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## Spore germination in *Hebeloma* stimulated by living plant roots[1]

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*Summary.* Basidiospores from 14 strains of *Hebeloma* (Agaricales) representing 5 groups of mycorrhiza-forming species were tested for germination on a nutrient agar medium. Germination occurred in 13 strains but never exceeded 0.1%. A 10-fold increase or more in germination percentage was obtained in 4 out of 7 tested spore collections only by placing the growing root of a pine seedling among the spores on the agar medium.

Basidiospores of mycorrhiza-forming Homobasidiomycetes generally do not germinate on ordinary agar media but require special conditions for germination[4]. However, in the genus *Hebeloma* (Cortinariaceae, Agaricales) where most species form ectomycorrhiza with trees, spore germination occurs readily within the section Denudata, subsection C, as was demonstrated by Bruchet[5]. In other sections of *Hebeloma* he observed no, or merely a very sparse and slow, germination.

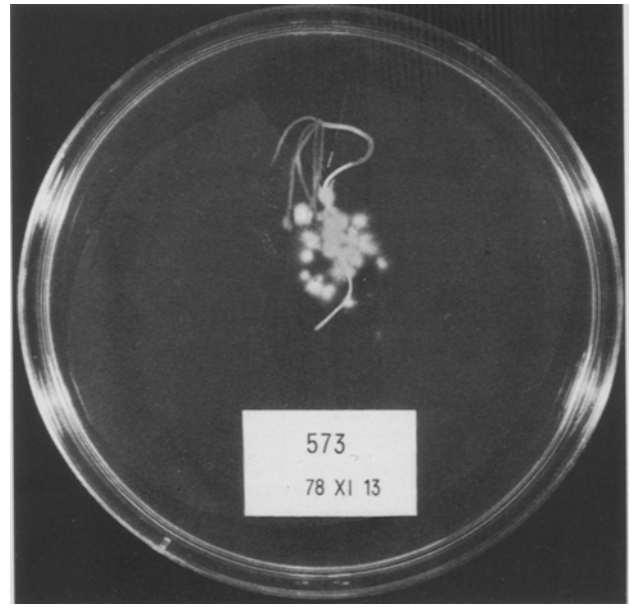
It was thought that increased knowledge of the conditions for spore germination in *Hebeloma* might also contribute to the understanding of the spore germination mechanism in other, chiefly micorrhiza-forming genera, e.g. *Inocybe* and *Cortinarius,* of the family Cortinariaceae, in which spore germination in vitro has not yet been achieved. In some other Homobasidiomycetes the germination rate could be increased by using *Rhodotorula* yeast as an activator organism and/or by adding activated charcoal[4]. Therefore these measures and some others were tested for their efficiency in improving the germination of spores from a number of *Hebeloma* species.

14 spore collections from fruit-bodies of *Hebeloma* were obtained in the autumn of 1978 and preserved in petri dishes under sterile conditions in darkness at 4 °C. Most of them came from habitats in the neighbourhood of Uppsala, Sweden. After spore-casting, the fruit-bodies were dried and sent to Dr G. Bruchet, Lyon, for determination. All of them could be identified as to section, subsection and stirpes, but some could not be given a definite species name because of the inadequacy of the material. However, the collections comprised at least 5 different species representing as many subsections or stirpes.

The spore germination tests were made on plates of a semisynthetic nutrient agar medium[4]. About 0.1 ml of a suspension containing 500,000–1,000,000 spores per ml was spread over the agar surface by means of a glass rod. In some experimental series a colony of *Rhodotorula glutinis* (Fres.) Harrison was inoculated onto each plate, or activated charcoal was dusted over half of the agar surface. The plates were sealed with parafilm and incubated in darkness at 25 °C. Because of the generally very sparse and varying germination, which did not take place simultaneously, no efforts were made to estimate the percentage germination exactly.

Spores of all tested collections, except No. 568, germinated at least in 1 experiment (cf. table). The first germinations could usually be observed only after 2 or 3 weeks. New ones then gradually appeared, even after an incubation time of up to 5 months. In all cases the percentage germination was below 0.1%. In certain spore collections, e.g., in Nos. 1 and 569, many of the small mycelia formed from the germinated spore died for unknown reasons before they had reached a size visible to the naked eye.

None of the 14 tested strains reacted positively to *Rhodotorula,* which confirms the results of Bruchet[5] gained with the




A pine seedling growing on an agar plate, the entire surface of which is evenly sown with spores of *Hebeloma mesophaeum,* strain No. 573. A thin cellophane foil covers almost the whole agar surface including the root of the seedling. The culture was incubated for 4 weeks by daylight at about 20 °C. Germinations have occurred chiefly close to the root. They have given rise to mycelia visible to the naked eye after 2–3 weeks.

