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Adaptation of ammonia fungi to urea enrichment environment

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Urease activities and growth responses of ammonia fungi to different concentrations of urea with varying pH were investigated to elucidate the ecophysiological characteristics of ammonia fungi. Urease activities were screened by modified Christensen media with peptone and without peptone. Most saprobic ammonia fungi occurring at early phase of succession (EP species) in the field and a few saprobic non-ammonia fungi showed strongly urease activities. Several ectomycorhizal ammonia fungi occurring at late phase of succession (LP species) in the field showed moderately urease activities but not most ectomycorrhizal non-ammonia fungi. The early stage EP species were significantly tolerant and the late stage EP species were tolerant to high concentration of urea. The EP species grew better under neutral to weak alkaline condition than saprobic non-ammonia fungi. The LP species were weak tolerant to high concentration of urea with wider range of spectrum for the urea concentration than the ectomycorrhizal non-ammonia fungi. The LP species grew better under weak acidic to neutral condition than the ectomycorrhizal non-ammonia fungi. These suggested that urea would be related to an important nitrogen source for colonization of early stage EP species for a short period after urea application, while, ammonium-nitrogen and/or nitrate-nitrogen originally derived from the urea would be related to another important factor(s) for the colonization of late stage EP species and LP species.

Key words: Ectomycorhizal fungi, Modified Christensen media, pH, Saprobic fungi· Urea concentration, Urease

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Introduction

Ammonia fungi are defined as a chemo-ecological group of fungi that sequentially develop reproductive structures exclusively or relatively luxuriantly on soil after a sudden addition of ammonia, some other nitrogenous materials that react as bases by themselves or on decomposition, or alkali (Sagara, 1975). Urea disturbance in the field is happened sporadically by mammalian urination (Schmidt-Nielsen, 1975; Heinonen-Tanski and Wiik-Sijbesma, 2005). Fertilizer urea application has been done in the various vegetations to examine not only mycobiota of ammonia fungi but also colonization mechanism of ammonia fungi (Sagara, 1975; Suzuki, 1992, 2000, 2006; Yamanaka, 1995a-c; Fukiharu *et al.,* 1997; Sato and Suzuki, 1997; Suzuki *et al*., 2002; He and Suzuki, 2004; Imamura and Yumoto, 2004, 2008). After a large amount of urea application in the filed, ammonium-nitrogen concentration rises rapidly associating with the increment of soil pH to 9-10. When the soil ammonium-nitrogen concentration gradually decreases in associated with a decrease in pH from 8 to 7, ammonia fungi starts to occur generally from anamorphic fungi and then followed by cup fungi in Ascomycota. These species occur at an early stage of the early phase of the succession are referred to early stage EP species. When ammonium-nitrogen concentration decreases in association with the decrement of soil pH from 7 to 6 by oxidation of ammonium-nitrogen, fungi in Basidiomycota with smaller fruiting bodies become dominant. These species occur at late stage in early phase of the succession referred to late stage EP species. Finally, ammonium-nitrogen concentration and pH return to similar level of control around one to two years after the urea application. During this final phase of ammonium-nitrogen concentration and pH declining, fungi in Basidiomycota with larger fruiting bodies occur. These late-phase occurring species are referred to LP species (Sagara, 1992; Yamanaka, 1995a-c; Suzuki, 2000; Suzuki *et al*., 2002). All EP and several LP species are saprobic and most LP species are ectomycorrhizal fungi (Sagara, 1995; Sagara *et al*., 2008). Ammonia fungi are known to utilize various nitrogen sources such as protein, amino acids, urea, ammonium-nitrogen, nitrite-nitrogen, and nitrate-nitrogen (Yamanaka, 1999; Suzuki, 2006; Sagara *et al*., 2008). No report has been done on urease activities in ammonia fungi as well as the effects of different concentrations of urea on the vegetative growth of ammonia fungi except for *Coprinopsis tuberosa* (Reported as *Coprinus stercorarius*; Morimoto *et al*., 1982).

Therefore, we conducted to investigate the urease activities of the ammonia fungi and the effects of different concentrations of urea with varying pH on the vegetative growth of ammonia fungi *in vitro* to elucidate the propagation strategy of ammonia fungi after urea disturbance in the field.

