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Gerronema nemorale (Basidiomycota, Agaricomycetes): anatomic-morphological, cultivational, enzymatic and molecular characteristics and its first records in the Republic of Korea

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The basidiomycetous agaric *Gerronema nemorale* Har. Takah. was collected at several localities in the Republic of Korea (South Korea). A macro- and microscopic description and cultivational characteristics are given. Also physiological studies were performed. These included measuring of extracellular ligninolytic enzymes and monitoring of protein and glucose concentration in media. Its placement in the genus *Gerronema* in the recent sense is proven using molecular methods.

Key words: *Gerronema*, Republic of Korea, cultural characteristics, ITS, ligninolytic enzymes, decolorisation test, protein assay.

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Lupenatá houba *Gerronema nemorale* Har. Takah. byla nalezena na několika lokalitách v Korejské republice (Jižní Korea). Autoři uveřejňují její podrobný makroskopický a mikroskopický popis, charakteristiku v kultuře a enzymatickou, proteinovou a glukozovou charakteristiku. Na základě molekulárních metod je potvrzeno její zařazení do rodu *Gerronema* v jeho současném pojetí.

INTRODUCTION

In July 2007, the authors made several joint field excursions in the northern and central parts of the Republic of Korea (South Korea). The main task was to collect material of marasmioid and gymnopoid fungi for a joint research project. At several excursions, an interesting omphalioid basidiomycetous fungus was col-

lected; it was collected also later by the Korean co-authors. Its microscopic studies showed that it represents *Gerronema nemorale* Har. Takah., a fungus recently described as new to science from Honshu, Japan (Takahashi 2000). These collections represent the first published records in the Republic of Korea.

MATERIALS AND METHODS

Macroscopic and microscopic studies. The macroscopic description is based on fresh basidiocarps of three collections made by the first author. Microscopic features are described from dried material mounted in H_2O, KOH, Melzer's reagent and Congo Red using an Olympus BX-50 light microscope with a magnification of 1000×. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. Authors of fungal names are cited according to Kirk and Ansell (1992), herbarium acronyms follow Holmgren and Holmgren (1998).

Cultural studies. The cultures of *Gerronema nemorale* were obtained from basidiospores of fresh basidiocarps. These isolates were incubated at 23 °C in the dark in both Petri dishes (90 × 18 mm) containing PDA (potato dextrose agar, Difco) and test tubes containing PDB (potato dextrose broth, Difco). Isolates were preserved for future reference in KACC (Korean Agricultural Culture Collection) with the following accession numbers: KACC 43598 (from specimens preserved in BRNM 709773, Antonín 07.64), KACC 43599 (BRNM 709772, Antonín 07.100) and KACC 43600 (collection R. Ryoo KG161). Cultures from the collections of Antonín 07.140 and KG137 were not successful. The culture mat was described using the terminology by Nobles (1965), Stalpers (1978) and Desjardin (1990). Microscopic features of the isolates were observed with an Olympus BX-51 light microscope and an Axio imager AI DIC-light microscope with a magnification of 1000×.

DNA extraction. DNA extraction was carried out according to the method proposed by Lee and Taylor (1990). Genomic DNAs were extracted using the mycelium of KACC 43599 and KACC 43600 and the basidiocarp of KG137. Small pieces of tissue were used to fill one-third of 1.5 ml Eppendorf microcentrifuge tubes. 500 µl of lysis buffer (0.5 M Tris-HCl, 0.5 M EDTA, 3 % SDS, and 1 % 2-mercaptoethanol) was added to each tubes before incubation for 3 hrs at 67 °C. 500 µl of buffer (phenol : chloroform : isoamyl alcohol ratio 25 : 24 : 1) was added to the DNA solutions. The samples were centrifuged at 14,000 rpm for 25 min. at 25 °C. 300 µl of aqueous phase containing the DNAs was transferred to new tubes. Each tube was supplemented with 10 µl of 3 M sodium acetate and 162 µl of isopropanol. The Eppendorf tubes containing the DNA solutions were centrifuged at 14,000 rpm for 15 min. at 4 °C. Supernatants were poured off and the pellets were centrifuged at 14,000 rpm for 5 min. at 4 °C. The DNA pellets were washed with cold 70 % ethanol, dried in a vacuum drier for 1 hr at 50 °C and resuspended in 50 µl of TE buffer (10 mM Tris-HCl and 0.1 mM EDTA). RNase was added to each sample and incubated at 37 °C for 1 hr.

PCR amplification. PCR amplification was performed according to the method described by Gardes and Bruns (1993). The forward primer ITS1-F and reverse primer ITS4-B were used for selective amplification of the complete ITS region of rDNA. PCRs were conducted in 50 µl of solutions containing 1.2 µl of template DNA mixture, 5 µl of 10x buffer (0.5 M KCl, 0.1 M Tris-HCl; pH 8.0, 0.1 % Triton X-100, 15 mM $MgCl_2$), 1 µl of 2.5 mM dNTP, 0.4 µl of 100 µM primer ITS1-F, 0.4 µl of 100 µM primer ITS4-B, 0.4 µl of Taq polymerase (5 unit/µl), and 36.6 µl of distilled water. Temperature cycling was performed with denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 1 min. at 72 °C. Thirty-five cycles were run with the first denaturation and last extension times extended to 5 min. at 72 °C. Purified DNAs were directly sequenced in an automatic sequencer (ABI Prism TM 377 DNA Sequencer) with the primers ITS1-F and ITS4-B for ITS rDNA.

Phylogenetic analysis. Sequences were edited with the DNASTAR computer package, whereby alignment of the sequences was performed using the CLUSTAL_X program (Thompson et al. 1997). Bayesian analysis was carried out using the MRBAYES computer program, version 3.1 (Ronquist

and Huelsenbeck 2003). The general time reversible model (GTR) was employed with gamma-distributed substitution rates for ITS alignment. Markov chains were run for 10^6 generations, saving a tree every 100th generation. Out of these, the first 1,000 trees were discarded. MRBAYES was used to compute a 50 % majority rule consensus of the remaining trees to obtain estimates for posterior probabilities of the groups. Branch lengths were computed as the mean values for the sampled trees.

