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FULL PAPER

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# Effects of pH, $NH_4$-N concentration, temperature, and storage period on basidiospore germination in an ectomycorrhizal ammonia fungus *Hebeloma vinosophyllum*

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**Abstract** Basidiospore germination in an ectomycorrhizal ammonia fungus *Hebeloma vinosophyllum* was stimulated by 10–500 mM $NH_4Cl$ aqueous solution at pH 4.5–9.0, but not by pure water. The basidiospores germinated at 10°–35°C with an optimum at 25°–30°C. The highest germination percentage (83.0%) was observed in 100 mM $NH_4Cl$ aqueous solution adjusted to pH 8.0 by KOH, when the basidiospores were incubated at a density of $10^6$ spores/ml at 30°C for 14 days. The percent germination value decreased with the increased duration of storage under both dry and wet conditions. Humidity and temperature affected the longevity of *H. vinosophyllum* basidiospores. The basidiospores maintained their germination ability longer under a dry condition than under a wet condition. The greatest longevity was accomplished by storage at 15°C under a dry condition.

**Key words** Ammonia fungi · $NH_4$-N concentration · pH · Spore germination · Spore longevity

## Introduction

"Ammonia fungi" are defined as a chemoecological group of fungi that sequentially develop reproductive structures exclusively or relatively luxuriantly on soil after a sudden addition of ammonia, and/or other nitrogenous materials that react as bases by themselves or on decomposition, or alkalis (Sagara 1975). According to the sequential occurrence of ammonia fungi in the field, they are divided into two types, early-phase fungi (EP fungi) and late-phase fungi (LP fungi). The former type species are saprobic fungi and most of the latter type species are ectomycorrhizal fungi (Sagara 1995).

The invasion sequence of the saprobic ammonia fungi (EP fungi) was examined by the cultivation of packed soils, which had been collected from the forest floor at different days after urea application, in sterilized test tubes with cotton plugs (Suzuki et al. 2002). However, the invasion sequence of ectomycorrhizal ammonia fungi has not been examined by this method, because the ectomycorrhizal ammonia fungi never form fruit bodies by urea-treated soil cultivation without their host plant(s) (Suzuki et al. 2002).

Suzuki et al. (1982) found that basidiospore germination in the EP fungi *Coprinopsis cinerea* and *C. phlyctidospora* was stimulated by the presence of $NH_4$-N under alkaline to slightly acidic conditions. Suzuki (1978) found that basidiospore germination in the LP fungus *Hebeloma vinosophyllum* was also stimulated by 0.1–100 mM aqua ammonia. Later, it was confirmed that the spore germination of the three EP fungi, namely, the conidium germination of *Amblyosporium botrytis*, ascospore germination of *Ascobolus denudatus*, and basidiospore germination of *C. phlyctidospora*, were markedly stimulated by the water extract of soil obtained 6 days after urea application. Moreover, it was revealed that basidiospore germination in the LP fungi *H. spoliatum* and *H. vinosophyllum* was also markedly stimulated by a water extract of the urea-treated soil (Suzuki 2006). These results suggest that the spore germination of ammonia fungi would be stimulated by the presence of $NH_4$-N under weak alkaline to weak acidic conditions.

The appearance of reproductive structures of ammonia fungi results either from mycelial growth that prefers or tolerates high concentrations of $NH_4$-N under a weak alkaline to a weak acidic condition, and/or from spore germination that is stimulated by $NH_4$-N under the same conditions (Suzuki 1989, 2006; Yamanaka 1999, 2003; Suzuki et al. 2002; Licyayo and Suzuki 2006).

The latent form(s) of ammonia fungi in the field has not yet been fully examined, but Sagara (1976) speculated that the spores and/or the fragment of the hyphae would be principal propagules for their rapid colonization. We have some preliminary data about the spatial distribution of

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living propagules of several EP fungi in the field (Suzuki et al. 2002; Suzuki 2006) and the spore longevity of the LP fungus *H. spoliatum* (Suzuki 2003). However, we do not yet have enough data to elucidate the propagation mechanism of each ammonia fungus. Therefore, it may be valuable to examine the longevity of spores in various environmental conditions and the effects of different environmental factors such as pH, $NH_4$-N concentration, and temperature, which would be principal factors for their colonization in the field, on spore germination to elucidate the colonizing mechanism of ammonia fungi.

