of MHB with low specificity could have unpredictable consequences for the composition of ectomycorrhizal fungal communities colonising roots and plant growth.

The morphology of mycorrhizal roots is known to be dramatically different from that of non-mycorrhizal roots (Linderman 1988). Both arbuscular mycorrhizal and ectomycorrhizal fungi have been shown to alter root architecture (Norman et al. 1996; Sen 2001). Ectomycorrhizal roots, particularly those of *Pinus* spp., are characterised by a lack of root hairs, thick mantle and extensive dichotomous branching of short roots. The ability of MHB to change the extent of dichotomous branching of roots has not been previously reported. Our observations on the *L. rufus* experiment indicate that the effects on mycorrhiza architecture were fungus-specific as only the strains EJP67 and EJP73, MHB isolated from the *P. sylvestris*–*L. rufus* symbiosis, changed the extent of dichotomous branching. Phytohormones, including auxins, cytokinin and ethylene have been implicated in producing morphological changes of roots during mycorrhiza formation (Slankis 1973; Kaska et al. 1999), including the formation of lateral roots and dichotomous branching of short roots (Barker and Tagu 2000). Although dichotomous short root branching can occur spontaneously in some *Pinus* sp. (Kaska et al. 1999), the regulation of auxin concentration and distribution in the mycorrhiza appears to be important for inducing changes to dichotomous branching of short roots (Gay et al. 1994). As many bacteria produce auxins (Costacurta and Vanderleyden

1995), this suggests a mechanism by which MHB could alter dichotomous short root branching. However, our data indicates that auxin is not involved in the induction of dichotomous short root branching by EJP73 and EJP67, suggesting an alternative mechanism was responsible.

To conclude, our work demonstrates that not all MHB–fungal interactions are specific when the helper effect is considered to be the enhancement of mycorrhiza formation. In which case, bacteria isolated from outside the mycorrhizosphere may have the ability to enhance mycorrhiza formation. However, we have found that with *P. sylvestris*, MHB can induce subtle changes in mycorrhiza architecture, particularly changes to dichotomous short root branching, which show fungus specificity. Therefore, MHB ‘helper effects’ should be clearly defined in future experiments as several parameters emerge to determine them. The non-specificity of MHB on mycorrhiza formation but specific effects on mycorrhiza architecture also suggests that different mechanisms operate for different MHB. Genomic approaches, which have recently been used to study specific genes involved in MHB interactions, could prove valuable in elucidating mechanisms by which different MHB operate (Schrey et al. 2005; Frey-Klett and Garbaye 2005).

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