Spot tests and dye decolorisation tests. Spot tests and dye decolorisation tests were carried out according to Stalpers (1978), Desjardin (1990) and Okino et al. (2000). The spot test was performed using an α-naphthol solution for laccase, a ρ-cresol solution for tyrosinase, and a pyrogallol solution for peroxidases. The reactions for laccase and peroxidases were conducted on advancing zones of marginal hyphae; the reaction for tyrosinase was performed on aerial mycelium of the culture mat.

The dye decolorisation test was performed using PDA with 0.2 g Congo Red of azo dyes, 0.5 g RBBR (Remazol Brilliant Blue R) of polymeric dyes, and 0.5 g methylene blue of heterocyclic dyes per 400 ml solution (Glenn and Gold 1983). Inoculum plugs of the KACC 43589, KACC 43590, KACC 43600 isolates were cut using a cork borer (4 mm diameter) and transferred to Petri dishes (90 × 18 mm) containing 20 ml PDA mixed with each dye compound. Triplicates of each isolate were incubated for 6 weeks at 25 °C in the dark.

Ligninolytic enzyme activity assay. Ligninolytic enzyme activity was analysed using Kirk's media (Kirk et al. 1978) and PDB. Kirk's media are composed of 1 ml of mineral solution per 1 l of basic media. The mineral solution contained 1.5 g of nitriliotriacetate, 3.0 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $MnSO_4 \cdot H_2O$, 1.0 g of NaCl, 0.1 g of $FeSO_4 \cdot H_2O$, 0.1 g of $CoCl_2 \cdot 6H_2O$, 0.1 g of $ZnSO_4 \cdot H_2O$, 0.01 g of $CuSO_4 \cdot 7H_2O$, 0.01 g of $AlK(SO_4)_2 \cdot 12H_2O$, 0.01 g of H_2BO_3 and 0.01 g of $Na_2MoO_4 \cdot 2H_2O$ per 1 l of distilled water. The basic media consisted of 2 g of KH_2PO_4, 0.5 g of $MgSO_4$, 0.1 g of $CaCl_2$, C-source (10 g of glucose) and N-sources (2.4 mM N of NH_4NO_3 and L-asparagine) per 1 l of distilled water. Erlenmeyer flasks (100 ml) containing 10 ml of Kirk's media or PDB were inoculated with five agar plugs (4 mm diameter). Cultures were incubated at 25 °C in the dark under stationary conditions. These were cultivated for 12, 60, 108, 156, 204, 252, and 300 hrs and the cultures were filtered through Whatman filter paper (No. 42).

Absorption spectra were recorded with a UV-Spectrophotometer (model: PowerWaveXS, Bio-Tek) for the activity of laccase, LiP (lignin peroxidases; E.C. 1.11.1.14) and MnP (manganese-dependent peroxidases), known as lignin degradation enzymes (Vares et al. 1995). Laccase activity tests were carried out using 33 µl of 5 mM ABTS [2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonate] as substrate and 250 µl of 0.1 M sodium acetate buffer (pH 4.6). LiP activity tests were carried out using 25 µl of 2 mM veratryl (3,4-dimethoxybenzyl) alcohol as substrate and 25 µl of 0.4 mM hydrogen peroxide in 225 µl of 50 mM sodium tartrate buffer (pH 4.0). MnP activity tests were performed using 10 µl of 1 mM guaiacol as substrate, 10 µl of 1 mM $MnSO_4 \cdot H_2O$, 10 µl of 1 mM hydrogen peroxide in 200 µl of 0.5 M sodium tartrate buffer (pH 3.0). Each kinetic measurement was determined in 420 nm (molar extinction coefficient ε = 36,000/Mcm) for laccase, 310 nm (ε = 9,300/Mcm) for LiP, and 525 nm (ε = 121,000/Mcm) for MnP. The enzyme activity was calculated using the following expression:

$$\text{Enzyme activity (Unit/ml)} = (\Delta A \times \Delta V \times 10^6) / (\varepsilon \times \Delta t \times \Delta S)$$

(ΔA: absorbance difference value, ΔV: total volume, Δt: reaction time in min., ε: molar extinction coefficient, ΔS: material volume).

Protein concentrations were measured using protein assay reagent (Bio-Rad), according to the method described by Bradford (1976). Bovine serum albumin (Bio-Rad) was the standard protein used. Reducing glucose and pH (pH meter: Model 750P, Istek) were simultaneously measured in Kirk's media. Glucose remaining in the media was measured according to the method by Miller (1958) using the following reagents: 10 g of 3-5-dinitrosalicylic acid, 2 g of phenol, 0.5 g of sodium sulphite, 200 g of potassium sodium tartrate, and 500 ml of 2 % NaOH buffer per 1 l of distilled water. Each absorbance was analysed at 595 nm and 575 nm with the same UV-Spectrophotometer.

RESULTS

Gerronema nemorale Har. Takah., Mycoscience 41: 16. 2000.

Description of collected basidiocarps

Basidiocarps (Figs. 1, 2) single or in groups. Pileus 6–17 mm broad, low convex with depressed centre and inflated margin when young, then applanate with (deeply) umbilicate centre and inflexed margin, finally with uplifted margin, almost entirely slightly translucently striate when moist, slightly striate and crenulate at margin in younger basidiocarps, finely tomentose when young, later innately finely radially fibrillose to striate-rugulose except for centre, greyish yellow to olive brown when young (4B4, 4E3–4), then greyish yellow to blond, olive brown or reddish blond (4B–D4, 5C4). Lamellae moderately distant to distant, L = 17–20, l = (1–)2–3 (irregular), broadly adnate to shortly decurrent when young, then decurrent, green-yellow (3–4A4, 4C5) when young, then greyish greenish yellow (3–4A–B5), edge concolorous, finely pubescent. Stipe 17–38 × 0.75–2 mm, cylindrical, broadened at apex, subbulbose (up to 3.5 mm) at base, straight, entirely pubescent, greyish greenish yellow (3A–B3–4) with pale yellowish base when young, then paler (3–4A3–4) with pale yellowish base; with white basal mycelium. Context hollow in stipe, yellowish (cream) in pileus, in stipe cortex with the same colour as its surface, without any special smell, taste slightly bitterish after longer mastication.