With this background, as the first step to elucidate the colonization mechanism of ammonia fungi, we examined the longevity of basidiospores of *H. vinosophyllum* as well as its basidiospore germination under different environmental conditions such as pH, $NH_4$-N concentration, and temperature.

## Materials and methods

### Organism

Stock culture of a dikaryotic isolate of *Hebeloma vinosophyllum* Hongo (isolate CHU Kiyosumi) was used in this study. The stock culture had been isolated from a *Quercus*- and *Castanopsis*-dominated forest in Chiba, Japan (Licyayo and Suzuki 2006).

### Preparation of basidiospores

The stock culture was inoculated on MY agar slant [10 g malt extract (Difco), 2 g yeast extract (Difco), 15 g agar (Nacalai Tesque), 1000 ml pure water] and the slants, placed upright, were incubated at 25° ± 0.5°C in the dark until fruit bodies were formed. Then, a piece of sterilized filter paper (no. 1, Advantec) was placed on the slant facing the pilei. Basidiospores were obtained aseptically from the filter paper having spore prints. Only the basidiospores discharged in the first 3 days were used for this study. Then, 20 µl of the spore suspension was centrifuged at 500 rpm for 5 min at 5°C, and the supernatant solution was decanted. After addition of another 50 ml of sterilized pure water, the spore suspension was again centrifuged and the supernatant decanted. This procedure was repeated three times. Finally, the water was removed and basidiospores that precipitated in the bottom of a centrifugation tube were collected for the following experiments. The basidiospores used for storage experiments were not rinsed with pure water.

### Cultivation and sampling methods for spore suspension

The aqueous solution of $NH_4Cl$ for culturing basidiospores was sterilized by filtration (acetate cellulose, 0.2 µm pore size; Advantec). The basidiospores were suspended in this solution. As a negative control, the basidiospores were suspended into pure water sterilized by the membrane filter filtration. Then, 20 µl of each suspension was poured into a sterile glass bottle (4 cm in diameter, 6.5 cm in height) sealed with a screw cap with silicon rubber for incubation. We designated various environmental conditions for this study.

For the examination of spore density, an aqueous solution containing 100 mM $NH_4Cl$ was adjusted to pH 8.0 with KOH and the rinsed basidiospores were suspended at different spore densities ($5 \times 10^5$ to $2 \times 10^7$ spores/ml). Spore densities were determined with a hemocytometer by the dilution method. The basidiospore suspensions were incubated for 14 days in the dark at different temperatures from 5° to 30°C at 5°C intervals. The precision of each designated temperature was ±0.5°C.

For the examination of $NH_4$-N concentration, an $NH_4Cl$ aqueous solution was adjusted at various concentrations (10–1000 mM) to pH 8.0 with KOH using a glass electrode. The rinsed basidiospores were suspended at a spore density of $10^6$ to $2 \times 10^6$ spores/ml. The basidiospore suspensions were incubated for 14 days in the dark at temperatures from 5° to 30°C at 5°C intervals. The precision of each designated temperature was ±0.5°C. After the end of the incubation, the final $NH_4$-N concentration of suspensions was measured by HPLC (Licyayo and Suzuki 2006) and the final pH was measured with a glass electrode. As a positive control, the rinsed basidiospores were also suspended in a 50 mM aqueous solution of $(NH_4)_2HPO_4$ (pH 8.0) at the density of $10^6$ to $2 \times 10^6$ spores/ml. The spore suspension was incubated for 14 days in the dark at 25°C.

For the examination of pH, the aqueous solution containing 100 mM $NH_4Cl$ was adjusted to a variety of pHs (pH 3.0–9.5) with KOH, NaOH, or aqua ammonia for pH above 6.0, and with HCl or $H_2SO_4$ for pH below 5.0. The precision of each designated pH was ±0.05. The rinsed basidiospores were suspended at a spore density of $10^6$ to $2 \times 10^6$ spores/ml, and incubated at 25° ± 0.5°C in the dark for 12 days. After the end of incubation, the final pH of the suspensions was measured with a glass electrode.

For the examination of temperature, the rinsed basidiospores, suspended at a spore density of $10^6$ to $2 \times 10^6$ spores/ml in 100 mM $NH_4Cl$ aqueous solution at pH 8.0, were incubated at various temperatures from 5° to 40°C at 5°C intervals in the dark for 14 days. The precision of each designated temperature was ±0.5°C. After the end of incubation, the final pH of suspensions was measured with a glass electrode.

