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FULL PAPER

Effects of environmental factors on basidiospore germination of ammonia fungi *Coprinopsis* spp. collected from different geographical areas

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Abstract Effects of pH, NH_4-N, and temperature on basidiospore germination in *Coprinopsis austrophlyctidospora* from New Zealand, *C. phlyctidospora* from Japan, *C.* aff. *rugosobispora* from Canada, and *C. echinospora* from Canada were investigated. The *Coprinopsis* spp. required the presence of ammonium-nitrogen under weak alkaline to neutral conditions for germination, regardless of their different areas of occurrence. The former two species had a wider concentration of NH_4Cl solution and pH range for germination in comparison to the latter two species. The optimum concentration of NH_4Cl solution for the germination was 0.01 M in *C. austrophlyctidospora* and 0.1 M in the other three species. The pH optimum for germination in the former two species was 8.0 whereas that for germination in the latter two species was 8.0–8.5. The temperature range (5.0–40.0°C) for the former two species was wider than that (5–30°C) for the latter two species. Temperature optima for the germination in the former two species, *C.* aff. *rugosobispora* and *C. echinospora*, were 30, 20–25 and 15°C, respectively. The germination abilities of these *Coprinopsis* species in a wide range of temperatures are relevant to their natural temperature regime, showing their potential ability to propagate in tropical to subarctic regions.

Keywords Ecophysiology · NH_4-N concentration · pH · Saprobic fungi · Temperature

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Introduction

Ammonia fungi are defined as a chemoecological group of fungi that sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after the sudden addition of ammonia, or of some other nitrogenous material that reacts as a base, or of alkalis (Sagara 1975). In nature, these fungi also occur after urination and the decomposition of dead bodies and feces of animals (Sagara 1992, 1995; Wang and Sagara 1997; Harmaja 2002; Sagara et al. 2008). The successive occurrence of ammonia fungi is divided into early phase (EP) and late phase (LP) (Yamanaka 1999; Tibbett and Carter 2003; Imamura and Yumoto 2004; Sagara et al. 2008). The former consists of saprobic species: anamorphic fungi (e.g., *Amblyosporium botrytis*), cup fungi (e.g., *Ascobolus* spp., *Peziza* spp.), and smaller agarics (e.g., *Lyophyllum* spp., *Coprinopsis* spp.). The latter group, the LP fungi, consists of larger agarics, mostly ectomycorrhizal species such as *Hebeloma* spp. (Sagara 1975, 1992, 1995; Yamanaka 1999, 2003; Suzuki et al. 2002b; Imamura and Yumoto 2004). The EP of ammonia fungi can be further divided into early stage (anamorphic and cup fungi) and late stage (smaller agarics) based on the observation of sequential occurrence of their fruit bodies. Some *Coprinopsis* species that appear in the late stage of EP of succession occur at high frequency after urea treatment (Sagara 1975; Fukiharu and Hongo 1995; Yamanaka 1995a,b,c; Fukiharu and Horigome 1996; Fukiharu et al. 1997; Sato and Suzuki 1997; Suzuki 2000; Suzuki et al. 2002a,b; He and Suzuki 2004; Imamura and Yumoto 2004) and some have high fruiting abilities in vitro (unpublished data). Moreover, their occurrence at the late stage of EP would be a useful point in determining their detailed physiology for better understanding of the successive occurrence of ammonia fungi in the field because the late stage of EP would be a starting point: from saprobic

ammonia fungus species to saprobic non-ammonia fungus species. *Coprinopsis* spp. in ammonia fungi have been widely reported from different parts of the world following application of a large amount of urea in the soil. They are distributed in Asia, Oceania (Australia and New Zealand), Europe, and North America (Fukiharu and Horigome 1996; Suzuki et al. 2003). In other words, they are found in subtropical to subarctic regions (Suzuki et al. 2003). In general, spore germination is a crucial event in the propagation of fungus species, and the study of the factors that control spore germination has, therefore, received serious consideration. The environmental factors affecting spore germination of ammonia fungi have been examined in several ammonia fungi: the saprobic species *Amblyosporium botrytis* (Suzuki 1989, 2006, 2009a,b), *Ascobolus denudatus* (Suzuki 1989, 2006, 2009a,b), *Peziza moravecii* (Suzuki 1989, 2009a,b), *Coprinopsis cinerea* (syn.: *Coprinus cinereus*) (Suzuki et al. 1982; Suzuki 2009a,b), *Coprinopsis phlyctodospora* (syn.: *Coprinus phlyctidosporus*) sensu stricto (Suzuki et al. 1982; Suzuki 1989, 1992, 2006, 2009a,b), *Coprinopsis echinospora* (syn.: *Coprinus echinosporus*) sensu lato (Suzuki 1992, 2009b), *Coprinopsis stercorea* (syn.: *Coprinus stercoreus*) (Suzuki 1992, 2009b), *Coprinopsis neolagopus* (syn.: *Coprinus neolagopus*) (Suzuki 2009b), *Lyophyllum tylicolor* (Suzuki 1989); and the ectomycorrhizal species *Hebeloma vinosophyllum* (Suzuki 1978, 1989, 1992, 2006, 2009a,b; Deng and Suzuki 2008), *Hebeloma spoliatum* (Suzuki 1989, 1992, 2006, 2009a,b), and *Hebeloma radicosoides* (Suzuki 2009b). However, the detailed physiological characteristics of spore germination in ammonia fungi have not been examined except for the ectomycorrhizal ammonia fungus *H. vinosophyllum* (Deng and Suzuki 2008). Furthermore, temperature greatly affects the geographical distribution of each species. Studies on the effect of temperature on the spore germination of ammonia fungi also have been only done in the ectomycorrhizal ammonia fungus *H. vinosophyllum* (Deng and Suzuki 2008) but not in any saprobic ammonia fungi. Therefore, as the first step to elucidate the elaborate physiological characteristics of spore germination in saprobic ammonia fungi, we have investigated the effects of pH, NH_4-N concentration, and temperature on basidiospore germination of the late-stage EP fungi *Coprinopsis* spp. collected from different geographical areas to reveal their universal characteristics and their contribution in the sequential colonization mechanism of ammonia fungi on the global scale.

Materials and methods

Collection of spore samples

Four *Coprinopsis* species, collected from urea-applied sites of different geographical regions, were used in this study (Table 1). The basidiospores were obtained from the stock cultures of these *Coprinopsis* species. The culture medium used to obtain basidiospores consisted of 10 g malt extract (Difco), 2 g yeast extract (Difco), 20 g agar (Nakalai), and 1,000 ml pure water (MY agar). About 8 ml MY agar was put in each 18-mm test tube, which was then autoclaved at 120°C for 15 min. After autoclaving, stock cultures of *Coprinopsis* spp. were inoculated on the agar slants and kept at 25.0 ± 0.5°C in a 12 h dark and 12 h light regime. After 2–3 weeks of incubation, basidiomata were formed, and spores put on the inner surface of the test tube were collected aseptically.