Materials and methods

Screening of urease activities

Modified Christensen medium consisting of glucose, 1 g; peptone (Bacto, Detroit, USA); 1 g; urea (Wako, Tokyo, Japan), 20 g; Na_2HPO_4, 2 g; NaCl, 5 g; phenol red, 12 mg; agar (Nacalai Tesque, Kyoto, Japan), 15 g; and distilled water 1000 mL were used for urease screening test. The medium without peptone was also conducted in the following experiments to avoid the role of peptone as nitrogen source. The double strength medium without agar was adjusted at pH 6.8 using 1 M NaOH. The double strength of the modified Christensen media without agar was sterilized by filtering through membrane filter (cellulose nitrate; 0.22 µm pore size; Advantec, Japan). The double strength of the plain agar medium was sterilized at 120°C for 15 minutes and kept at 60°C in a water bath in order to mix together with either of the same volume of the double strength liquid medium. Twenty millilitres of the modified Christensen media with and without peptone were poured separately into a Petri dish (90 mm in diameter) under aseptic condition. Mycelium agar discs (5 mm diameter) were cut from the sub-peripheral region of actively growing mycelial colonies of each fungal isolate grown on MY agar medium [malt extract (Difco, Detroit, USA), 10 g; yeast extract (Difco, Detroit, USA); 2 g; agar (Nacali Tesque, Kyoto, Japan), 15 g; and 1000 mL distilled water sterilized at 120°C for 15 minutes]. The discs were then inoculated separately on the center of the modified Christensen agar media, keeping the mycelium surface attaching to the medium surface. The cultures were incubated at 25.0±0.5°C in darkness, except for 1 hour light irradiation at ca. 700 lux with a white light florescent lamp (Hitachi, Tokyo, Japan) at 3 days interval for the observation. Five replicates were prepared for urease screening test of the each species. In these experiments, 53 isolates of saprobic fungi and 8 isolates of ectomycorrhizal fungi were used (Table 1). The tests were done in five replications. Urease activity was evaluated as follows: strong activity (color in Christensen media were changed to deep pink), moderate activity (color in Christensen media were changed to pink), weak activity (color in Christensen media were changed to light pink), and no urease activity (color in Christensen media were not changed).

Growth responses of fungi to different concentrations of urea

Basal medium consists of glucose, 22.22 g; KH_2PO_4, 0.33 g; $MgSO_4 \cdot 7H_2O$, 0.33 g; $CaCl_2 \cdot 2H_2O$, 0.11 g; $ZnSO_4 \cdot 7H_2O$, 0.33 mg; $FeSO_4 \cdot 7H_2O$, 0.15 mg; $CuSO_4 \cdot 5H_2O$, 0.10 mg; $MnSO_4 \cdot 5H_2O$, 0.10 mg;

$Na_2MoO_4 \cdot 2H_2O$, 0.02 mg; thiamine hydrochloride, 0.50 mg; nicotinic acid, 0.10 mg, and 1000 mL distilled water (Kitamoto *et al*., 1972). Urea was added as nitrogen source to the basal medium at different concentrations (0, 0.046, 0.13, 0.46, 1.3, 4.6, 13.9, 23.3, 46.6, 69.9 g N/L). The basal medium with urea was adjusted at pH 7.0 using 1 M NaOH and then sterilized by filtering through membrane filter (cellulose nitrate; 0.22 µm pore size; Advantec, Japan). Mycelial agar discs (4 mm diameter) were cut from the sub-peripheral region of actively growing mycelial colony of each fungal isolate grown on the MY agar medium, the disc was then inoculated in the liquid medium. The cultures were incubated at 25.0±0.5^{o}C in darkness. The mycelium was harvested on 14 days of incubation for saprobic fungi and on 28 days of incubation for ectomycorrhizal fungi, and weighed after 48 hours drying at 60^{o}C. Five replicates were prepared for each treatment.

Growth responses of fungi to different pHs

The basal medium was prepared according to the optimal concentrations of urea for each ammonia fungus and adjusted pH from 2 to 9 at 1 pH interval to determine the effects of different pH on the vegetative growths. The pH was adjusted by 1 M NaOH and 1M HCl. Other cultivation procedures and weighing of mycelium biomass were done as described above. Five replicates were prepared for each treatment.

Statistical analysis

Data were analyzed by one-way ANOVA, and significant differences between treatments were determined by Tukey-Kramer test. All statistical analyses were performed using Statcel2 software (OMS Publishing Co, Tokorozawa, Japan).

Results and discussion

Urease activities

The presence or absence of peptone did not affect the results of screening of urease activity in each ammonia fungus (Table 1). All ammonia fungi except for *Lyophyllum abustum,* grew significantly on the modified Chirstensen media. All isolates of the early stage, EP species *Amblyosporium botrytis, Doratomyces putredinis, Ascobolus denudatus, Peziza moravecii, Pseudombrophila petrakii* and *Humaria velonovskyi* showed strongly positive activities of extracellular urease. The late stage EP species *Coprinopsis* spp.

and *Lyophyllum tylicolor* showed strongly or moderately activities of extracellular urease. The late stage EP species *Crucispora rhombisperma* and *Lyophyllum mephiticum* showed weakly activities of extracellular urease. All isolates of the saprobic LP species *Lepista* spp. and ectomycorrhizal LP species showed positive activities of extracellular urease. Among them, *Alnicola lactariolens* and *Hebeloma vinosophyllum* showed moderately urease activities whereas other tested isolates of LP species showed weak urease activities. A saprobic LP species *L. abustum* did not grow on the modified Christensen media and not show extracellular urease activity (Table 1). In contrast, 62% of non-ammonia fungi did not grow on the modified Christensen media. Among the saprobic non-ammonia fungi grown on the modified Christensen media, most species did not show significant positive activities of extracellular urease on the media, except for *Amblyosporium spongiosum*, *Isaria farianosa, Thricoderma viride, Ascobolus carbonarius, Ascobolus epimyces, Coprinopsis gonophylla,* and *Warcupia terrestris* (Table 1).