Basidiospores 8.0–10(–11) × (4.5–)5.0–6.0 µm, average = 9.2 × 5.6 µm, E = 1.5–2.0, Q = 1.7–1.8, broadly ellipsoid to broadly ellipsoid-fusoid, thin-walled, smooth, hyaline. Basidia easily collapsing (only one found), e.g. 30 × 8.0 µm, 4-spored, clavate. Basidioles 15–45 × 3.0–9.0(–12) µm, clavate, cylindrical or subfusoid. Cheilocystidia abundant, (25–)32–50(–65) × 6.0–16 µm, variable in shape, clavate, subcylindrical, lageniform, subutriform or (sub)fusoid, sometimes rostrate, irregular or with projection(s), thin-walled. Pleurocystidia absent. Trama hyphae ± cylindrical, both thin- and thick-walled (then walls up to 1.0 µm), branched, 2.0–13 µm wide. Pileipellis a cutis consisting of ± cylindrical, radially arranged, thin- to slightly thick-walled, smooth or scatteredly diverticulate, up to 8.0 µm wide hyphae with non-incrusted, pale olivaceous grey walls in KOH; terminal elements slightly to distinctly swollen (broadly clavate), often irregular or with projection(s); true pileocystidia absent. Stipitipellis a cutis made up of cylindrical, parallel, slightly thick-walled (walls up to 1.0 µm), smooth, up to 6.0 µm wide hyphae. Caulocystidia numerous, 21–50 × (4.0–)6.0–10 µm, cylindrical, fusoid, clavate or subutriform, sometimes irregular, rostrate or branched, thin- to slightly thick-walled, sometimes 2(–3)-celled, pale yellowish in H_2O and KOH. Clamp-connections present in all tissues (Fig. 3).

Chemical reactions. All parts of the basidiocarps are inamyloid and non-dextrinoid.

Fig. 1. *Gerronema nemorale.* Guri, Donggureung , 17 July 2007. Photo: V. Antonín.

Fig. 2. *Gerronema nemorale.* Chuncheon, Dongsan-myon, Bongmyeong-ri, 22 July 2007. Photo: V. Antonín.

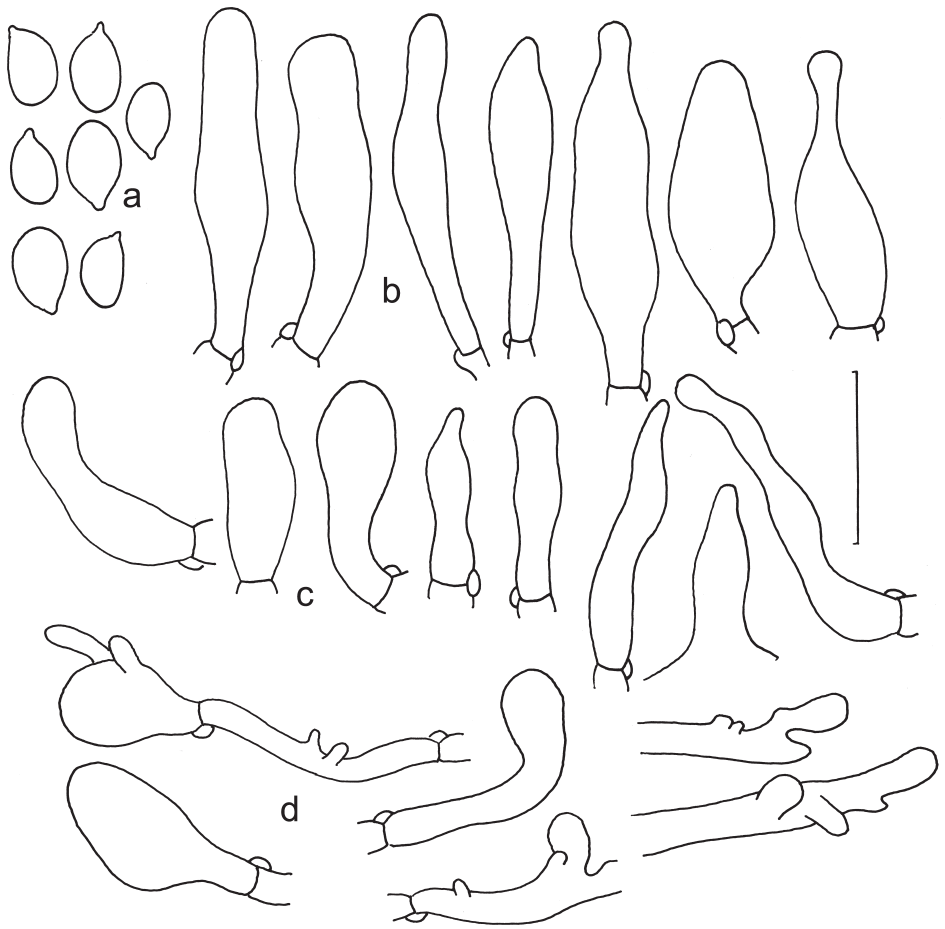


Fig. 3. *Gerronema nemorale*. a) basidiospores, b) cheilocystidia, c) caulocystidia, d) pileipellis elements. Scale bar = 20 µm.

E c o l o g y. Saprotrophic, lignicolous; growing on stems, branches and twigs of *Quercus mongolica* and an unidentified broadleaved tree. At the type locality in Japan, it was collected in a lowland forest with dominating *Pasania edulis*, *Quercus myrsinaefolia* and *Ligustrum japonicum* (Takahashi 2000).