For the examination of spore longevity, the spore prints made on the sterilized filter paper (no. 1; Advantec) were placed in a large glass bottle (4 cm in diameter, 6.5 cm in height). A small glass bottle (1.5 cm in diameter, 4 cm in height) was put into the large bottle and fixed by filling the gap between the two bottles with paper. For the dry condition, the small bottle (inner bottle) was filled with silica gel as a desiccant. Containers were sterilized by dry heating at 140°C for 40 min. For the wet condition, after sterilization by dry heating, 8 ml sterilized pure water was poured into the small bottle. After placing the spore prints into the gap between the small and the large (outer) bottles, the large bottle was sealed with a silicon rubber cap sterilized by

autoclaving and stored at various temperatures from 5° to 30°C at 5°C intervals in the dark. During the storage period, a part of the nonrinsed spores (intact spores) were taken out at different sampling dates and then suspended at a spore density of $10^6$ to $2 \times 10^6$ spores/ml in 100 mM $NH_4Cl$ aqueous solutions adjusted to pH 8.0 with KOH. Thereafter, the spore suspensions were incubated for 12 days at 25°C in the dark. The precision of each designated temperature was ±0.5°C.

For sampling, just after the stirring of basidiospore germination, 0.5 ml of each suspension was removed for microscopic examination at different incubation periods. All procedures were done under aseptic conditions.

### Microscopic observation of spore germination

Germination of a basidiospore in the present study was defined as the protrusion of hypha(e) [germ tube(s)] discernible under a microscope. In each treatment, 100 spores were examined in random microscope fields to count the germination percentage. All results are shown as the average of three replications with standard error.

### Statistical analysis

All statistical analyses were performed by the Tukey–Kramer method.

## Results and discussion

### Effect of spore density on germination

The optimum density for the germination of this fungus was from $10^6$ to $2 \times 10^6$ spores/ml, under all temperature conditions (Fig. 1). The highest germination percentage (83.0%) was observed when the basidiospores were incubated at a density of $10^6$ spores/ml at 30°C for 14 days (Fig. 1). A high spore density may cause deficiency of nutrient supply and/or harmful secretion from the spores whereas a low spore density may be disadvantageous for the conditioning of the germination by spores themselves, e.g., the contact chance of each sexual spore. In the following experiments, we adjusted the spore density to the optimum, namely, $10^6$ to $2 \times 10^6$ spores/ml.

### Effect of $NH_4$-N concentration on germination

Basidiospore germination of *H. vinosophyllum* was stimulated by 10–500 mM $NH_4Cl$ aqueous solution, but not by 1000 mM $NH_4Cl$ aqueous solution adjusted to pH 8.0, irrespective of temperature conditions (10°–30°C) (Fig. 2). The highest germination percentage, 74.3%, was obtained in 100 mM $NH_4Cl$ solution at 30°C. The concentration of $NH_4$-N decreased with increasing germination percentage (Table 1, Fig. 2). The 50 mM $(NH_4)_2HPO_4$ aqueous solution (pH 8.0) also stimulated the basidiospore germination of

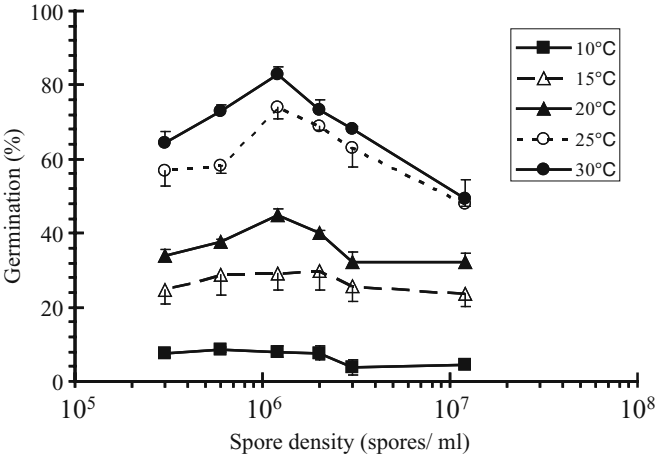


**Fig. 1.** Effect of spore densities on basidiospore germination of *Hebeloma vinosophyllum.* The basidiospores were suspended at various spore densities ($5 \times 10^5$ to $2 \times 10^7$ spores/ml) in 100 mM $NH_4Cl$ aqueous solution adjusted to pH 8.0 by KOH. The spore suspensions were incubated at 10°–30°C in the dark for 14 days. *Bar* is standard error