Preparation of spore suspension

The NH_4Cl and KCl aqueous solutions were sterilized by using a 0.2-μm-pore-size cellulose nitrate membrane filter (Advantec, Japan) after preparing tested solutions as described below. The spore density of each suspension was fixed aseptically at the range of 10^5–10^6 spores/ml by using a hemacytometer at 100× magnification.

Experiments for basidiospore germination at different pHs

Basidiospore suspensions were made in 0.1 M NH_4Cl aqueous solution for each stock cultures and adjusted at different pHs (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0) by pH adjustment reagents 1 M HCl, 0.5 M H_2SO_4, 1 M NH_4OH, 1 M NaOH, or 1 M KOH. Different pH adjustment reagents were used to check their substance-specific effects. The precision of each designated pH was ±0.05. Then, the spore suspensions were incubated at 25.0 ± 0.5°C under the dark condition. Basidiospores were suspended in pure water as a control. After the end of incubation, the final pH of the each spore suspension was measured with a glass electrode pH meter (F-23; Horiba, Japan). All procedures were done under aseptic conditions.

Experiments for basidiospore germination at different NH_4Cl concentrations

Basidiospore suspensions were made at different concentrations (0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 2 M) of NH_4Cl aqueous solutions. Initial pH of each suspension was adjusted at their corresponding optimum value by using 1 M NaOH or 1 M HCl. As one control, the basidiospores of *C. austrophlyctidospora* and the other three tested *Coprinopsis* species were suspended in 0.01 M KCl adjusted at pH 8.0 by 1 M KOH and 0.1 M KCl adjusted at pH 8.0 by 1 M KOH, respectively. Basidiospores were also suspended in pure water as

Table 1 Fungal stock cultures used in this study

Fungus species	Stock culture no	Location	Dominant species in vegetation	Reference
Coprinopsis austrophlyctidospora	CHU3007[a]	Riverhead, North Island, New Zealand	*Pinus radiata* (plantation)	Suzuki et al. (2002a), Fukiharu et al. (2011)
Coprinopsis phlyctidospora sensu stricto	NBRC30478[a]	Iwakura, Kyoto, Japan	*Castanopsis cuspidata*	Suzuki et al. (2002a)
Coprinopsis aff. *rugosobispora*	CHU 2016[b]	Chipman, Alberta, Canada	*Populus tremuloides*	
Coprinopsis echinospora sensu lato	CHU 2020[a]	Nojack, Alberta, Canada	*Pinus contorta* var. *latifolia*	

[a] Stock culture isolated from a basidioma obtained by urea treatment in the field experiment

[b] Stock culture isolated from a basidioma obtained by urea treatment in the laboratory experiment

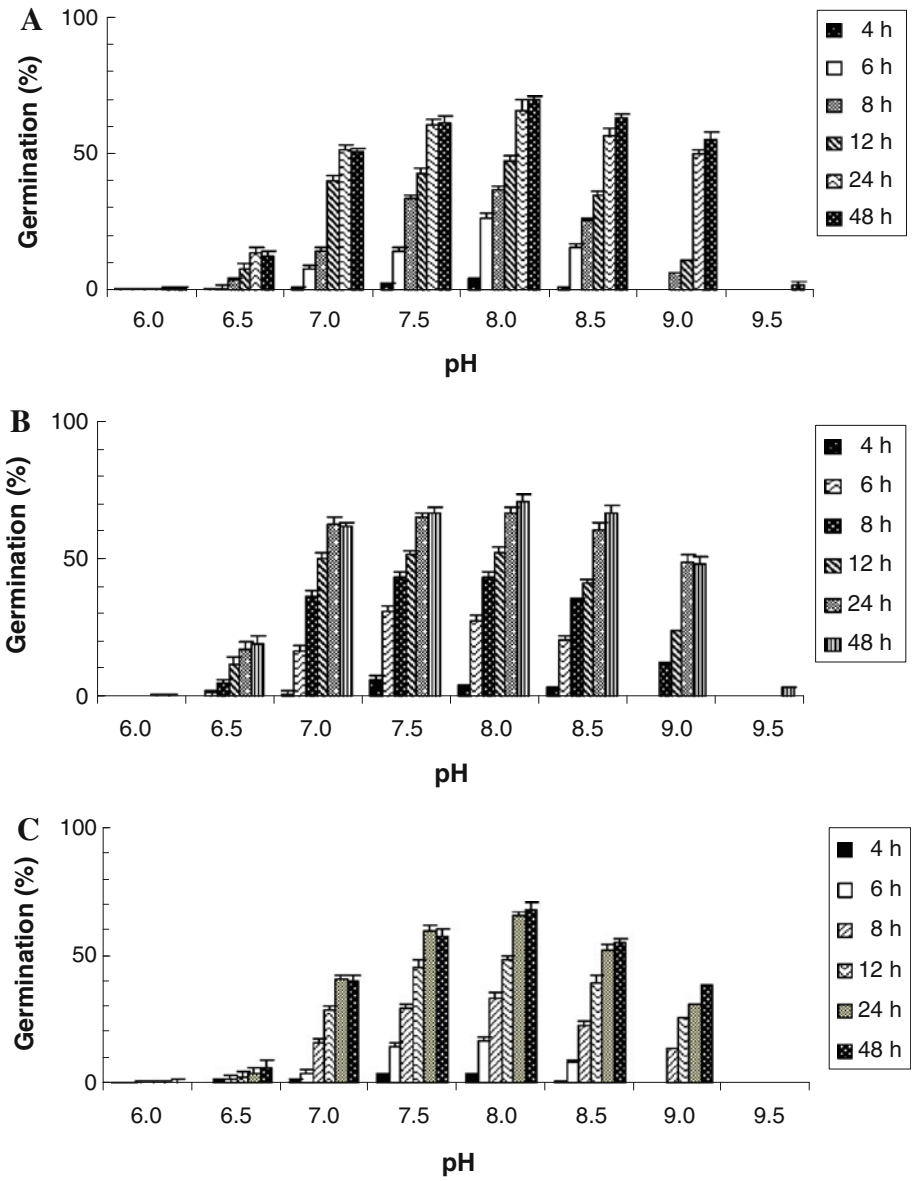

Fig. 1 Basidiospore germination in *Coprinopsis austrophlyctidospora* at different pHs. The spore density of each suspension was fixed at the range of 10^5–10^6 spores/ml in 0.1 M NH_4Cl aqueous solution. The spore suspension was incubated at 25.0 ± 0.5°C under dark conditions. The initial pH of each suspension was adjusted by using different chemical reagents. **a** pH values adjusted by 1 M HCl for 5.5–6.0 and 1 M NaOH for 6.5–10.0. **b** pH values adjusted by 0.5 M H_2SO_4 for 5.5–6.0 and 1 M KOH for 6.5–10.0. **c** pH values adjusted by 1 M NH_4OH for 6.5–10.0. *Bars* represent standard error of mean values

