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A Possible Role of Oxalate Produced in the Symbiotic Culture System with a Host Plant Pinus densiflora and a Mycorrhizal Fungus Lactarius hatsudake*1

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Introduction
Mycorrhizal fungi in mycorrhizae of terrestrial plants are believed to play an important role in taking up water and nutrients such as nitrogen, phosphorus, minerals from soil1). As a possible mechanism facilitating phosphorus uptake by the host plant, oxalate secreted by ectomycorrhizal fungi has been proposed to liberate phosphate from fixed phosphate in calcium and aluminum precipitates2,3). However, the growth promotion of woody plants by the addition of oxalate has not been reported. Furthermore, few reports have been published on the production of organic acids in an axenic symbiotic culture of the host plant with the ectomycorrhizal fungi4,5).

We have established the symbiotic system of Pinus densiflora associated with Lactarius hatsudake in vermiculite culture. A possible role of oxalate is discussed in relation to symbiotic interactions between P. densiflora and L. hatsudake.

Materials and Methods
Pinus densiflora seedlings were transplanted into the culture of L. hatsudake in vermiculite culture system containing glucose (1%) and yeast extract (0.1%), and was grown at 21°C under 16 hr-light (3,000 lx) and 8 hr-dark conditions. The organic acids extracted according to the modified method of Yoshida et al.6) were analyzed by GC-MS. The effect of oxalate on the growth of P. densiflora was investigated.

Results and Discussion
Ectomycorrhizae were synthesized on the roots of P. densiflora associated with L. hatsudake during cultivation of 82 days. As a result, a thick mantle and a well-developed epidermal Hartig net were observed.

Compared with non-inoculated seedlings as a control, seedlings inoculated with L. hatsudake showed significant increase in total dry weight (150%), seminal root length (150%) and the number of lateral roots longer than 10 mm (160%) (Table 1). The extent of the growth enhancement recognized in the present investigation was almost similar to that previously reported for Sitka spruce and Japanese Larch inoculated with Paxillus involutus or Suillus grevillei7), and for Pinus wallichiana and P. densiflora inoculated with Suillus granulatus8).

The amounts of organic acids accumulated in the three different cultures, i.e. free-living fungal culture, symbiotic culture of P. densiflora associated with L. hatsudake, and non-inoculated control P. densiflora culture were compared. Lactate, oxalate, succinate, malate and citrate were identified in the symbiotic culture. Oxalate accumulated as the major organic acid in both free-living fungal culture and symbiotic culture. However, there was no statistically significant difference in the amounts of oxalate

Table 1. Changes in root length and dry weight of Pinus densiflora associated with and without (control) L. hatsudake during cultivation.

<table>
<thead>
<tr>
<th>Culture days</th>
<th>Conditions</th>
<th>Length (mm)</th>
<th>Number of lateral root longer than 10 mm</th>
<th>Dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Seminal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Control</td>
<td>29.2±5.8</td>
<td>147.7±15.7</td>
<td>4.5±1.7</td>
</tr>
<tr>
<td></td>
<td>Symbiotic</td>
<td>31.9±6.5**</td>
<td>221.4±45.2*</td>
<td>7.4±2.2*</td>
</tr>
</tbody>
</table>

The significance of differences between means of treatments and controls (n=5) was tested by Student's t-test. The author repeated the experiment three times. Similar results were always observed. *: P<0.05; **: P<0.01; NS: not significant.

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accumulated between free-living and symbiotic cultures. Thus, it is necessary to compare the amounts of oxalate accumulated near the ectomycorrhizae and the other parts in the same vermiculite culture in order to elucidate the effect of ectomycorrhizal symbiosis on the production of organic acids. However, more than 100 times the amount of oxalate accumulated in symbiotic culture compared to the non-inoculated *P. densiflora* culture. Furthermore, the seminal root length and dry weight of *P. densiflora* were enhanced by 160% and 150%, respectively, by the addition of oxalate (0.3 mM) whose concentration was almost the same as that determined in the symbiotic culture. Thus, the results suggest that oxalate produced by the fungi was involved in the growth promotion of *P. densiflora* in the late stage during symbiotic cultivation.

Further research is needed to elucidate the mechanism for oxalate biosynthesis and the role of organic acid in the symbiosis between woody plants and ectomycorrhizal fungi.

References