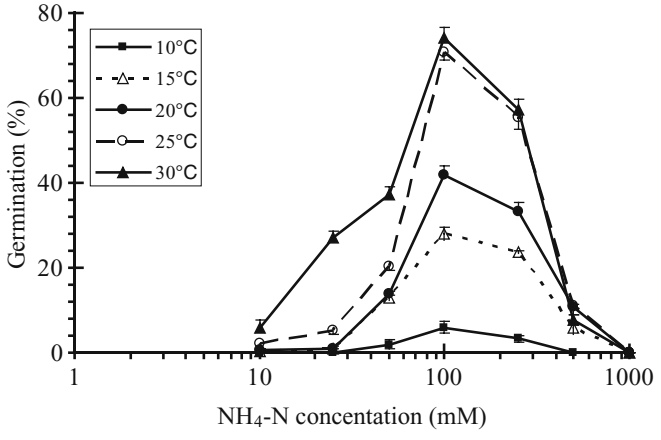


**Fig. 2.** Effect of $NH_4$-N concentrations on basidiospore germination of *Hebeloma vinosophyllum*. The basidiospores were suspended at a density of $10^6$ to $2 \times 10^6$ spores/ml in $NH_4Cl$ aqueous solution at 10–1000 mM. The spore suspensions were adjusted to pH 8.0 with KOH and incubated at 10°–30°C in the dark for 14 days. *Bar* is standard error

**Table 1.** Changes in pH and $NH_4$-N concentration of the medium for basidiospore suspension of *Hebeloma vinosophyllum* after a 14-day incubation

| Initial $NH_4$-N concentration (mM) | Final pH/Final $NH_4$-N concentration (mM) of the spore suspensions incubated at different temperatures (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 |
| 10 | 6.5/7 | 6.9/7 | 6.8/6 | 6.8/5 | 7.1/5 | 6.9/4 |
| 25 | 6.9/14 | 7.1/14 | 7.0/10 | 7.0/10 | 7.2/9 | 7.2/8 |
| 50 | 7.1/25 | 7.3/24 | 7.2/23 | 7.2/21 | 7.4/20 | 7.3/14 |
| 100 | 7.4/65 | 7.3/55 | 7.4/53 | 7.3/48 | 7.4/43 | 7.4/25 |
| 250 | 7.5/149 | 7.5/120 | 7.5/118 | 7.4/112 | 7.5/106 | 7.4/100 |
| 500 | 7.5/496 | 7.6/424 | 7.5/376 | 7.5/323 | 7.5/241 | 7.4/351 |
| 1000 | 7.7/886 | 7.7/946 | 7.6/818 | 7.7/837 | 7.9/758 | 7.5/608 |

The basidiospores were suspended ($10^6$ – $2 \times 10^6$ spores/ml) in $NH_4Cl$ aqueous solution at different $NH_4$-N concentrations (initial pH 8.0); the spore suspensions were incubated at different temperatures in the

*H. vinosophyllum* (80.7% by a 14-day incubation at 25°C in the dark). Suzuki (1978) reported that basidiospore germination of *H. vinosophyllum* was markedly stimulated by 0.5–5 mM aqua ammonia, but not in pure water. These results indicate that basidiospore germination of *H. vinosophyllum* is stimulated by $NH_4$-N, but not by chloride ions. The $NH_4$-N concentration markedly decreased at the $NH_4$-N concentration that gave a higher germination percentage, although the $NH_4$-N concentration decreased in all experiments (see Table 1). This result suggests that a part of the decrease in $NH_4$-N concentration may be caused by the volatilization of ammonia. Decline of $NH_4$-N concentration became more pronounced when the germination percentage became higher. This effect may be mainly derived from the absorption of $NH_4$-N by the basidiospore as a nitrogen source for germ tube growth and not simply as a germination-triggering substance.