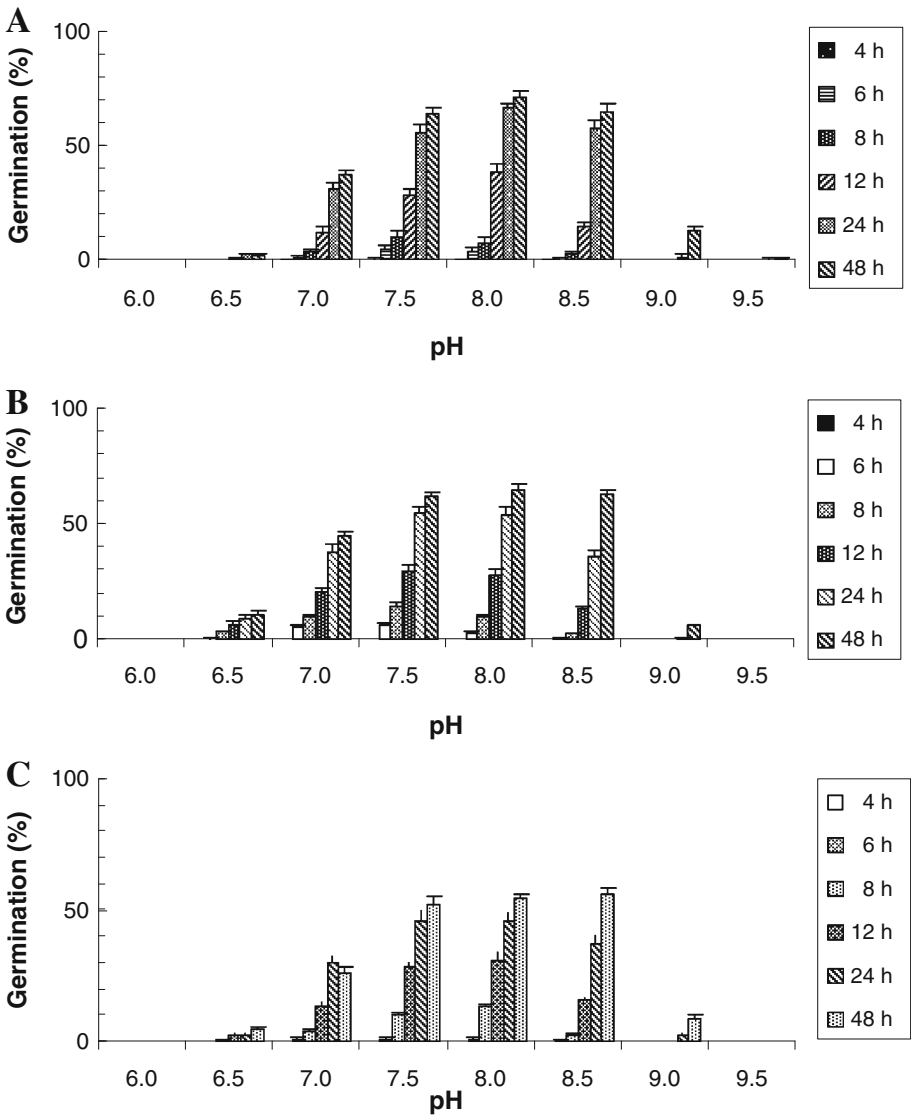

Fig. 2 Basidiospore germination in *Coprinopsis phlyctidospora* sensu stricto at different pHs. The spore density of each suspension was fixed at the range of 10^5–10^6 spores/ml in 0.1 M NH_4Cl aqueous solution. The spore suspension was incubated at 25.0 ± 0.5°C under dark conditions. The initial pH of each suspension was adjusted by using different chemical reagents. **a** pH values adjusted by 1 M HCl for 5.5–6.0 and 1 M NaOH for 6.5–10.0. **b** pH values adjusted by 0.5 M H_2SO_4 for 5.5–6.0 and 1 M KOH for 6.5–10.0. **c** pH values adjusted by 1 M NH_4OH for 6.5–10.0. *Bars* represent standard error of mean values

another control. Then, the spore suspensions were incubated at 25.0 ± 0.5°C under the dark condition. After the end of incubation, the final pH of each spore suspension was measured with a glass electrode pH meter (F-23; Horiba). All procedures were done under aseptic conditions.

Experiments for basidiospore germination at different temperatures

The basidiospore suspension of each stock culture was made at its optimum NH_4Cl aqueous solution adjusted at its optimum pH and then incubated at different temperatures (5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, or 45.0 ± 0.5°C) under dark conditions. All procedures were done under aseptic conditions.

Sampling of spore suspension and microscopic observation

Each spore suspension (0.5 ml) was sampled aseptically after the designated time intervals of cultivation. Germination of the spores was observed immediately after sampling. In cases where spore germination was not observed at once, the spore suspensions were kept at −20°C and observed later. The spore suspensions were stirred and 10 μl of sample was mounted on a glass slide. Germination of the spores was observed through a light microscope (Olympus CH-2; Olympus Optical, Japan) at 400× magnification. In each treatment, at least 50 basidiospores were examined in random microscopic fields to examine the germination percentage. Germination of the basidiospore is defined as the protrusion of germ tube(s) [hypha(e)]