The urease activity was observed in the early stage EP species, the late stage EP species and the ectmycorrhizal LP species in 3 days of incubation, 6 days of incubation, and 15 days of incubation, respectively (Table 1).

Urea has been known an important nitrogen source for many fungi (Patemen and Kinghorn, 1976) and distribution of urease in fungi is very common (Navarathna *et al*., 2010). However, the positive extracellular urease activity in 37% was detected in the isolates of non-ammonia fungi. The ecological role of urease has been closely examined in human pathogenic fungi as a virulence factor (Cole, 1981; Granger *et al*., 1985), but not well examined in other ecological group of fungi. It is expected that production of the extracellular urease would be related to the important factor for colonization of ammonia fungi in the field as described below, but the ecological role(s) of urease in the non-ammonia fungi with strong activities could not be easily presumed, except for fungicolous fungi *Trichoderma viride* (Harman *et al*., 2004) and *A. spongiosum* (Pirozynski, 1969), which colonizing in urea rich substrata (Casimir and Heinemann, 1953; Dennis *et al*., 1960).

Our results indicated that distribution of urease in fungi is not rare, but not prevalent with high percentage (Table 1). Urea would be mainly decomposed by bacteria and utilized as inorganic nitrogen by many microbes including fungi (Fries 1955, Gottlieb and Legator, 1953). The potential urease activity of the O layer was significantly highest among the soil layers (Imamura *et al*., 2006). These suggested that most ammonia fungi, especially early stage EP species would contribute to decompose urea with urea splitting bacteria after urea application in the field. Early stage EP species as well as urea splitting bacteria would have an advantage for other microbes in their quick

colonization for a short period after urea application. However, if EP species can not produce or not enough biomasses after urea application, they would not much contribute to the decomposition of urea in the field, in spite of their enough ability to produce extracellular urease.

Growth responses of ammonia fungi to different concentrations of urea

For the growth tests in different urea concentrations, four EP species of saprobic fungi and two LP species of ectomycorrhizal fungi *Hebeloma* spp. which occur at high frequency in the field were selected as also reported by Sagara (1975), Suzuki (1992, 2000), Fukiharu and Hongo (1995), Yamanaka (1995a-c), Fukiharu *et al.* (1997), Sato and Suzuki (1997), Suzuki *et al.* (2002), He and Suzuki (2004) and Imamura and Yumoto (2004, 2008). Three saprobic non-ammonia fungi showed positively activities of extracellular urease, and two common wood rotting fungi and one common ectomycorhizal fungus which did not show extracellular urease activities (Table 1).

Urea concentration optima for biomass growth of *A. botrytis* and other tested EP species were 13.9 g N/L and 1.3 g N/L, respectively. The upper limit of urea concentrations for biomass growth of *A. botrytis* and other three tested EP species were 46.6 g N/L and 23.3 g N/L, respectively (Fig. 1A-D). Urea concentration optima for biomass growth of the LP species *Hebeloma radicosoides* and *H. vinosophyllum* were 0.046-0.13 g N/L and 0.46 g N/L, respectively (Fig. 1E, F). The upper limit of urea concentration for biomass growth of LP species *H. radicosoides* and *H. vinosophyllum* was 13.9 g N/L (Fig. 1E, F). Urea concentration optima for biomass growth of the saprobic non-ammonia fungus *A. spongiosum* and other four tested saprobic non-ammonia fungi were 4.6 g N/L and 0.46 g N/L, respectively. The upper limit of urea concentration for biomass growth of the saprobic non-ammonia fungus *A. spongiosum* and other four tested saprobic non-ammonia fungi were 46.6 g N/L and 4.6 g N/L, respectively (Fig. 1G-K). Urea concentration optimum for biomass growth an ectomycorrhizal non-ammonia fungus *Lyophyllum shimeji* was 0.46 g N/L and the upper limit of urea concentration for biomass growth was 4.6 g N/L (Fig. 1L). The saprobic ammonia fungi *A. botrytis, Coprinopsis phlyctidospora, L. tylicolor*, and *H. vinosophyllum* showed remarkably higher biomass than the non-ammonia fungi (Fig. 1A, C, D, G-K).

Final pH increased in the cultures according to the high urease activities of each isolates. These suggested that the increment of pH level would be mainly caused by ammonium ion that derived from the decomposition of urea by urease producing isolates. These indicated that ammonia fungi, especially the early stage EP species were better adapted to high concentration of urea

and/or ammonium-nitrogen than non-ammonia fungi, except for a fungicolous fungus *A. spongiosum*.