## Effect of pH on basidiospore germination

Basidiospores germinated at pH 4.5–9.0 with the optimum at pH 8.0, in the presence of 100 mM (optimal concentration) of $NH_4Cl$ (Fig. 3). The highest germination percentage, 73.0%, was observed at pH 8.0 after a 12-day incubation at 25°C. There was no significant difference in percentage germination of the basidiospores among reagents used for pH adjustments (Fig. 3). The final pH values rose slightly in acid solution and decreased slightly in a neutral or basic solution (Table 2). Therefore, the actual optimum pH for spore germination of *H. vinosophyllum* should be slightly lower than pH 8 (Fig. 3, Table 2).

Basidiospore germination of EP fungi *C. cinerea* and *C. phlyctidospora* is stimulated by the presence of $NH_4$-N under alkaline to slightly acidic conditions (Suzuki et al. 1982). This finding suggests that the effect of pH on spore germination of LP fungi is similar to that on spore germination of EP fungi such as *Coprinopsis* spp. Suzuki (2006) confirmed that basidiospore germination of both *H. vinosophyllum* and *C. phlyctidospora* was stimulated by the water extract of the forest soil (820 mM $NH_4$-N, pH 9.2) collected 6 days after urea application. In red pine (*Pinus densiflora*) forest, the pH value of the forest floor after application of a large amount of urea increased up to about 8, associated with the increment of $NH_4$-N concentration up to about 1000 mM at the 7th day. Then, the $NH_4$-N concentration and pH decreased gradually, reaching about 10 mM and 4.5, respectively, at the time (about 190 days after the urea application) of the occurrence of *H. vinosophyllum*. The $NH_4$-N concentration and pH value of the urea-untreated soil of the forest are 3–7 mM and 3.6–4.4, respectively (Yamanaka 1995). The $NH_4$-N concentrations and pH values of the urea-treated soils of the mixed forests (*Abies firma* and *Quercus* sp(p).-dominated forests) sometimes reached above 1000 mM and 8.5, respectively, and then gradually declined to less than 100 mM and about 5.5, respectively, by the time of the occurrence of LP fungus. The $NH_4$-N concentrations and pH values of the urea-untreated soils of the mixed forests are 2–20 mM and 4.5–5.5, respectively (Suzuki 2000; Suzuki et al. 2002). The basidiospore germination of the EP fungus *C. phlyctidospora* is stimulated by 1–100 mM $(NH_4)_2HPO_4$ aqueous solution (pH 7.5–8.3), 0.01–10 mM aqua ammonia (pH 7.5–10.2), 0.1–1000 mM $NH_4Cl$ aqueous solution (pH 6.5–4.8), and a few other ammonium salts in aqueous solution (Suzuki et al. 1982). The upper limits concentration of $NH_4$-N for vegetative growth of *H. vinosophyllum* and *C. phlyctidospora* is 600 mM and the optima is 3 mM. They grow weakly even at 0.1 mM $NH_4$-N (Licyayo and Suzuki 2006). The vegetative mycelia of *H. vinosophyllum* grow at pH 3.0–8.0 and *C. phlyctidospora* at pH 5.0–9.0 (Yamanaka 2003). The optimal pHs for the vegetative growth of *H. vinosophyllum* and *C. phlyctidospora* are 5.0–7.0 and 6.0–7.0, respectively (Yamanaka 2003; Suzuki 2006). These data indicate that basidiospore germination of this fungus requires a remarkably high concentration of $NH_4$-N whereas vegetative growth of this fungus was tolerant to a markedly higher

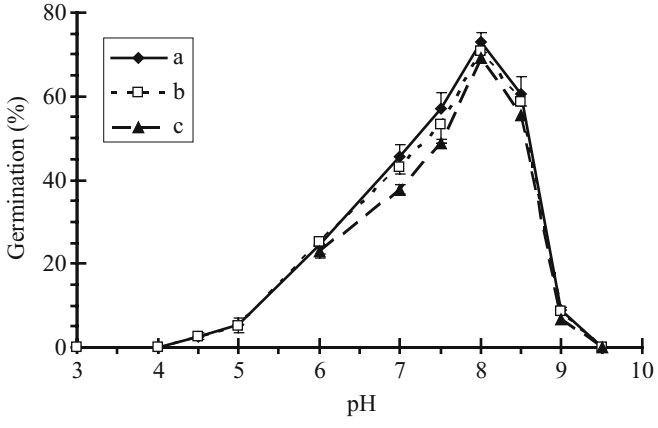


**Fig. 3.** Effect of pH on basidiospore germination of *Hebeloma vinosophyllum.* The basidiospores were suspended at a density of $10^6$ to $2 \times 10^6$ spores/ml in 100 mM $NH_4Cl$ adjusted at different pHs (3.0–9.5). The spore suspensions were incubated at 25°C in the dark for 12 days. *a* pH values adjusted by HCl for 3.0–5.0 and KOH for 6.0–9.5 (*solid line*); *b* pH values adjusted by $H_2SO_4$ for 3.0–5.0 and NaOH for 6.0–9.5 (*dotted line*); *c* pH values adjusted by $NH_4OH$ for 6.0–9.5 (*broken line*). *Bar* is standard error