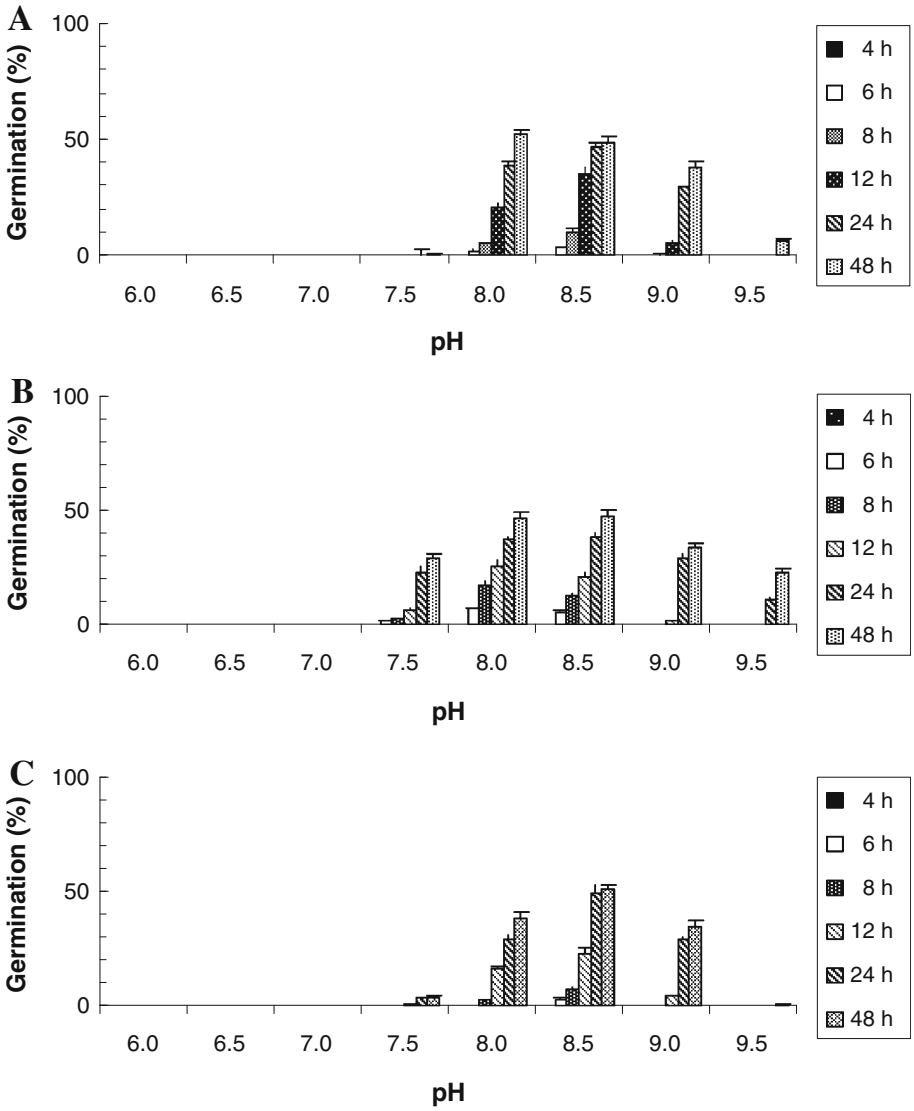

Fig. 3 Basidiospore germination in *Coprinopsis* aff. *rugosobispora* at different pHs. The spore density of each suspension was fixed at the range of 10^5–10^6 spores/ml in 0.1 M NH_4Cl aqueous solution. The spore suspension was incubated at 25.0 ± 0.5°C under dark conditions. The initial pH of each suspension was adjusted by using different chemical reagents. **a** pH values adjusted by 1 M HCl for 6.0 and 1 M NaOH for 6.5–10.0. **b** pH values adjusted by 0.5 M H_2SO_4 for 6.0 and 1 M KOH for 6.5–10.0. **c** pH values adjusted by 1 M NH_4OH for 6.0–10.0. *Bars* represent standard error of mean values

distinguishable under the light microscope. Spore germination percentage was computed using the following formula:

$$\text{spore germination percentage} = (\text{number of spores germinated} / \text{total number of spores observed}) \times 100.$$

Results are shown as the average of seven replicates.

Statistical analysis

All statistical analyses were performed by using Statcel2 software (OMS Publishing, Japan), and significant differences among treatments were determined by the Tukey–Kramer test at the 5% level.

Results and discussion

Effect of pH and NH_4Cl concentrations on basidiospore germination

The effective pH ranges for germination for *C. austrophlyctidospora*, *C. phlyctidospora* sensu stricto, *C.* aff. *rugosobispora*, and *C. echinospora* sensu lato were 6.0–9.5, 6.0–9.0, 7.5–9.5, and 7.5–9.0, respectively, when they were cultured in 0.1 M NH_4Cl aqueous solution. pH optima for the spore germination of *C. phlyctidospora* sensu stricto and *C. austrophlyctidospora* were 8.0 (maximum percentage germination: 71% and 70%, respectively; $P < 0.05$), whereas those of *C. echinospora* sensu lato and *C.* aff. *rugosobispora* were 8.0–8.5 (maximum percentage germination: 62% and 51%, respectively; $P < 0.05$).