Growth responses of ammonia fungi to different pHs

The optimum pH for biomass growth of the EP species *A. botrytis, A. denudatus, C. phlyctidospora* and *L. tylicolor* was pH 7 or 8, and grew poorly at pH 4, and did not grow at all at pH 3 (Fig. 2A-D). The optimum pH for biomass growth of the LP species *H. radicosoides* and *H. vinosophyllum* was pH 6 or 7 (Fig. 2E, F). In contrast, the optimum pH for biomass growth of saprobic non-ammonia fungi *A. spongiosum, A. carbonarius*, *Lyophyllum carbonarium, Grifola frondosa,* and *Lentinula edodes* and an ectomycorrhizal non-ammonia fungus was pH 5 to 6, and grew poorly at pH 2, whereas *L. carbonarium* did not grow even at pH 3 (Fig. 2G-K). The optimum pH for biomass growth of all tested saprobic ammonia fungi were higher than that for biomass growth of the saprobic non-ammonia fungi except for *A. carbonarious* (Fig. 2A-D, G-K), but the former grew less than the latter under acidic condition (Fig. 2A-D).

The culture pH of the EP species and saprobic non-ammonia fungi increased from 0.8 to 2.7. The culture pH of the LP species and ectomycorrhizal non-ammonia fungus *L. shimeji* increased from 2.4 to 4.1. These suggested that the saprobic EP species adapted to high concentration of urea and/or ammonium ion under a neutral and weak alkaline conditions whereas the ectomycorrhizal LP species adapted to relatively high concentration of urea and/or ammonium ion under weak alkaline to neutral conditions (Figs. 1, 2A-F). In contrast, saprobic non-ammonia fungi and the ectomycorrhizal non-ammonia fungus would not adapt well both high concentration of urea and/or ammonium ion and weak alkaline to neutral condition (Fig. 1G-L, 2G-L).

The ammonia fungi naturally occur after decomposition of animal wastes such as urine, feces, and animal corpse (Sagara, 1995; Fukiharu *et al*., 2000a, b). Urea concentration of urine in mammalian such as bat (Studier and Wilson, 1983), deer (Robbinson *et al*., 1974), dog (Prause and Grauer, 1998), pig (Schmidt-Nielsen, 1975) and human being (Costa, 1968; Johnson *et al*., 1972) is in between 17 and 660 mg urea/L. Our results suggested that optimum concentration of urea for all tested saprobic ammonia fungi would be shown higher than urea concentration in the mammalian urination sites.

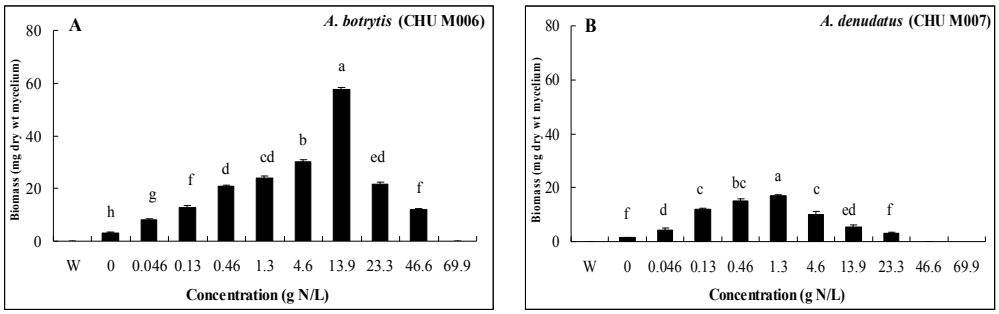
Most saprobic EP species had strongly extracellular urease activities (Table 1) and grew well at high concentration of urea (1.3-13.9 g N/L) under neutral to weak alkaline condition (Table 1; Figs. 1A-D, 2A-D). In contrast, less than 25% of non-ammonia fungi had moderately or strongly urease activity, but

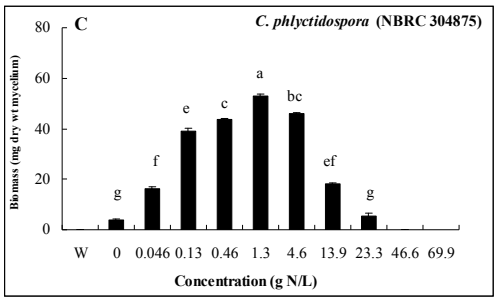
was not tolerant to high concentration of urea (Table 1; Figs. 1G-L, 2G-L). In contrast, early stage EP species were tolerant to higher concentration of urea (Fig. 1A, B). Licyayo and Suzuki (2006) reported that mycelia of early stage EP species were tolerant to higher concentration of ammonium-nitrogen. Probably, the mycelia of early stage EP species would survive, but not most non-ammonia fungi, on the sites where urea enrichment by mammalian urination in the field would be done. In mammalian urination sites, early stage EP species as well as urea-splitting bacteria (Araj *et al*., 1989) may cause arising of soil pH above 9 and would make their advantage for late stage EP species, LP species, and non-ammonia fungi, etc.