**Table 2.** Changes in pH of basidiospore suspension of *Hebeloma vinosophyllum* after a 12-day incubation

| Initial pH | Final pHs of the spore suspension adjusted at different initial pHs | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3.0 | 4.0 | 4.5 | 5.0 | 6.0 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 |
| Reagent[a] | HCl | 3.2[b] | 4.1 | 4.6 | 5.4 | | | | | | | |
| | $H_2SO_4$ | 3.1 | 4.1 | 4.6 | 5.4 | | | | | | | |
| | KOH | | | | | 5.7[b] | 6.9 | 7.3 | 7.6 | 8.1 | 8.9 | 9.2 |
| | NaOH | | | | | 5.9 | 6.3 | 6.9 | 7.8 | 8.2 | 8.9 | 9.4 |
| | $NH_4OH$ | | | | | 5.6 | 6.4 | 7.3 | 7.7 | 8.1 | 8.8 | 9.3 |

The basidiospores ($10^6$ – $2 \times 10^6$ spores/ml) suspended in 100 mM $NH_4Cl$ aqueous solution at different initial pHs were incubated at 25°C in the dark

[a] Chemical reagents used for pH adjustment

[b] Final pH

concentration of $NH_4$-N, but did not prefer it. These findings suggest that the EP fungus *C. phlyctidospora* and the LP fungus *H. vinosophyllum* germinate one after another when both $NH_4$-N concentration and pH of the urea-treated soil decline below the threshold values, which would be between 500 and 800 mM and 8 and 9, respectively, in the field, and establish their territory by their vegetative growth. The vegetative growth of ammonia fungi such as *C. phlyctidospora* and *H. vinosophyllum* following spore germination would accelerate the absorption of $NH_4$-N in the soil with the activities of other soil microbes (immobilization of $NH_4$-N), resulting in the lowering of $NH_4$-N concentration and declining of the pH value of the soil; this may rapidly make more suitable conditions for the vegetative growth of *H. vinosophyllum*.

## Effect of temperature on basidiospore germination

The basidiospores germinated at 10° to 35°C with optima at 25° to 30°C (Fig. 4). The basidiospores did not germinate at 5°C and 40°C. At the optimal temperature, the spore germination initiated on the second day after the treatment and increment of the germination percentage reached a plateau about 12 days after the start of the treatment (Fig. 4).

*Hebeloma vinosophyllum* occurs at high frequency in the field after urea application in both summer and winter (Sagara 1975). The occurrence of *H. vinosophyllum* in the field, irrespective of the season of urea application, may be partially explained by the wide temperature range for spore germination of *H. vinosophyllum* (Fig. 4). Moreover, the foregoing results suggest that *H. vinosophyllum* has the potential ability to propagate even in tropical and cool temperate regions. However, the occurrence of *H. vinosophyllum* has been recorded in the area from Kanto district to the middle Kyushu Island in Japan and San Ming in China, but not in the cool temperate and subtropical regions in Japan (Sagara 1975; Suzuki 1992, 2000; Yamanaka 1995; Fukiharu and Horigome 1996; Hongo 1996; Fukiharu et al. 2000a,b). The biogeographic distribution of *H. vinosophyllum* would be affected by the temperature through each stage of its morphogenesis, including spore germination and interaction with other microbes, including other ectomycorrhizal ammonia fungi. The biogeographic distribution of host trees would be mainly determined by several environmental factors such as temperature, rainfall, and pH.