D i s t r i b u t i o n. The species was originally described from central Honshu, Japan, where it is said to be common (Kanagawa, Tokyo; Takahashi 2000). Recently, it has been collected at five localities in the northern and central part of the Republic of Korea. However, it has probably also been collected on the South Korean island

of Jeju (Kim et al. 2005, see discussion below). It seems to be rather common in this country. It is probably also widely distributed in the East-Asian region.

Collections examined. Republic of Korea: Guri, Donggureung (East Nine Royal Grave), 17 July 2007 leg. V. Antonín 07.64 (BRNM 709773). – Chuncheon, Dongsan-myon, Bongmyeong-ri, Experimental Forest of Kangwon National University, 22 July 2007 leg. V. Antonín (07.100), R. Ryoo and H. D. Shin (BRNM 709772). – Hongcheon, Bukbang-myon, Seongdong-ri, 27 July 2007 leg. V. Antonín (07.140), R. Ryoo and H. D. Shin (BRNM 709771). – Hongcheon, Bukbang-myon, Seongdong-ri, 20 Aug. 2007 leg. R. Ryoo (KG 137). – Muju, Deogyusan National Park, Cheon-yeon falls, 24 Aug. 2007 leg. R. Ryoo (KG 161).

Cultural characteristics

Nobles Code: 2. 3. 9. 32. 36. 39. 44. 45. 54.

Stalpers Code: 1. 3. 8. 13. 25. (30). 31. 38. 39. 45. 52. 89.

Macromorphological characteristics. On PDA (n=3), diameter 12–15 mm in one week, 80–85 mm in 4–5 weeks; culture mat tightly interwoven, initially felty, pale yellow or (pale) cream colour; advancing zone submerged and translucently yellow or dark yellow; plug densely felty and yellowish white; reverse coloration more or less dark yellow; odour not distinctive (Fig. 4a).

Micromorphological characteristics. On PDB (n=3), hyphae 2.1–2.9 µm wide, thin-walled, differentiated, straight and more or less irregular in outline, rarely branched, hyaline (Fig. 5a). Clamp-connections 3.7–4.1 × 2.1–2.5 µm, the clamp making an angle of about 90° with the hypha (Fig. 5b). Crystals scattered on PDA (Fig. 5c).

Phylogenetic characteristics

Three isolates of *G. nemorale* (KACC 43599, KACC 43600 and KG137) were compared with sequences within the omphalioid species. Except for *G. nemorale*, all sequences were obtained from GenBank (Tab. 1). These clades showed a high probability value (1.00 in all branches) in the MCMC tree (Fig. 6), in which all sequences of *G. nemorale* formed a monophyletic group among which no sequence difference is found. These sequences formed the *Gerronema* group with *G. subclavatum* and *G. strombodes*, strongly supported by a high probability value of 1.00. *G. strombodes* was reallocated to *Chrysomphalina strombodes* by Clémençon (1982). However this species was significantly distant from the *Chrysomphalina* clade including *C. chrysophylla* and *C. grossula*. Species of the genus *Omphalina* formed a sister clade with *Gerronema* distantly separated from the *Arrhenia* clade. The *Chrysomphalina* clade was distant from both of them and formed an unrelated genus.

We obtained a sequence of *Megacollybia platyphylla* from GenBank (according to Moncalvo et al. 2002), and tried to re-analyse the phylogenetic relationship with our fungi. Unfortunately, this sequence is more closely related with the *Gerronema* group than the omphalioid group (results not shown). Therefore, we think *Megacollybia platyphylla* is not appropriate as an outgroup. Based on a study by

Tab. 1. Strains of GenBank accession numbers and DNA source used for this research.

Taxon	GenBank accession no.	DNA Source
Arrhenia epichysium	U66442	Lutzoni, 1997 as *Omphalina*
Arrhenia philonotis	U66449	Lutzoni, 1997 as *Omphalina*
Arrhenia sphagnicola	U66453	Lutzoni, 1997 as *Omphalina*
Arrhenia velutipes	U66455	Lutzoni, 1997 as *Omphalina*
Chrysomphalina chrysophylla	U66430	Lutzoni, 1997
Chrysomphalina grossula	DQ486689	Matheny, 2006
Chrysomphalina strombodes	U66433	Lutzoni, 1997 as *Gerronema*
Gerronema subclavatum	U66434	Lutzoni, 1997
Omphalina pyxidata	U66450	Lutzoni, 1997
Omphalina rivulicola	U66451	Lutzoni, 1997
Megacollybia platyphylla	AF498289	Matheny, 2006
Megacollybia platyphylla	DQ 249275	Matheny, 2006
Multiclavula corynoides	U66440	Lutzoni, 1997
Multiclavula vernalis	U66439	Lutzoni, 1997

Redhead (2002), we selected two sequences, *Multiclavula corynoides* and *M. vernalis*, as the outgroup. However, the position of the main groups (*Arrhenia*, *Omphalina*, *Chrysomphalia* and *Gerronema*) is consistent in both analyses.

Spot tests and dye decolorisation tests

The results of spots test using α-naphthol solution and a pyrogallol solution were positive, but the test using ρ-cresol solution was negative. Evaluation of positive results showing colour changes was made 30 min after treating solutions: purple for laccase and yellowish-brown for peroxidases (Fig. 4b).

The colour of media containing Congo Red was changed from red to purple or dark red after 10 days of incubation, and the colour of media containing RBBR was changed from blue to orange after 10 days of incubation for the dye decolorisation test (Figs. 4c, d). However the media containing methylene blue were not obtained from decolorisation test results, because methylene blue did not support mycelial growth.

Ligninolytic enzyme activity

The ligninolytic enzyme activity of *G. nemorale* showed similar curves on Kirk's media and PDB for laccase, LiP and MnP, but it showed a higher peak on PDB than on Kirk's media. All samples of laccase activity reached a maximum yield after 108 hrs, whereas the maximum yield of LiP activity was shown after 156 hrs. On the other hand, the maximum point of MnP activity was reached after 204 hrs on Kirk's media and 252 hrs on PDB (Figs. 7a-c).