Results of germination experiments with spores of *Hebeloma*

| Taxonomical unit[a] and isolation number | | Species to which the strain is related | Germination[b] Early tests without roots[c] | Later tests in presence of roots[d] |
|---|---|---|---|---|
| Section Denudata | | | | |
| Sub-section A | | | | |
| Stirpes crustuliniforme | 1 | *H. crustuliniforme* | + | |
| | 3 | *H. crustuliniforme* | + + | 3 |
| | 551 | *H. crustuliniforme* | +[e] – – | 1[e] |
| | 553 | *H. crustuliniforme* | + | |
| | 603 | *H. crustuliniforme* | + | |
| Stirpes pusillum | 568 | *H. pusillum* | – | |
| Stirpes alpinum | 569 | *H. ingratum* | + + | 2,2,2,3,3 |
| | 576 | *H. ingratum* | + | |
| | 613 | *H. ingratum* | + | 1,1 |
| Sub-section C | | | | |
| | 506 | *H. edurum* | + | 1,1 |
| Section Hebeloma | | | | |
| | 552 | *H. mesophaeum* | + | |
| | 570 | *H. mesophaeum* | + | 3,3 |
| | 573 | *H. mesophaeum* | + | 2,2 |
| | 586 | ? | + | |

[a] According to Bruchet[5]; [b] for each strain the germination tests are recorded in time order, i.e. the age of each stored spore collection increases from left to right; [c] –, no germination; +, germination; [d] 1, germination but no root effect; 2, germination stimulated by the root; 3, germination only in the presence of the root; [e] only a few germinations among a million spores.

same technique. *Rhodotorula* and activated charcoal combinded proved to inhibit rather than stimulate germination in some species. The presence of a *Hebeloma* mycelium among the spores of the same strain did not affect germination, in contrast to the mode of response in, e.g., *Leccinum* species and *Paxillus involutus*[4].

Earlier observations by Melin[6] in, e.g., *Boletus, Amanita, Russula,* and *Lactarius,* as well as by Fries and Birraux (unpublished) in *Thelephora terrestris* indicated that exudates from plant roots may stimulate spore germination in some mycorrhiza-forming fungi. Similar findings by Heinemann and Gaie[7] in *Russula,* although cautiously interpreted, may point in the same direction. The fact that mycorrhiza formation has sometimes been found to occur when spore suspensions have been added to axenically grown tree seedlings also suggested that living roots may induce spore germination under appropriate conditions[8,9]. This possibility was investigated by placing 15-day-old pine seedlings (*Pinus silvestris* L.) on agar plates with *Hebeloma* spores. Although most of the *Hebeloma* species tested here prefer deciduous trees to conifers in nature, the capacity for forming mycorrhiza with pine seems to be common within this genus[10]. The axenically grown pine seedlings were placed either in their entirety on the agar plate in the petri dish (⌀8 cm), 1 per plate, or with only the root system on the agar plate, the shoot protruding through a hole drilled in the wall of the petri dish. The root system and the surrounding spores were covered by a thin cellophane foil. Spores from 7 collections were used in these experiments. They had been stored for 5–10 weeks and their germination power on the nutrient agar medium, as just described, was on the decline.

The table shows that in 4 of these 7 spore collections germination was improved by the presence of a living pine seedling root. These 4 strains belonged to the species (or species groups) of *H. crustuliniforme, H. ingratum,* and *H. mesophaeum.* As can be seen in the figure, the germinations in certain cases started close to the upper (proximal) part of the root and then spread over the agar surface. The percentage germination was at least 10 times higher than in the best earlier experiments without roots. The observed effect suggests that one or more substances which stimulate spore germination are being exuded from the root surface. It should be noted that this strong germination stimulation also occurred in cases (strains No. 3, 569 and 570), where the spores had apparently lost their ability to germinate alone on the agar medium after a long storage period.

The inborn prerequisites for spore germination differ considerably from one group of Homobasidiomycetes to another. The *Hebeloma* basidiospore shares the very sparse and slow germination with other mycorrhiza-forming genera, but is distinguished by its insensitivity to inhibiting compounds in the agar, lack of response to *Rhodotorula* and, in some species, a positive response to living plant roots.

The ecological implications of the root effect are obvious. For a fungus, which must form a mycorrhizal symbiosis with the roots of a tree in order to accomplish its life cycle, a spore germination reaction like the one now described in some of the *Hebeloma* species, ought to increase the survival chances of the species in question. The degree of specificity of the reaction remains to be elucidated.

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