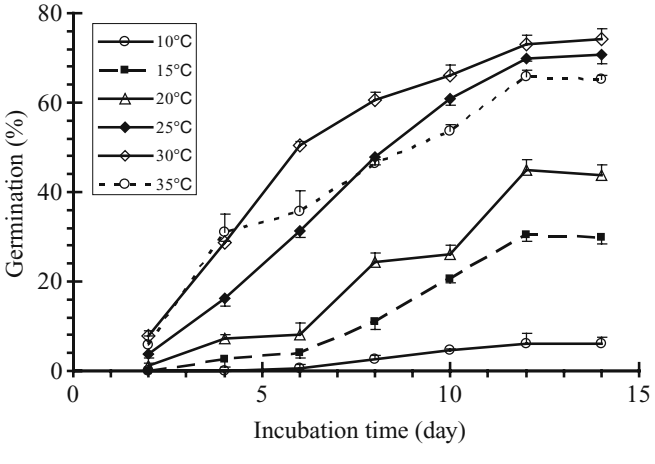


**Fig. 4.** Effect of temperature on basidiospore germination of *Hebeloma vinosophyllum.* The basidiospores were suspended at the density of $10^6$ to $2 \times 10^6$ spores/ml in 100 mM $NH_4Cl$ adjusted to pH 8.0 by KOH. The spore suspensions were incubated at 10°–35°C in the dark for 14 days. *Bar* is standard error

## Longevity of basidiospores of *H. vinosophyllum* at different temperatures in both wet and dry conditions

Germination ability of the spores gradually decreased with increasing storage period in both dry and wet conditions. Storage at 25°C and 30°C markedly lowered their germination ability (Fig. 5). Above 10°C, the germination ability of the basidiospores was maintained for a longer period in a dry condition than in a wet condition (Fig. 5). In a wet condition, a high temperature caused conspicuous loss of spore viability. The basidiospores completely lost their germination ability within a 150-day storage in a wet condition. However, the basidiospores stored in a dry condition for 150 days at 10°C and 15°C still germinated, at the rate of 13.0% and 19.3%, respectively. In a wet condition, a lower temperature seems to be more favorable for the storage of the basidiospores. In a dry condition, a temperature around 15°C would be better for the storage of basidiospores (Fig. 5).

The nonstored basidiospores rinsed by pure water (see Fig. 3; at pH 8.0) and basidiospores stored for only 1 day without rinsing (see Fig. 5) showed similar germination percentages when they were incubated under optimal environmental conditions, namely, at 25°C in 100 mM $NH_4Cl$ adjusted at pH 8.0. This result indicates that rinsing the spores with pure water and centrifugation of the spores have no significant effect on the germination rates of this fungus. Basidiospore germination of ectomycorrhizal fungi is not easily induced or stimulated (Horikoshi and Suzuki 1990). The germination rates of basidiospores of ectomycorrhizal fungi do not reach high percentages even when stimulated by specific environmental factor(s) (Ohta 1986; Horikoshi and Suzuki 1990). In contrast, the germination percentage of the ectomycorrhizal ammonia fungus *H. vinosophyllum* reached above 70.0% by incubation under optimal conditions (see Figs. 1–4). This result suggests that the stage of basidiospore germination would be at least one of the principal characteristics for categorizing *H. vinosophyllum* as a member of the ammonia fungi. Ammonia fungi may not always have access to an ammonium nitrogen-rich condition in the field. In other words, *H. vinosophyllum* may successfully colonize in the field even when the fungus is exposed by chance to a large amount of $NH_4$-N derived from urine or the decomposition of feces, corpses, or urine.

Sagara (1976) supposed that both hyphae and spores would be latent forms of ammonia fungi in the field, but no research has been done to clarify his assumption. The short