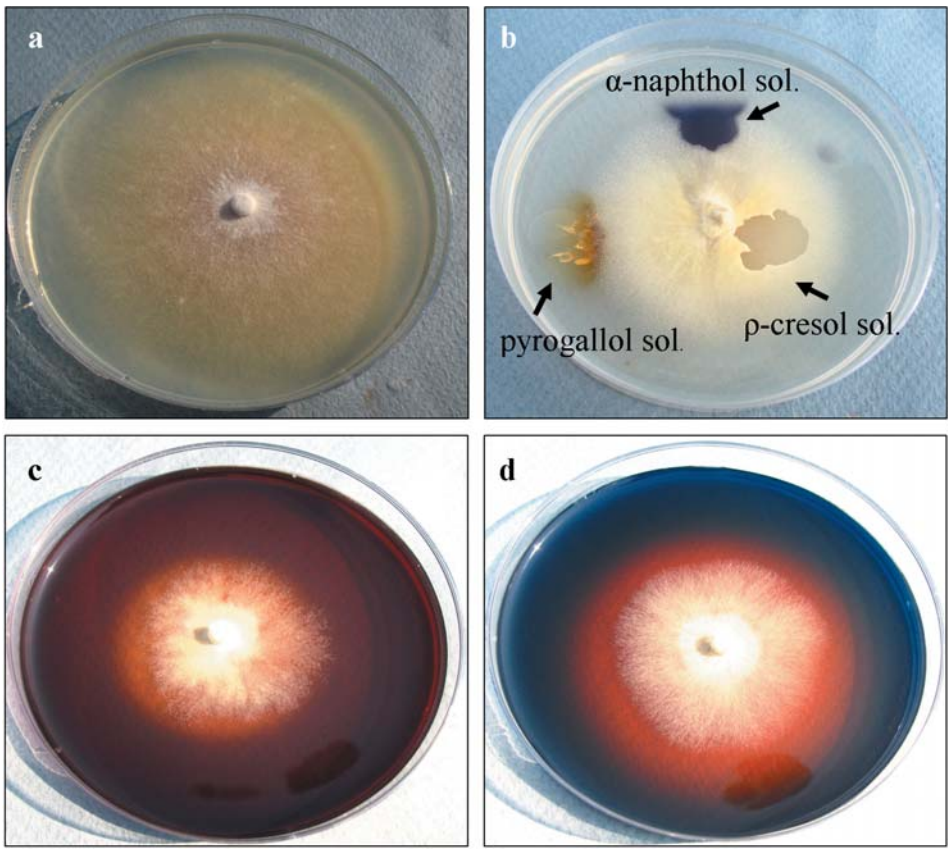


Fig. 4. Macromorphological culture characteristics of *Gerronema nemorale* (KACC 43600). a) on PDA after 5 weeks at 25 °C in the dark, b) spot test: on PDA after 3 weeks at 25 °C in the dark, c) on PDA containing Congo Red after 3 weeks at 25 °C in the dark, d) on PDA containing RBBR after 3 weeks at 25 °C in the dark. Photo: R. Ryoo.

Total protein concentration of *G. nemorale* showed a similar curve as laccase activity (Fig. 7d). In all cases, protein concentration showed a peak after 108 hrs. The peaks of PDB showed a more or less abundant protein concentration in contrast to Kirk's media. It was a rising curve similar to the protein sigmoid curve between 12 hrs and 156 hrs. However the dry weight showed a rapid increase after 156 hrs and had its maximum after 204 hrs (Fig. 7e). Glucose was rapidly reduced after 156 hrs, and pH gradually decreased (Fig. 7f). This was an inverse trend between dry weight of the mycelium and glucose remained in the media.