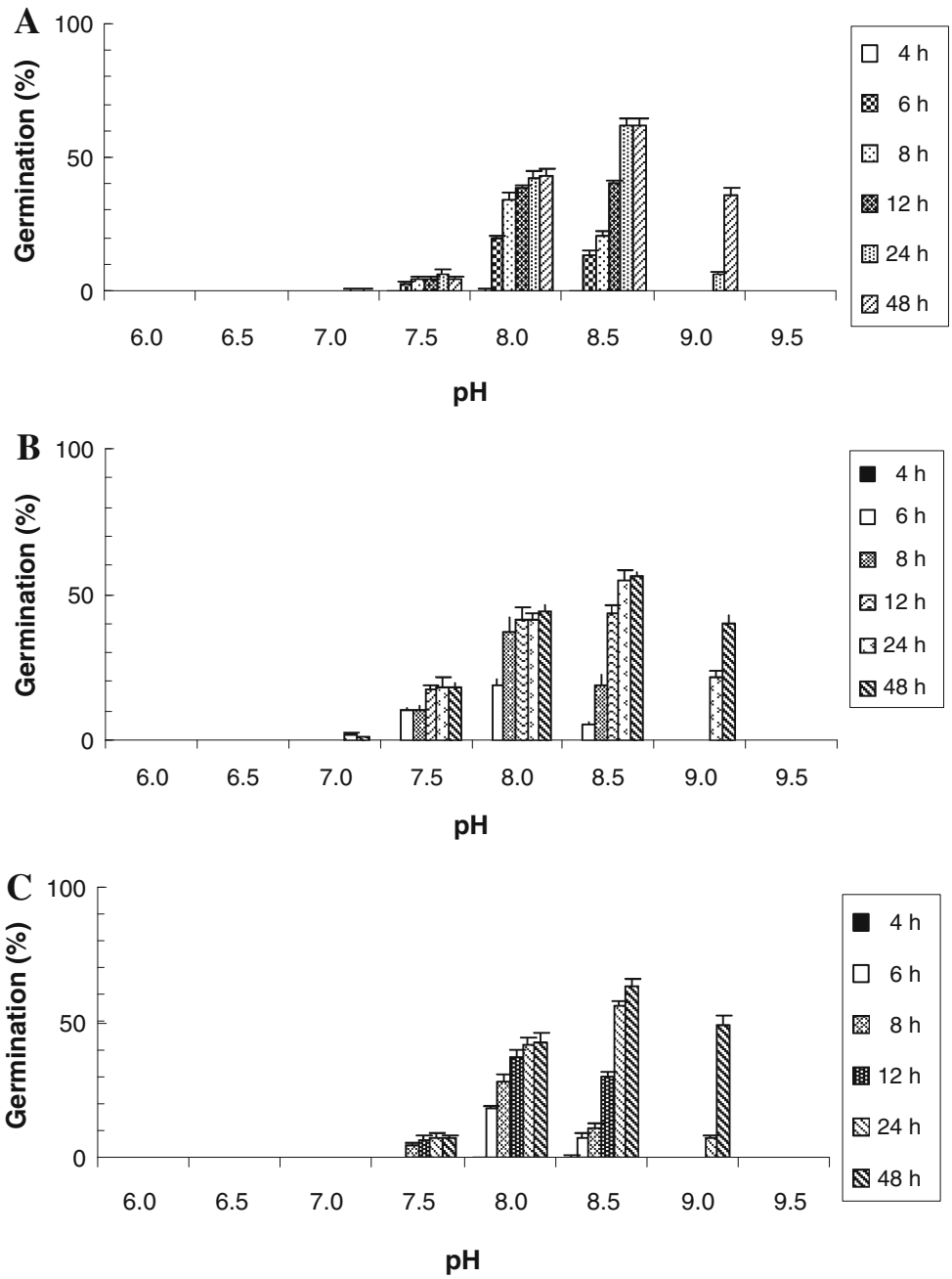

Fig. 4 Basidiospore germination in *Coprinopsis echinospora* sensu lato at different pHs. The spore density of each suspension was fixed at the range of 10^5–10^6 spores/ml in 0.1 M NH_4Cl aqueous solution. The spore suspension was incubated at 25.0 ± 0.5°C under the dark condition. The initial pH of each suspension was adjusted by using different chemical reagents. **a** pH values adjusted by 1 M HCl for 5.5–6.0 and 1 M NaOH for 6.5–10.0. **b** pH values adjusted by 0.5 M H_2SO_4 for 5.5–6.0 and 1 M KOH for 6.5–10.0. **c** pH values adjusted by 1 M NH_4OH for 6.5–10.0. *Bars* represent standard error of mean values

Notable decline of germination occurred when pH increased from 8.5 to 9.5 (Figs. 1, 2, 3, 4). *C. austrophlyctidospora*, *C. phlyctidospora* sensu stricto, *C.* aff. *rugosobispora*, and *C. echinospora* sensu lato did not germinate below 5.5 and above 10.0, below 5.5 and above 9.5, below 7.0 and above 10.0, and below 7.0 and above 9.5, respectively (Figs. 1, 2, 3, 4). The final pH of all treatments declined slightly from their original values, irrespective of pH adjustment reagents, except for most cases of initial pH 5.5 (Table 2). Therefore, the actual optimum pH for the spore germination in each *Coprinopsis* species should be slightly lower than the optimum value based on initial pH value. Basidiospore germination of *C. cinerea* is stimulated weakly by potassium ion (Suzuki et al. 1982). However, no significant difference in basidiospore germination was observed among reagents used for pH adjustments in the present study (see Figs. 1, 2, 3, 4). Thus, not only potassium and sodium ions but also chloride and sulfate ions showed no substance-specific effects on the germination of the *Coprinopsis* species. That is, the ranges and optima of pH and NH_4Cl for the spore germination shown in Figs. 1, 2, 3, and 4 simply reflect the effect of NH_4Cl on spore germination without any effects of the pH adjustment reagents.

Table 2 pH changes in the basidiospore suspension of *Coprinopsis* spp. after a 10-day incubation

Fungus species	Reagent[a]	Final pHs of the basidiospore suspensions adjusted at different initial pHs:									
		5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
C. austrophlyctidospora	HCl	5.7	5.8								
	H_2SO_4	5.8	6.5								
	NaOH			5.9	6.4	6.9	7.5	7.9	8.5	9.3	9.9
	KOH			5.9	6.6	7.3	7.5	8.2	8.7	9.2	9.8
	NH_4OH			5.7	6.4	6.7	7.5	8.1	8.7	9.2	9.8
C. phlyctidospora sensu stricto	HCl	4.1	4.3								
	H_2SO_4	4.4	4.3								
	NaOH			4.9	5.0	6.1	6.7	7.6	8.4	9.3	9.8
	KOH			4.9	6.2	6.4	6.9	7.5	8.5	9.3	9.8
	NH_4OH			4.7	5.4	6.3	6.9	7.6	8.4	9.1	9.7
C. aff. *rugosobispora*	HCl		5.3								
	H_2SO_4		5.3								
	NaOH			5.2	5.7	6.4	7.2	8.0	8.6	9.1	9.9
	KOH			5.4	5.8	6.4	7.3	8.0	8.7	9.2	9.9
	NH_4OH			5.2	5.7	6.2	7.0	8.1	8.5	9.0	9.9
C. echinospora sensu lato	HCl	5.2	5.4								
	H_2SO_4	5.1	5.4								
	NaOH			5.5	6.1	6.6	7.3	7.9	8.6	9.2	9.6
	KOH			6.0	6.3	7.2	7.3	8.0	8.5	9.1	9.7
	NH_4OH			5.8	6	6.7	7.4	7.9	8.5	9.1	9.7

The spore density of each suspension was fixed at the range of 10^5–10^6 spores/ml in 0.1 M NH_4Cl aqueous solution at designated pHs; the spore suspensions were incubated at 25.0 ± 0.5°C under dark conditions

[a] Reagents used for initial pH adjustment

The effective ranges of NH_4Cl concentration for the spore germination of *C. austrophlyctidospora*, *C. phlyctidospora* sensu stricto, *C.* aff. *rugosobispora*, and *C. echinospora* sensu lato were 0.0003–0.3, 0.0001–1.0, 0.03–0.3, and 0.01–0.3 M, respectively (Fig. 5). Optimum NH_4Cl concentration for spore germination were 0.01 M in *C. austrophlyctidospora* and 0.1 M in the other three *Coprinopsis* species ($P < 0.05$). *C. austrophlyctidospora*, *C.* aff. *rugosobispora*, and *C. echinospora* sensu lato (maximum percentage germination: 62%, 51%, and 70%, respectively) did not germinate below 0.0001 M and above 1 M, below 0.003 M and above 1 M, and below 0.001 M and above 1 M, respectively (see Fig. 5). *C. phlyctidospora* sensu stricto did not germinate above 2 M. The final pHs of all treatments declined slightly from their original values, irrespective of pH adjustment reagents (Table 3). It is, therefore, expected that the results of the NH_4Cl concentration experiments in *Coprinopsis* spp. (Fig. 5) mainly reflect the effect of different concentrations of NH_4Cl but not that of changing pH during cultivation of the spore suspensions. All the *Coprinopsis* species tested did not germinate in KCl aqueous solutions and pure water, suggesting that the presence of ammonium-nitrogen is the essential factor for spore germination for *Coprinopsis* spp. under weak acidic to weak alkaline conditions, regardless of their geographically different areas.

Some interspecific and geographic variation was observed among the tested *Coprinopsis* species. The *Coprinopsis* species from Canada had slightly narrower effective ranges of NH_4-N concentration and pH with lower optimum NH_4-N concentration in comparison with the *Coprinopsis* species from Japan and New Zealand (see Figs. 1, 2, 3, 4, 5). Among the four species, *C.* aff. *rugosobispora* showed the lowest maximum germination percentage (51%) in comparison with others whereas the highest germination percentage (71%) occurred in *C. phlyctidospora* sensu stricto.