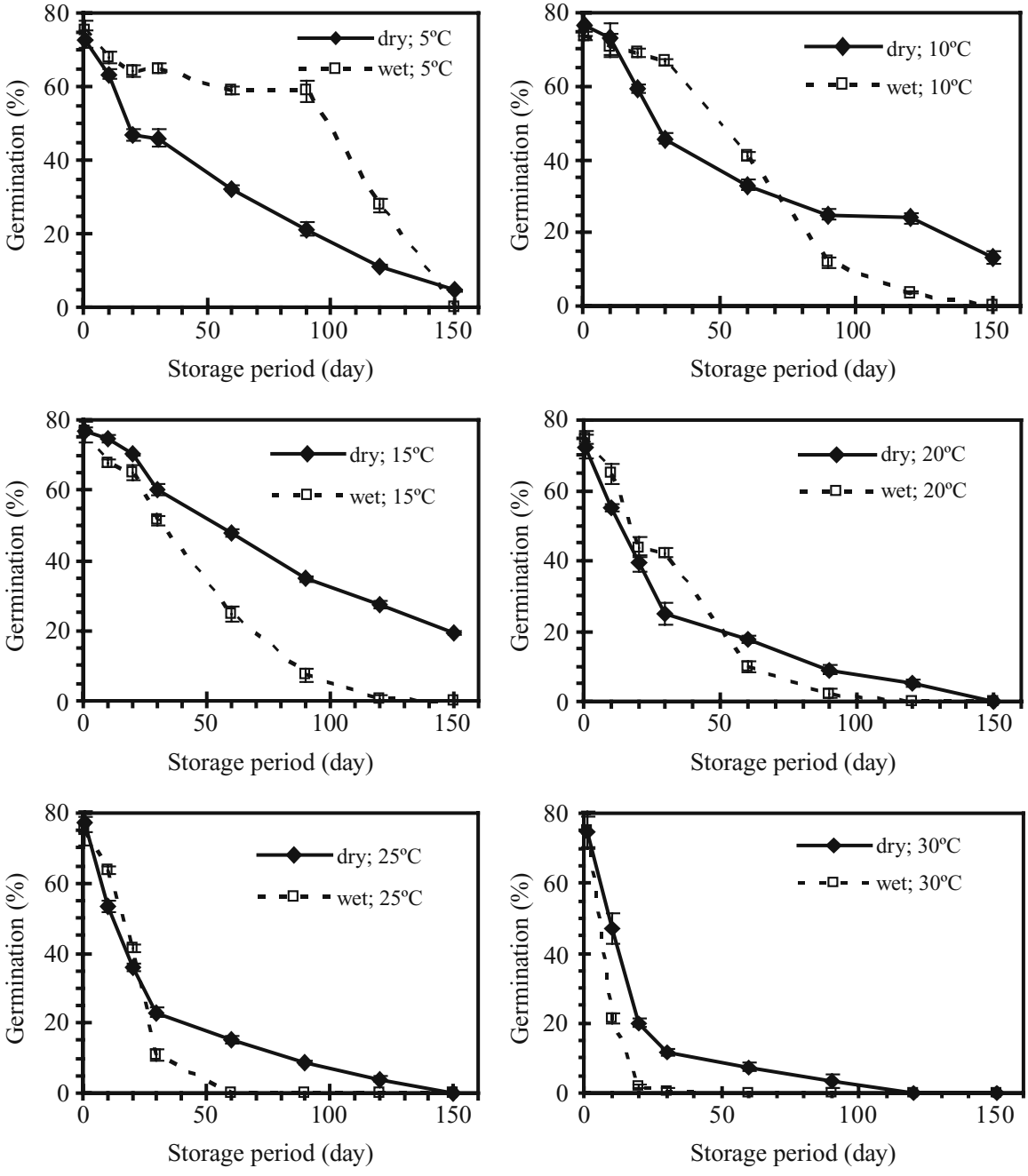


**Fig. 5.** Longevity of basidiospores of *Hebeloma vinosophyllum* under various temperatures in wet and dry conditions. The basidiospores were stored at 5°–30°C for 150 days in dry and wet conditions. The stored spores were suspended in 100 mM $NH_4Cl$ aqueous solution adjusted to pH 8.0. The density of the spore suspension was adjusted to $10^6$ to $2 \times 10^6$ spores/ml. The spore suspensions were incubated at 25°C in the dark for 12 days. *Bar* is standard error

longevity of the basidiospores suggests that the principal form of colonization of *H. vinosophyllum* would be the hyphae, not the basidiospores. Probably the principal role of the basidiospores of *H. vinosophyllum* would be the establishment of genetic diversity of this species, rather than resistance to severe environmental conditions.

Sood and Sackston (1971, 1972) found that daylength and light intensity had little effect on the germination of some basidiospores, but higher light intensity had an adverse effect on the germination of the spores. Maddison and Manners (1973) proposed that the nucleic acids and proteins of spores were affected by high-intensity light. The light intensity on the forest floor and in the litter may have no significant inhibitive effects on the spore germination of *H. vinosophyllum*, although we cannot completely deny the possibility that sunlight filtering down through the trees affects the spore germination of this fungus, e.g., shortening of the longevity of the spores.

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