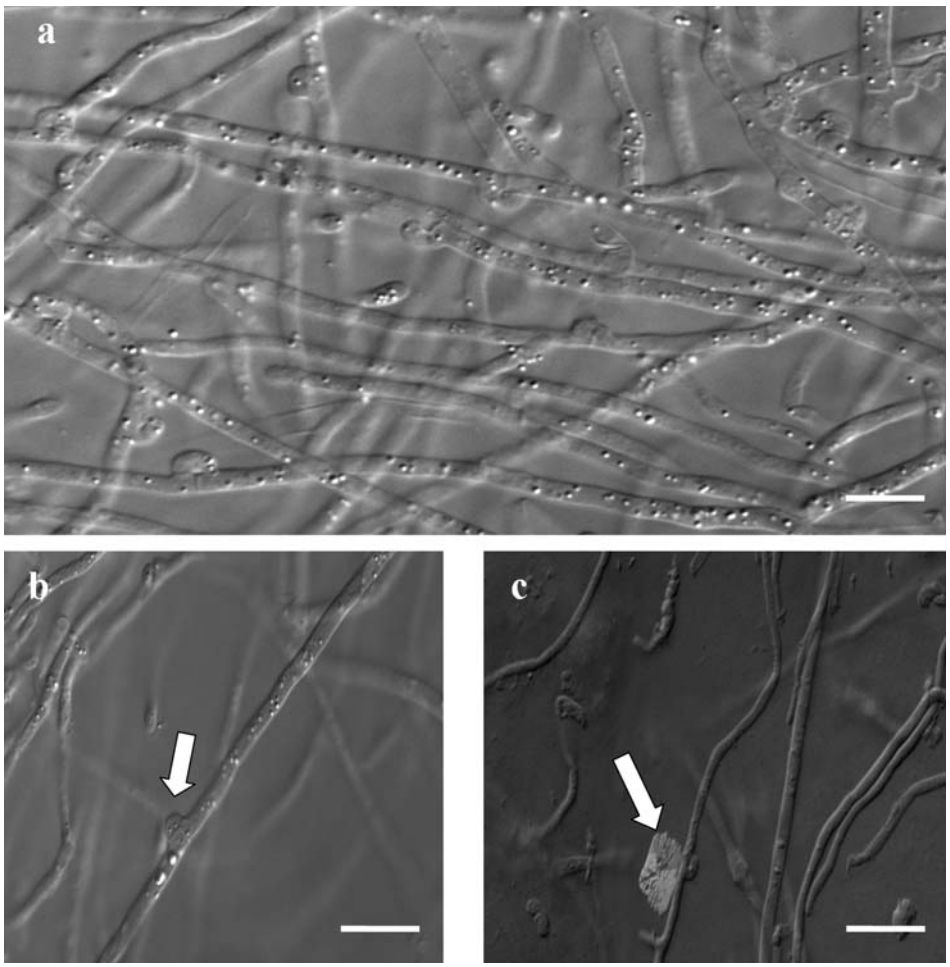


Fig. 5. Micromorphological culture characteristics of *Gerronema nemorale* (KACC 43599). a) hyphae on PDB, b) clamp-connections on PDB, c) crystal on PDA. Photo: R. Ryoo. Scale bar = 10 µm.

DISCUSSION

Gerronema nemorale is characterised by having small omphalioid basidiocarps with innately fibrillose, olivaceous coloured pilei, abundant variable cheilocystidia, often clavate terminal cells in the pileipellis, and being lignicolous. According to Singer´s concept (Singer 1964), it belongs to sect. *Xanthophylla* Singer because of well-developed clamp-connections, and, according to Singer (1986), to subgen. *Gerronema*, sect. *Xanthophylla* Singer. For a more detailed discussion, see

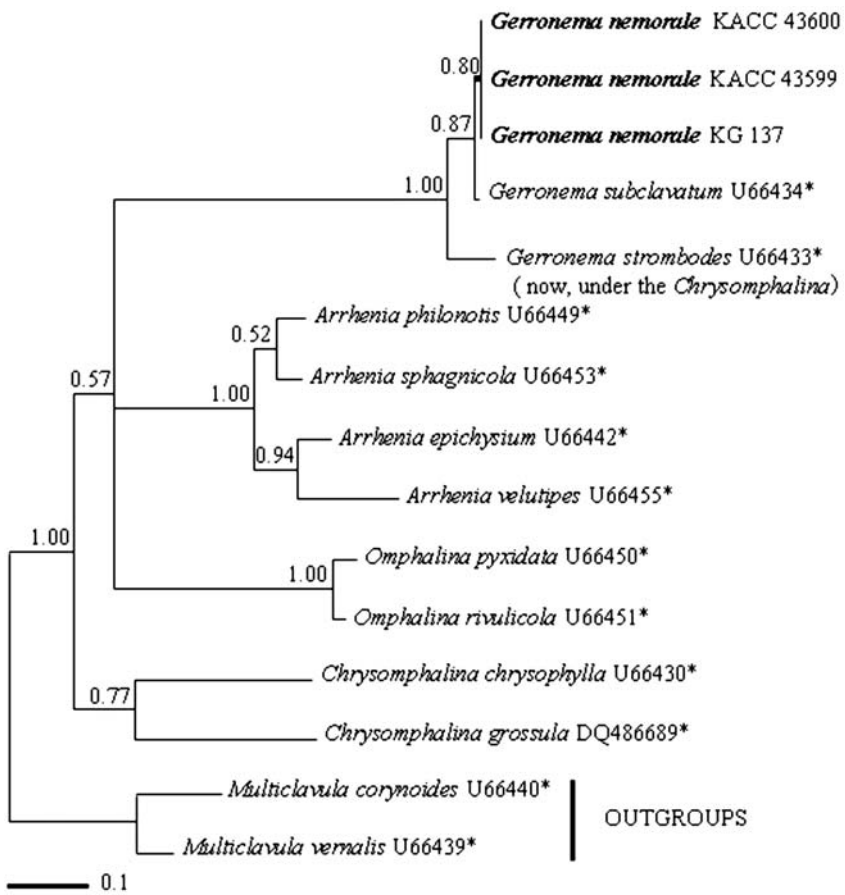
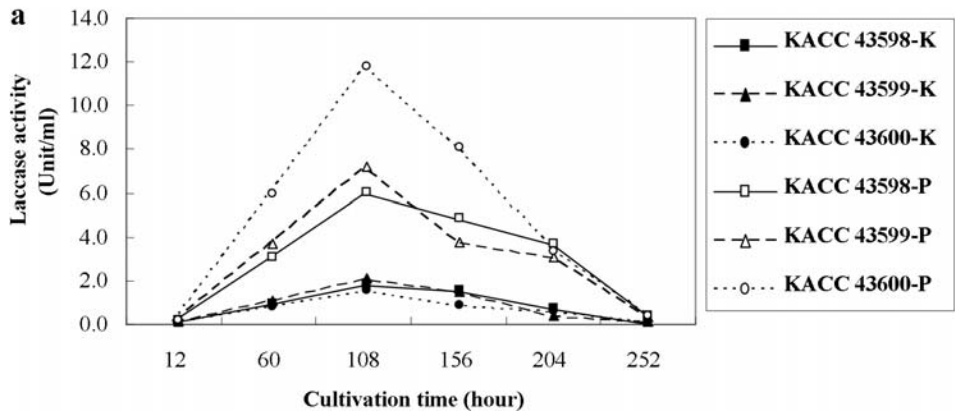