Effect of temperature on basidiospore germination

The temperature optima for the spore germination in *C. austrophlyctidospora*, *C. phlyctidospora* sensu stricto, *C.* aff. *rugosobispora*, and *C. echinospora* sensu lato were 30, 30, 20–25, and 15°C, respectively (Fig. 6; $P < 0.05$). *C. austrophlyctidospora* and *C. phlyctidospora* sensu stricto did not germinate at 45°C and failed to resume

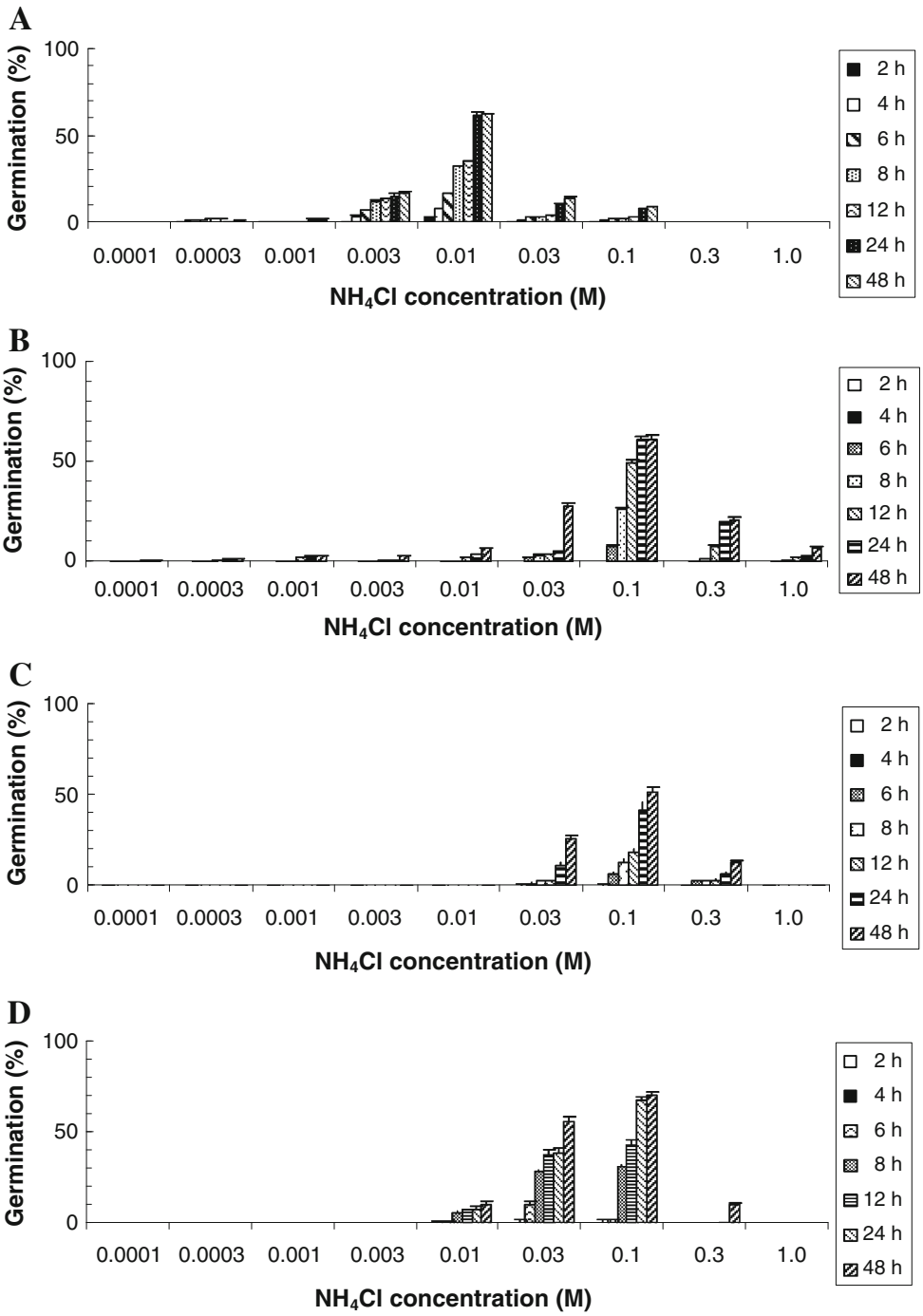

Fig. 5 Basidiospore germination in *Coprinopsis* spp. at different NH_4Cl concentrations. The basidiospores were suspended at a density of 10^5–10^6 spores/ml in different concentrations of NH_4Cl aqueous solution. The initial pH of the spore suspension was adjusted to the optimum value of each by using 1 M NaOH or 1 M HCl. The spore suspension was incubated at 25.0 ± 0.5°C under dark conditions. *Bars* represent standard error of mean values. **a** *Coprinopsis austrophlyctidospora* (pH 8.5). **b** *Coprinopsis phlyctidospora* sensu stricto (pH 8.0). **c** *Coprinopsis* aff. *rugosobispora* (pH 8.0). **d** *Coprinopsis echinospora* sensu lato (pH 8.5)

germination after 10 days of additional incubation at the optimum temperature. *C.* aff. *rugosobispora* and *C. echinospora* sensu lato did not germinate at 35°C and failed to resume germination after 10 days of additional incubation at each optimum temperature. Temperature responses were also slightly different among these species showing their geographic and interspecific variations. Namely, Canadian species (*C.* aff. *rugosobispora* and *C. echinospora* sensu lato) had a little narrower effective temperature range (5–30°C) in comparison with Japanese species (*C. phlyctidospora* sensu stricto) and New Zealand species (*C. austrophlyctidospora*) (5–40°C; Fig. 6). *C. echinospora* sensu lato had the lowest temperature optimum (15°C) and narrower effective temperature range (5–30°C). In contrast, *C. austrophlyctidospora* and *C. phlyctidospora* sensu stricto had a broader effective temperature range (5–40°C) with higher temperature optimum at 30°C. This finding suggests that the *Coprinopsis* spp. collected from subarctic region are well adapted to lower temperatures than those collected from

Table 3 pH changes of basidiospore suspensions in the *Coprinopsis* spp. after a 30-day incubation under different NH_4Cl concentrations

NH_4Cl concn. (M)	Initial and final pHs of basidiospore suspensions in							
	C. austrophlyctidospora		*C. phlyctidospora* sensu stricto		*C.* aff. *rugosobispora*		*C. echinospora* sensu lato	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0.0001	8.5	7.5	8.0	7.9	8.0	7.7	8.5	7.1
0.0003	8.5	7.5	8.0	7.8	8.0	7.6	8.5	6.6
0.001	8.5	7.1	8.0	7.5	8.0	7.7	8.5	7.5
0.003	8.5	7.5	8.0	7.4	8.0	7.4	8.5	7.2
0.01	8.5	8.0	8.0	7.4	8.0	7.5	8.5	7.7
0.03	8.5	8.3	8.0	7.4	8.0	7.5	8.5	7.9
0.1	8.5	8.3	8.0	7.3	8.0	7.3	8.5	8.1
0.3	8.5	8.3	8.0	7.3	8.0	7.8	8.5	8.2
1.0	8.5	8.3	8.0	7.9	8.0	7.8	8.5	8.3

The basidiospores of each species were suspended at a density of 10^5–10^6 spores/ml in different concentrations of NH_4Cl aqueous solution. The initial pH of each treatment was adjusted to the optimum value of each species by using 1 M NaOH or 1 M HCl and the spores suspensions were incubated at 25.0 ± 0.5°C under dark conditions

temperate region. The germination responses of these *Coprinopsis* species to a wide range of temperatures (see Fig. 6) suggest that they would germinate all seasons of the year, except for freezing conditions in Canada, when they were exposed to soil containing a high amount of NH_4-N associated with neutral to weak alkaline conditions. Therefore, further experiments about not only the effects of temperature on the basidiospore germination of many stock cultures collected from different biogeographical areas but also the effects of temperature on their vegetative and reproductive growth are required to elucidate the physiological background of their biogeographical distribution.