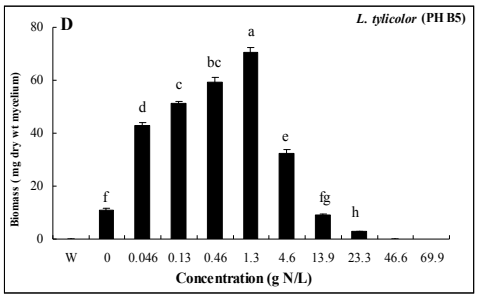
Ammonium-nitrogen and pH have been reported as key factors for the vegetative growth of ammonia fungi (Enokibara *et al*., 1993; Morimoto *et al.,* 1982; Suzuki, 1989, 2006, 2009a, b; Suzuki *et al*., 1982, 2002; Yamanaka, 1999, 2003; He and Suzuki, 2003; Licyayo and Suzuki, 2006; Sagara *et al*., 2008). Ectomycorrhizal ammonia fungi grow well in nitrate *in vitro* (Yamanaka, 1999; Suzuki, 2006) and their occurrence are observed during high nitrate-nitrogen concentration (Yamanaka, 1995a-c; Suzuki, 2000; Suzuki *et al.,* 2002).

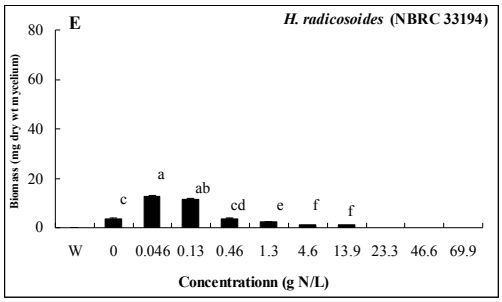
These suggested that not only ammonium-nitrogen and pH but also urea would be related to an important factor for colonization of early stage EP species in urea amended soil. Moreover, ammonium- and/or nitrate-nitrogen originally derived from the urea would be related to another important factor(s) for the colonization of late stage EP species and LP species as proposed by Yamanaka (1999) and Suzuki *et al.* (2002).