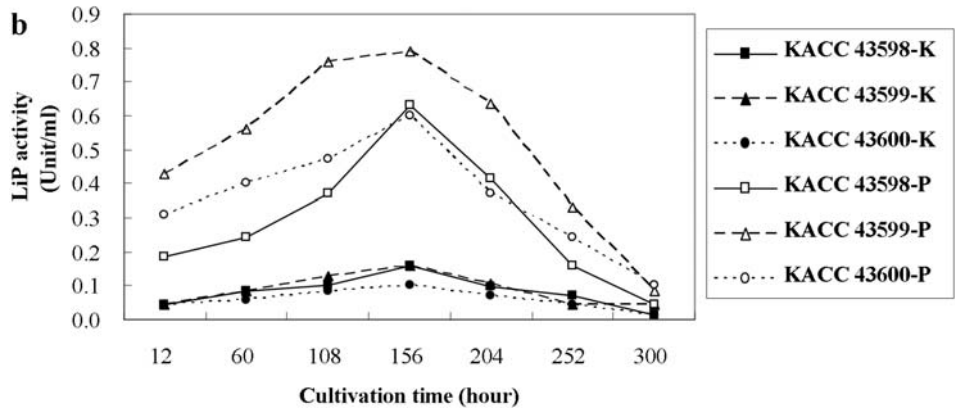
Fig. 6. Phylogenetic tree for *Gerronema nemorale* isolates (KACC 43599, KACC 43590, KG137) inferred from Bayesian analysis of the complete ITS region (ITS1, 5.8S rDNA, and ITS2), showing mean branch lengths of a 50 % majority rule consensus tree, obtained from an MCMC analysis of one million generations. An asterisk (*) denotes a sequence from GenBank.

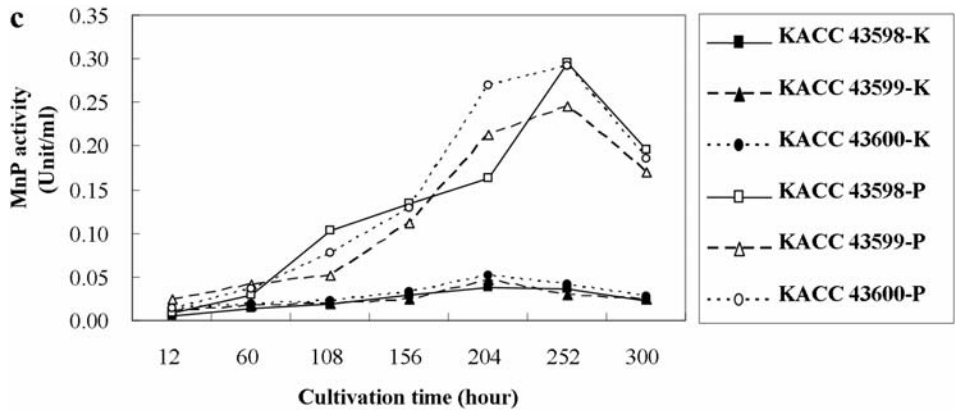
Takahashi (2000). Another lignicolous *Gerronema* species, *G. corticiphilum* L. N. Vassiljeva, described (as *G. corticiphila*) from the Russian Far East, differs especially by white, wood-coloured, pale, rarely sulphurous coloured pilei and distinctly longer and narrower (13–17 × 4–5 µm), subulate basidiospores (Vassiljeva 1973).

Outside of Asia, *G. nemorale* has been reported from Mexico (Veracruz; López and García 2002). Although the Mexican fungus seems to be macroscopically similar to our species, it has distinctly smaller basidiospores (7–8 × 6–7 µm) and probably represents a different species.

Kim et al. (2005) published two photographs of *G. nemorale* from Mount Halla (Jeju Island) as *Omphalina epichysium*. Although both photographs represent


a
Laccase activity (Unit/ml)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
12
60
108
156
204
252
Cultivation time (hour)
KACC 43598-K
KACC 43599-K
KACC 43600-K
KACC 43598-P
KACC 43599-P
KACC 43600-P


b
LiP activity (Unit/ml)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12
60
108
156
204
252
300
Cultivation time (hour)
KACC 43598-K
KACC 43599-K
KACC 43600-K
KACC 43598-P
KACC 43599-P
KACC 43600-P


c
MnP activity (Unit/ml)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
12
60
108
156
204
252
300
Cultivation time (hour)
KACC 43598-K
KACC 43599-K
KACC 43600-K
KACC 43598-P
KACC 43599-P
KACC 43600-P