Ecophysiological aspects of the *Coprinopsis* species

The basidiospore germination of EP fungi *C. cinerea* and *C. phlyctidospora* sensu stricto from Japan is greatly enhanced by the presence of NH_4-N [0.001–0.1 M $(NH_4)_2HPO_4$ aqueous solution] associated with alkalinity (pH 7.5–8.3; Suzuki et al. 1982). Similar effects are reported in some other saprobic (EP) ammonia fungi from Japan. Basidiospore germination in *C. neolagopus* and *C. stercorea* is significantly stimulated with 0.03–0.15 M NH_4Cl at pH 8–10 and 0.1 M $(NH_4)_2HPO_4$ at pH 8, respectively (Suzuki 2009b). Similarly, basidiospore germination in *L. tylicolor* is stimulated with 0.00001–0.3 M NH_4-N at pH 5.0–12.0 (Suzuki 1989). Conidium germination of *Amblyosporium botrytis* is stimulated with 0.0003–0.6 M NH_4Cl aqueous solution adjusted at pH 5.0–9.0 (Licyayo 2007). Ascospore germination of *Ascobolus (As.)denudatus* and *Peziza moravecii* is remarkably stimulated with 0.01–0.03 M NH_4Cl aqueous solution adjusted at pH 8–10 and 0.01 M NH_4Cl aqueous solution adjusted at pH 8–10, respectively (Suzuki 2009b). Spores of these species, except for *Am. botrytis*, do not germinate at all in distilled water (Suzuki et al. 1982; Suzuki 1992, 2006; Licyayo 2007; Deng and Suzuki 2008). All these EP fungi appear well adapted to high NH_4-N concentration under weak acidic to weak alkaline conditions at their basidiospore germination stage.

The effective pH ranges for spore germination of all tested *Coprinopsis* species were narrower (see Figs. 1, 2, 3, 4) than that of *Hebeloma* (*H.) vinosophyllum* (Deng and Suzuki 2008) whereas the pH optimum for the basidiospore germination in all tested *Coprinopsis* species (pH 8.0 or 8.5; see Figs. 1, 2, 3, 4) was similar to that in *H. vinosophyllum* (pH 8.0; Deng and Suzuki 2008). In contrast, the effective NH_4Cl concentration ranges of the *Coprinopsis* species (0.0001–1.0 M in *C. phlyctidospora* sensu stricto and 0.0003–0.1 M in the other three *Coprinopsis* species; Fig. 5) tested in the present study was broader than that of *H. vinosophyllum* (0.01–0.5 M; Deng and Suzuki 2008). The spore germination of the three *Coprinopsis* species might be possible under lower NH_4-N concentration compared to *H. vinosophyllum*. However, the optimum NH_4Cl concentration for spore germination of all those *Coprinopsis* species except for *C. austrophlyctidospora* (Fig. 5) was the same as that (0.1 M) of *H. vinosophyllum* (Deng and Suzuki 2008), whereas the optimum NH_4Cl concentration for spore germination in *C. austrophlyctidospora* (0.01 M; Fig. 5) was lower than that of *H. vinosophyllum* (Deng and Suzuki 2008).

These results indicate that NH_4-N concentration and pH are the principal environmental factors for the germination of saprobic ammonia fungi including *Coprinopsis* spp., but the sequential occurrence of the saprobic ammonia fungi cannot be explained simply by their ranges and optima for NH_4-N concentration and pH.