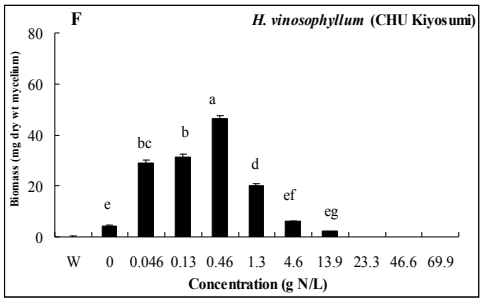
Ammonia fungi



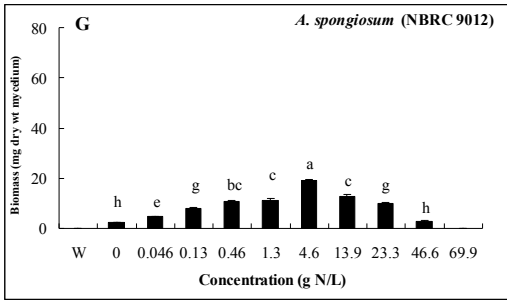


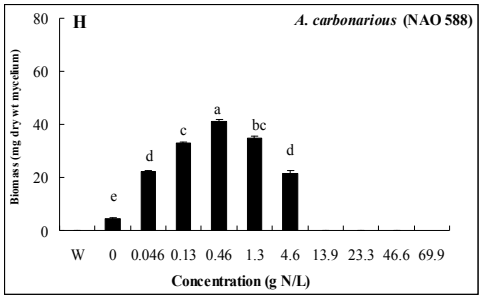


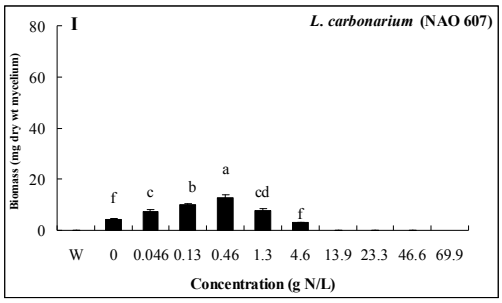


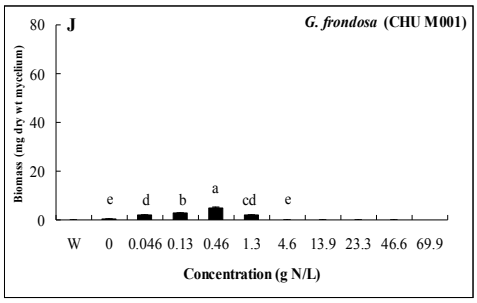


Non-Ammonia fungi









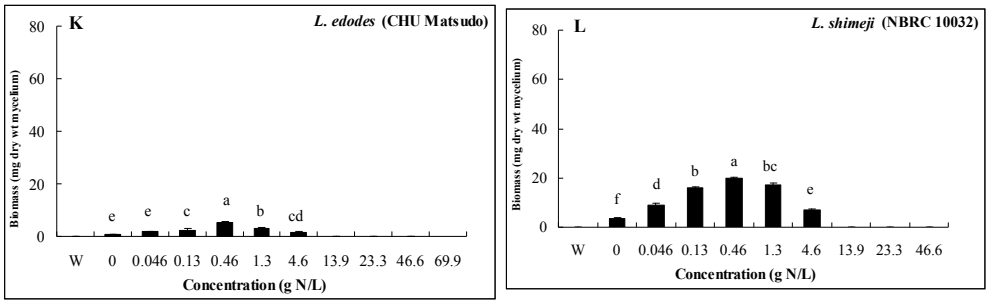
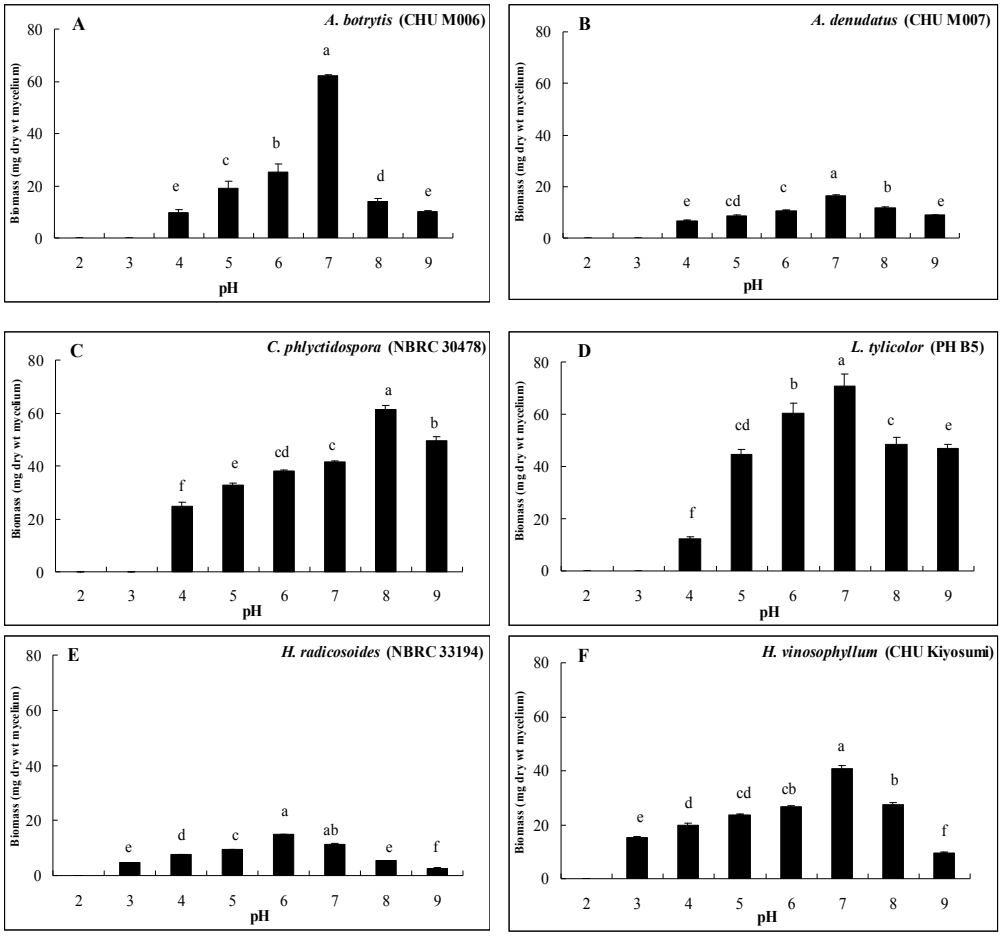


Fig. 1. The effects of different concentrations of urea on biomass growth of ammonia fungi. A, B: early stage EP species; C, D: late stage EP species; E, F: LP species; G-K: saprobic non-ammonia fungi; L: an ectomycorrhizal non-ammonia fungus; vertical bar indicates SE of means (n=5). Different letters on the columns show significantly differences at $P < 0.05$ according to the Tukey-Kramer test.