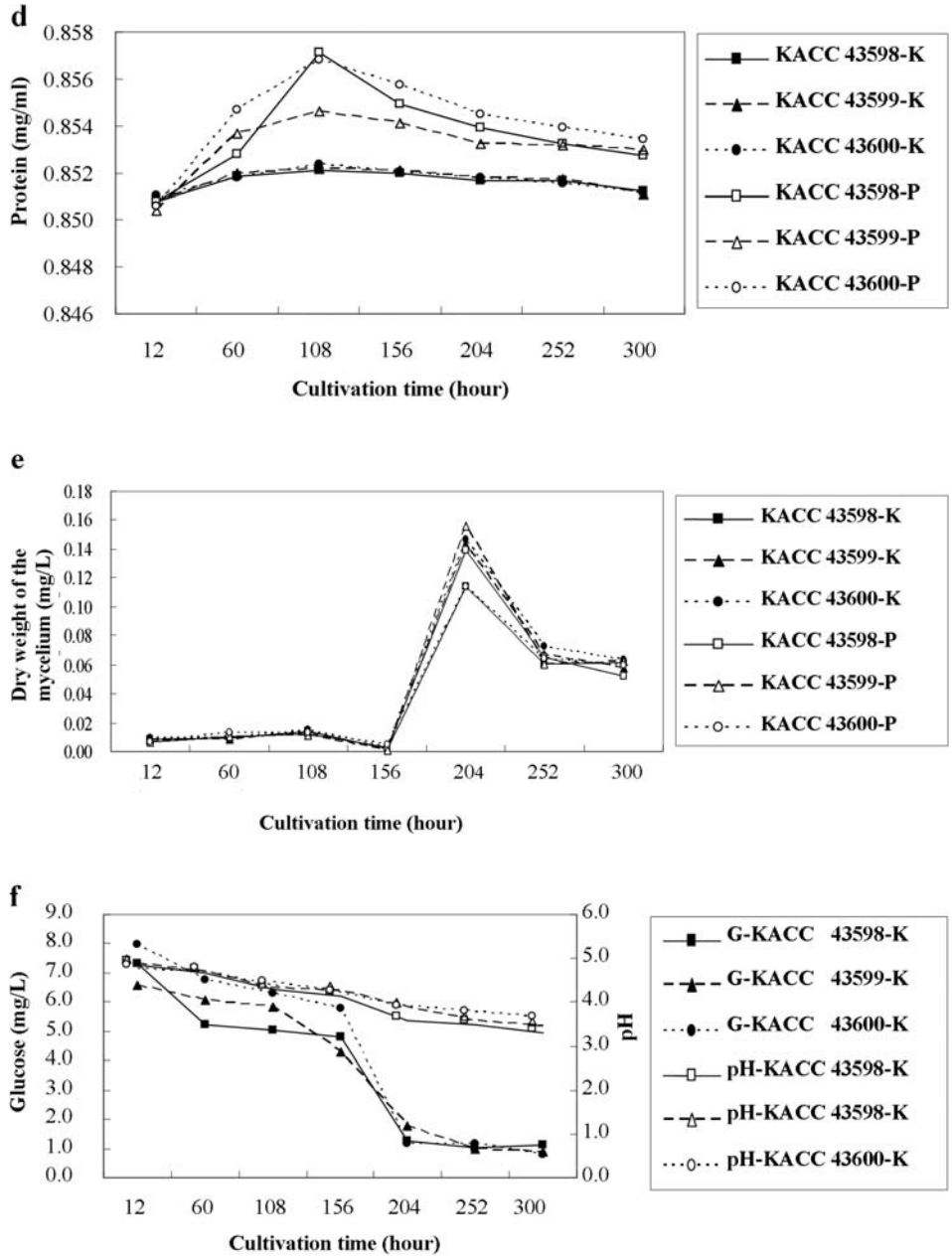


Fig. 7. Ligninolytic enzyme activity of *Gerronema nemorale* (KACC 43598, KACC 43599, KACC 43600). a) laccase activity, b) LiP activity, c) MnP activity, d) protein concentration, e) dry weight of the mycelium, f) remaining glucose and pH in media. K: Kirk's media. P: PDB. G: glucose.

our *Gerronema* species, the mentioned size of the basidiospores (6.0–8.2 × 3.5–4.8 µm) is distinctly smaller with values around the lower limit of the basidiospore variability of *O. epichysium*. *Omphalina epichysium* (Pers.: Fr.) Quél. is a lignicolous species with a dark grey-brown to ash-coloured pileus and stipe, (7–)8–9(–10) × (3.5–)4–4.5(–6) µm large basidiospores which grows on decaying wood (stems, trunks) of conifers (Bon 1997).

The genus *Gerronema* in the sense of Singer (1964, 1986), however, is polyphyletic (Moncalvo et al. 2002). Norvell et al. (1994) restricted the genus *Gerronema* to lignicolous species with thin-walled basidiospores and typical sarcodimitic tissue. In this concept, *Gerronema* is monophyletic and belongs to the /hydropoid clade together with *Hydropus* s. str., *Megacollybia*, *Clitocybula* and *Porotheleum fimbriatum* (Moncalvo et al. 2002). Matheny et al. (2006) also included *Hydnopolyporus fimbriatus*, *Henningsomyces candidus* and some *Mycena* species (*M. auricoma*, *M. amabilissima* and *M. aurantiidisca*) in the /hydropoid clade which belongs to the large marasmioid clade. However, they did not include any *Gerronema* species in their studies.

Having a sarcodimitic tissue structure, *G. nemorale* suits in the currently restricted concept of the genus *Gerronema*.

Cultivational characteristics were used for the identification and classification of *Aphyllophorales* (Nobles 1965, Stalpers 1986), and later successfully applied to *Agaricales* (Desjardin 1990). A group with omphalioid basidiocarps includes *Omphalina* (*Omphalia*), *Xeromphalina*, *Chrysomphalina*, *Arrhenia*, *Gerronema*, *Rickenella* and many species of *Clitocybe*. The cultivational characteristics of *G. nemorale* are more or less similar to those of *Xeromphalina campanella* (Nobles 1965, as *Omphalia campanella*) but differentiated by the hyphal structure and the growth rate. On the other hand, the cultivational characteristics of *G. nemorale* are similar to those of *Collybia radicata* (= *Xerula radicata*) and *Collybia velutipes* (= *Flammulina velutipes*) according to the species codes described by Nobles (1965). This is supported by the phylogenetic data of omphalioid genera obtained by molecular analyses (Fig. 6). The *Gerronema* and *Omphalina* species represent the monophyletic group of two sister clades, which is different from other groups. *G. nemorale* forms the *Gerronema* group along with *G. subclavatum* and "*Chrysomphalina*" *strombodes*, which is quite distant from *Arrhenia* and true *Chrysomphalina*. The *Chrysomphalina strombodes* sequence used in this study corresponds to the concept of Norvell et al. (1994) and Redhead et al. (2002). This was proven through the molecular analyses by Lutzoni (1997). According to them, *Chrysomphalina strombodes* is restricted to an American species belonging to the genus *Gerronema*, so its correct name should be *Gerronema strombodes* (Berk. & Mont.) Clémençon, whereas the correct name for the European taxon usually named *Chrysomphalina* (*Gerronema*) *strombodes* should be *Gerronema xanthophyllum* (Bres.) Norvell, Redhead & Ammirati.

In addition, one of the ecological characteristics of the genus *Gerronema* is the lignicolous habitat (Norvell et al. 1994). *G. nemorale* inhabits the part of sapwood under the bark. The ligninolytic enzymes laccase, LiP and MnP were required for effective degradation of the lignin within the woody cell wall (Schmidt 2006). Moreover, although the combination of MnP and laccase was usually studied in white-rot fungi, the combination of LiP and MnP was researched only in 40 % of the white-rot fungi (Schmidt 2006). Therefore it is inspiring to see the results of measuring the ligninolytic enzyme activity of *G. nemorale*. Laccase, LiP and MnP were detected in *G. nemorale* as extracellular enzymes. This study supports that *G. nemorale* belongs to lignicolous basidiomycetes.

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