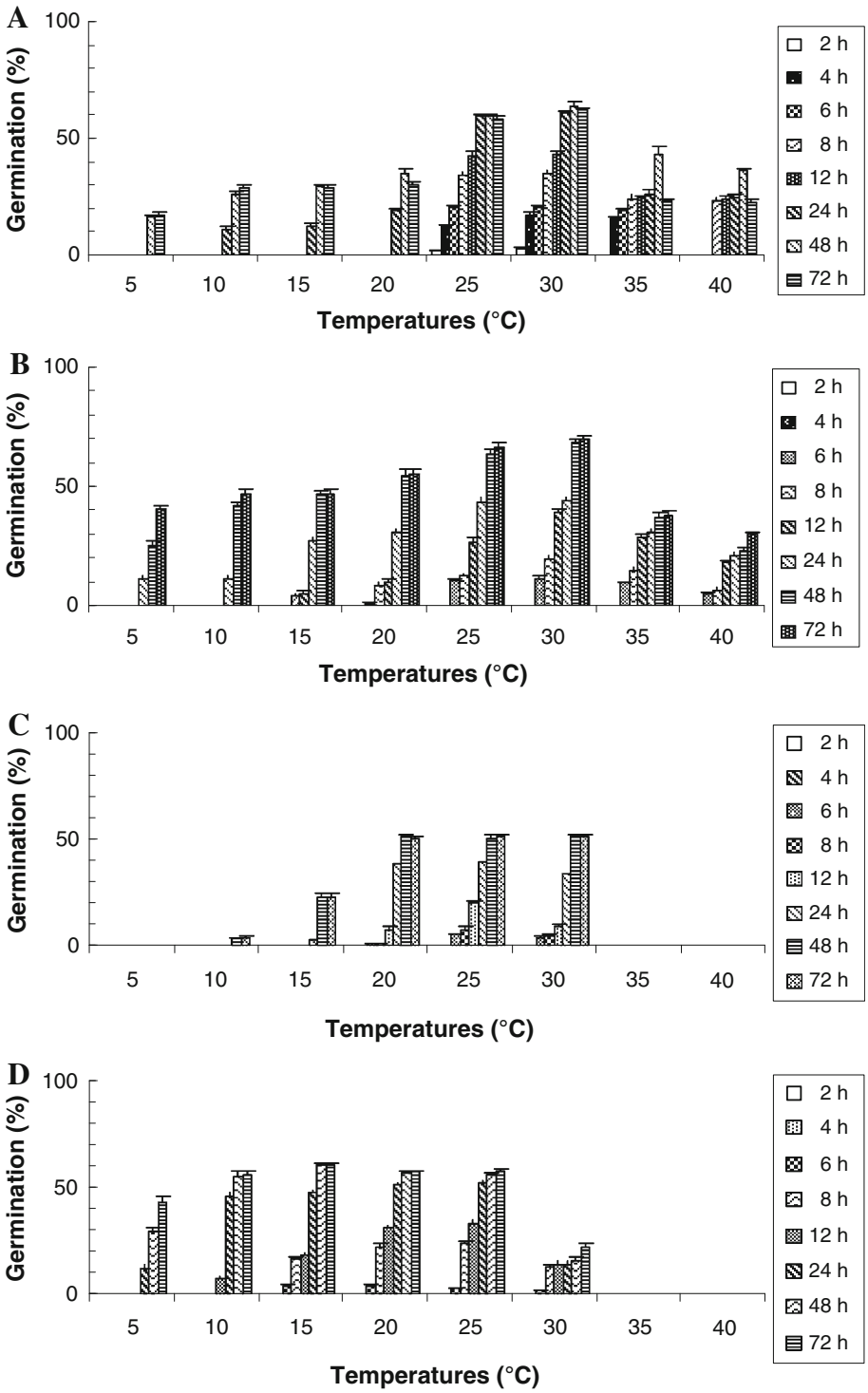

Fig. 6 Basidiospore germination in *Coprinopsis* spp. at different temperatures. The basidiospores were suspended at a density of 10^5–10^6 spores/ml in the optimum NH_4Cl concentration and pH for each species. The initial pH of the spore suspension was adjusted to the optimum value of each by using 1 M NaOH or 1 M HCl. The spore suspension was incubated at 25.0 ± 0.5°C under dark conditions. *Bars* represent standard error of mean values. **a** *Coprinopsis austrophlyctidospora* (0.01 M NH_4Cl, pH 8.5). **b** *Coprinopsis phlyctidospora* sensu stricto (0.1 M NH_4Cl, pH 8.0). **c** *Coprinopsis* aff. *rugosobispora* (0.1 M NH_4Cl, pH 8.0). **d** *Coprinopsis echinospora* sensu lato (0.1 M NH_4Cl, pH 8.5)

The basidiospores of the tested *Coprinopsis* species germinated at 5–40°C with optima at 15–30°C (Fig. 6) whereas the basidiospores of *H. vinosophyllum* germinate at 10–35°C with optima at 25–30°C (Deng and Suzuki 2008). *C. phlyctidospora* sensu stricto and *C. echinospora* sensu lato used in this study is reported as a late-stage EP fungi (Yamanaka 1999; Suzuki et al. 2002b; Sagara et al. 2008). Similarly, *C. austrophlyctidospora* and *C.* aff. *rugosobispora* are also reported as late-stage EP fungi (unpublished data). These results indicate that sequential occurrence of the ammonia fungi cannot be explained simply by the effects of pH, NH_4-N, and temperature on their spore germination.

Suzuki (2006) reported that the basidiospore germination of *C. phlyctidospora* sensu stricto and some other

ammonia fungi was stimulated by a sterilized water extract of the forest soil (0.82 M NH_4–N, pH 9.2) collected after 6 days of urea application, which agrees well with the present results (Figs. 1, 2, 3, 4, 5). Similar to previous studies, we also did not find any germination in the KCl solutions and pure water in the present study, which suggests that NH_4-N would be a principal factor for the tested *Coprinopsis* species to induce basidiospore germination and initiate propagation in the field under weak acidic to weak alkaline conditions, regardless of their geographically different areas of occurrence.

In addition, NH_4-N content of the L–F horizon of a *Castanopsis*- and *Quercus*-dominated mixed forest after 9 days of urea application rises to 40 mg N/g dry soil (~1.25 M NH_4-N), then declines gradually, and the pH value of the soils rises to above pH 9.0 within 3 days and remains above 9.0 for the next 24 days, then gradually declines to those of control soils until 125 days after the urea application (Suzuki et al. 2002b). A similar tendency in changes in soil pH and NH_4-N concentration is observed in urea plots in other different forests. Moreover, pH and NH_4-N content of soil are reported to be around 6.6–9.4 and 0.7–10.0 mg N/g dry soil (~0.02–0.31 M NH_4-N), respectively, during the occurrence of *C. echinospora* sensu lato and *C. phlyctidospora* sensu stricto (Yamanaka 1995a,b,c; Suzuki 2000; He and Suzuki 2004). Similarly, the pH and NH_4-N content of soil are reported to be around 4.5–5.0 and 0.1–10.0 mg N/g dry soil (~0.0031–0.31 M NH_4-N), respectively, during occurrence of the ectomycorrhizal (LP) ammonia fungus *H. vinosophyllum* (Yamanaka 1995c). Yamanaka (1995c) reported the fruiting of *H. vinosophyllum* is twofold following urea application in red pine forest. After the first flush (which occurs before 200 days after urea application) and spore discharge, the soil retains a suitable pH and NH_4-N concentration for stimulation of basidiospore germination. Consequently, the second flush is observed before 600 days after urea application, and thereafter pH of the soil is still suitable for stimulation of basidiospore germination but the NH_4-N content is lower than the lower limit for the stimulation of the germination. As a result, the basidiospore cannot germinate in the prevailing soil conditions. These results suggest that the basidiospores of *Coprinopsis* spp. and *H. vinosophyllum* would be able to germinate from just after urea application to the periods of their occurrence; that is, they would have the ability to germinate and colonize even at a later period of their occurrence.

Moreover, the basidiospore germination of *C. austrophlyctidospora* initiated within 2 h and the germination percentage reached 60–70% after 2 days of incubation at optimal conditions, whereas in the three other *Coprinopsis* species germination initiated within 6 h and germination percentage reached 51%–71% after 2 days of the incubation at optimal conditions (see Figs. 1, 2, 3, 4, 5, 6). In contrast, the basidiospore germination of *H. vinosophyllum* initiates after 2 days and germination percentage reaches more than 70% in 2 weeks when incubated at optimal conditions (Deng and Suzuki 2008). These results indicate that the tested *Coprinopsis* species can be characterized by rapid germination with high germination percentage in the presence of ammonium-nitrogen at neutral to weak alkaline conditions. Rapid germination with a higher germination percentage is an important feature of *R*-selected fungal species (Cooke and Rayner 1984). Therefore, the tested *Coprinopsis* species have characteristics of *R*-selection at basidiospore germination stage.

In conclusion, the foregoing study suggests that NH_4-N concentration and pH are the essential conditions for spore germination of the *Coprinopsis* species tested, despite their different areas of occurrence around the world and interspecific differences; namely, both NH_4-N concentration and pH have indispensable roles in causing the spore germination of *Coprinopsis* spp. and other ammonia fungi at the same time just after urea application. However, the effects of NH_4-N concentration, pH, and temperature on spore germination cannot be the root cause for the biogeographical distribution of the tested *Coprinopsis* spp., as described here.

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