Ammonia fungi



Non-Ammonia fungi

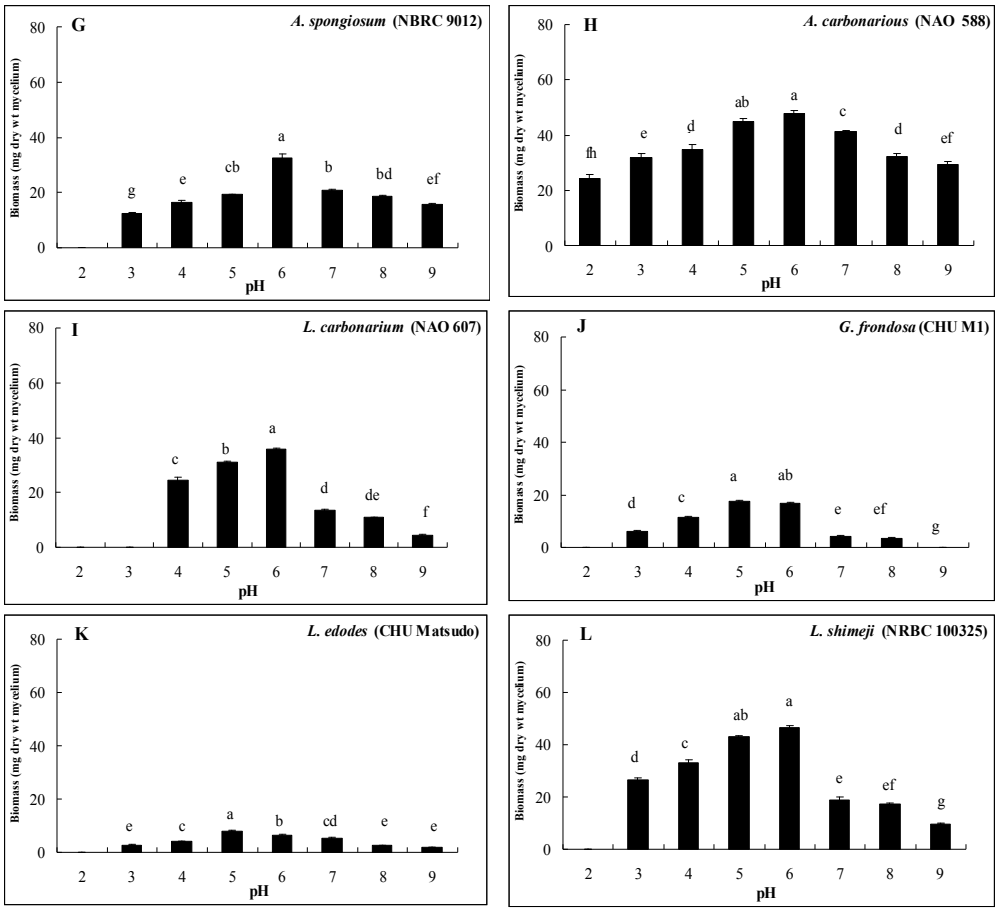


Fig. 2. The effects of pHs on biomass growths of ammonia fungi. A, B: early stage EP species; C, D: late stage EP species; E, F: LP species; G-K: saprobic non-ammonia fungi; L: an ectomycorrhizal non-ammonia fungus; vertical bar indicates SE of means (n=5). Different letters on the columns show significantly differences at $P < 0.05$ according to the Tukey-Kramer test.

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Table 1. Urease activities of ammonia fungi and non-ammonia fungi

Fungal species	Isolate no.	Activity		Incubation time[e]	Incubation time[f]	Colony diameter[g]	Recovery time
		(+ Peptone)	(- Peptone)	(day)	(day)	(mm)	(day)
Anamorphic fungi							
Early stage EP species							
Anamorphic fungi							
Amblyosporium botrytis	CHU M006	+++	+++	3	9	77.8 ± 0.9	*
Doratomyces putredinis	CHU 348	+++	+++	3	9	41.0 ± 1.0	*
Ascomycota							
Ascobolus denudatus	CHU M007[j]	+++	+++	3	9	79 .0± 0.7	*
Humaria velenovskyi	CHU 6567	++	+++	3	9	14.9 ± 0.7	*
Peziza moravecii	CHU A 6001	+++	+++	3	12	61.0 ± 0.8	*
Psudombrophila petrakii	Yamanaka 236[k]	+++	++	3	10	15.0 ± 0.3	
Late stage EP species							
Basidiomycota							
Coprinopsis cinerea	NBRC 10011	+[c]	++	6	21	11.0 ± 1.6	*
Coprinopsis echinosporus	NAO 630[l]	++	+++	6	21	60.0 ±0.4	*
Coprinopsis neolagopus	NBRC 100013	++	+++	6	15	22.0 ± 2.0	*
Coprinopsis phlyctidospora	NBRC 30478	+++	+++	6	12	61.6 ± 1.0	*
Crucispora rhombisperma	Fukiharu 248[m]	+	+	6	13	14.0 ± 0.3	*
Lepista sordida	NAO 561	+	+	6	30	6.6 ± 0.3	*
Lepista tarda	NAO 564	+	+	6	22	12.6 ± 0.5	*
Lyophyllum abustum	NAO 599	-[d]	-	6	30	0 ± 0	9
Lyophyllum tylicolor	PH (B5)[n]	+++	+++	6	21	43.8 ± 4.0	"
Lyophyllum mephitacum	PH (B3)	+	+	6	18	15.2 ± 0.3	*
Late phase species							
Basidiomycota							
Alinicola lactariolens	Fukiharu 14299	++	++	15	24	24.4 ± 0.7	*
Hebeloma radicosoides	NBRC 33194	+	+	15	30	18.2 ± 0.3	*
Hebeloma spoliotum	NAO 664	+	+		30	28.0 ± 0.4	*
Hebeloma vinosophyllum	CHU Kiyosumi	++	++	15	24	20.7 ± 1.7	"
Non-ammonia fungi							
Anamorphic fungi							
Amblyosporium spongiosum	NBRC 9012	+++	+++	3	9	80 .0± 1.0	*
Arthrobotrys superba	MS05[o]	-	-		30	17.9 ± 0.4	*
Isaria farianosa	MH 199044[p]	+++	+++	6	24	83.0 ± 1.0	*
Isaria japonica	MH 199012	-	-		30	79.0 ± 0.4	*
Thricoderma viride	IFM 40938	++	+++	6	12	51.0± 1.0	*
Zygomycota							

Mucor mucedo	CHU M1-1	-	-		6	76.8 ± 1.0	
Ascomycota							
Ascobolus carbonarius	NAO 588	+++	++	6	12	23.0 ± 0.2	*
Ascobolus epimyces	CHU 74-5H-42F	+++	++	12	24	36.6 ± 0.8	*
Cordyceps militaris	MH 199006	-	-		30	77.0± 0.6	*
Cordyceps takaomontana	MH 199017	+	+	6	27	68.0 ± 0.5	*
Trichophaea abundans	CHU 94-5H-50D	-	-		18	29.6 ± 0.9	*
Warcupia terrestris	CBS-891-69	+++	+++	3	15	16.9 ± 0.4	*
Basidiomycota							
Coprinellus angulatus	NBRC 30971	-	-		30	0 ± 0	6
Coprinellus flocculosus	CHU AO	-	-		30	0 ± 0	15
Coprinopsis atramentaria	NBRC 30626	-	-		30	0 ± 0	15
Coprinopsis gonophylla	NAO 216	++	+++	3	9	81.0 ± 0.2	*
Coprinopsis lagopus	CHU AO	+	+	21	30	14.8 ± 0.9	*
Coprinopsis radiata	NBRC 30118	-	-		30	0 ± 0	15
Coprinus comatus	CHU F074	+	+	21	30	11.8 ± 0.10	*
Coprinus ochraceo–velatas	NBRC 30121	-	-		30	0 ± 0	15
Coprinus sterquilinus	CHU S2	+	+	6	18	14.0 ± 0.2	*
Flammulina velutipes	CHU 2009	-	-		30	18.0 ± 0.5	*
Grifola frondosa	M51[q]	-	-		30	0 ± 0	15
Lyophyllum carbonarium	NAO 607	+	+	15	30	14 .0± 0.3	*
Lentinula edodes	CHU Matudo	-	-		30	0 ± 0	6
Marasmius sp.	CHU Kiyosumi	++	+	9	26	26.0 ± 0.3	*
Omphalotus japonicus	CHU 2001	-	-		30	0 ± 0	30
Polyporus arucalarius	CHU 69B	-	-		30	18.8 ± 0.4	*
Pholiota carbonaria	H16225	-	-		30	0 ± 0	30
Pholiota highlendesis	CHU9003	-	-		30	0 ± 0	30
Phaeolepiota aurea	CHU 9001	-	-		30	0 ± 0	30
Polyporellus satommosus	CHU 2006	-	-		30	0 ± 0	30
Schizophyllum commune	CHU 2007	-	-		30	17.0 ± 0.6	*
Basidiomycota							
*Hebeloma radicosum***	NBRC 32938	-	-		30	0 ± 0	12
Hebeloam crustuliniforme	NBRC 3097	+	+	21	30	10.0 ± 0.4	*
Lyophyllum shimeji	NBRC 100325	-	-		30	0 ± 0	15
Tricholoma matsutake	NBRC 33137	-	-		30	0 ± 0	18

[a] Strong urease activity; [b] Moderate urease activity; [c] Weak urease activity; [d] No urease activity; [e]Days required for the first detection of urease activity;
[f] Days required for the first detection of maximum urease activity; [g]Maximum colony diameter (mm) on the day of the first detection of maximum urease activity;
[h]Means ± SE (n=5);
[i] Survival test has not been done since the isolate grew in urea;
[j] Isolates number CHU indicates the stock cultures of Faculty of Education, Chiba University, Japan.

[k] Isolate number Yamanaka indicates the stock cultures donated by Dr. Takashi Yamanaka (Forestry of Forest Products Research Institute, Ibaraki, Japan) to Faculty of Education, Chiba University, Japan.
[l] Isolates number NAO indicates the stock cultures donated from Naohiko Sagara (Prof. Emeritus, Kyoto University) to Faculty of Education, Chiba University, Japan.
[m] Isolates number Fukiharu indicates the stock cultures donated from Dr. Toshimitsu Fukiharu (Natural History Museum and Institute Chiba, Japan) to Faculty of Education, Chiba University, Japan.
[n] Isolates number PH indicates stock culture of Microbiology Lab, Biotechnology Center of HoChiMinh City, Vietnam.
[o] Isolates number MS indicates the stock culture donated from Minoru Saikawa (Prof. Emeritus, University of Tokyo Gakugei) to Faculty of Education, Chiba University, Japan.
[p] Isolates number MH indicates the stock cultures of Mushroom Research Laboratory, Hokuto Corporation, Nagano, Japan.
[q] Isolate number M indicates the stock cult ure of Mori & Co, LTD, Gunma, Japan.
* Sometimes occur at late stage; **: Postputrefaction fungus, but not ammonia fungus.
Ammonia fungi: Isolates obtained from fruiting body occurred by urea treatment, except for *L. sordida* and *L.tarda* which were obtained from water grass wastes.