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Noble strain of *Sparassis latifolia* produces high content of β-glucan

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ABSTRACT

Objective: To classify four new *Sparassis* strains (CLM1, CKM1, CKM2, and KJM1) using the internal transcribed spacer sequence and to elucidate their β-glucan content and mycelial growth.

Methods: Two different microbiological media were used to determine growth rate. The β-glucan contents were analyzed using the Megazyme Mushroom and Yeast Beta-Glucan kit. To determine the genetic relationships, phylogenetic trees were constructed using ClustalX. Multiple sequence alignments were printed and shaded with the BOXSHADE 3.21 program.

Results: In this study, four new *Sparassis* strains were isolated from the southern region of the Korea Peninsula. They were all classified into the *Sparassis latifolia* clade as a monophyletic group based on the internal transcribed spacer sequence. Mycelial growth rate of the CLM1 strain was highest in potato dextrose agar and potato dextrose agar larch. The β-glucan content of the CLM1 strain was highest at 29.5% (w/w). A high degree of sequence divergence was detected in the RNA polymerase second largest subunit II gene (RPB2) within *Sparassis* spp. tested. The putative amino acid sequences of the RPB2 had a distinct sequence. The nucleotide sequences of the RPB2's intron were also divergent among *Sparassis* spp., even though their nucleotide length was well conserved within *Sparassis latifolia*.

Conclusions: These results indicate that the nucleotide sequences and the amino acid sequences of RPB2 can be used to identify individual *Sparassis* sp. The *Sparassis* strain CLM1 may be best for developing a remedy to prevent or treat cancer and other chronic diseases.

1. Introduction

Sparassis Fr. species are distributed in Europe, Eastern Asia, North America, and Australia. They were known as brown-rot producers with a bipolar mating system [1]. These species primarily grow on the stumps of coniferous trees such as pine (*Pinus densiflora*), larch (*Larix kaempferi*), and Korean pine (*Pinus koraiensis*). The edible mushroom, *Sparassis* spp., plays significant industrial and economic role as sources of pharmaceutics, therapeutics, health supplements and biotechnological products. Phylogenetic analyses of mushrooms using molecular-based methods have increased dramatically in the last decade. The phylogenetic relationships among *Sparassis* spp. have been studied using nucleotide sequence data from ribosomal DNAs (rDNA), mitochondrial rDNAs, and partial RNA polymerase subunit II gene (RPB2) [2–6]. These nucleotide markers as the primary fungal barcode genes have been used to correctly identify mushroom.

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At least eight clades were reported in *Sparassis* spp. [*Sparassis brevipes* (*S. brevipes*), *Sparassis crispa* (*S. crispa*), *Sparassis cystidiosa* (*S. cystidiosa*), *Sparassis latifolia* (*S. latifolia*), *Sparassis miniensis* (*S. miniensis*), *Sparassis radicata* (*S. radicata*), *Sparassis spathulata* (*S. spathulata*), and *Sparassis subalpina* (*S. subalpina*)]. *S. crispa* in Europe, *S. spathulata* in Eastern North America, and *S. radicata* Weir in Western North America are the main species found. Asian collections such as *S. crispa* are morphologically different from European collections. New *Sparassis* species such as *S. subalpina* in China was isolated [6], in which the flabellae are very broad, zonate and with only slightly contorted and thickened margins. This species originates from subalpine regions in Southwestern China [6].

S. latifolia was classified as separate species from *S. crispa* and *S. radicata* by Dai *et al*. [2]. *S. latifolia* is very widespread in Asia. The basidiocarps of *S. latifolia* are composed of numerous loosely arranged flabellae that are morphologically large, broad, dissected, and slightly contorted. The nuclear gene phylogeny, morphological differences, geographic distribution, and host shifts of *S. latifolia* are distinct from those of other *Sparassis* Fr. sp. Three clamp connection-producing species (*S. latifolia*, *S. crispa*, and *S. radicata*) form a clade, for which there is no sequence divergence in the *ATP6* gene. The nuclear gene data tree divides the three taxa into two highly divergent clades, one of which contains only *S. latifolia*. Korean collections also were redistributed into *S. latifolia* from *S. crispa* by molecular sequence analysis of the internal transcribed spacer (ITS) rDNA regions [4]. The 39 isolates collected from southern regions of Korea were grouped into subclade A of *S. latifolia*.

Medicinal mushrooms have a long history of frequent use in traditional Asian therapy. The extracted materials from various mushrooms have been used as a remedy for treatment of cancers or other diseases [7–9]. Polysaccharides represent the major class of bioactive compounds found in mushrooms. *Sparassis*, an edible mushroom, is popular worldwide because it shows potentially great pharmaceutical properties. The purified β-glucan from *Sparassis* sp. is a polysaccharide, which exhibits various biological activities, such as immune stimulation, enhancement of the hematopoietic response, and anticancer effects (antiangiogenic and antimetastatic) [7,9,10]. Kwon *et al*. reported that oral administration of *S. crispa* can improve the impaired healing of diabetic wounds by increasing the migration of macrophages and fibroblasts, and β-glucan from *S. crispa* directly increases the synthesis of type I collagen [11]. Thus, *Sparassis* sp. extracts have been applied as health supplements in food, drinks, and drugs.

Four new cauliflower mushrooms were collected in the southern region of the Republic of Korea, and their mycelia were induced and cultured. They were all identified as a *S. latifolia* strain by molecular sequence analyses. We wanted to look for and identify *Sparassis* strains that produce high amount of β-glucan. This study was carried out to assess which strain of *S. latifolia* can grow faster and produce more amount of β-glucan.

2. Materials and methods

2.1. Fungus growth

Fruiting body parts of four *Sparassis* strains were sterilized, isolated, and incubated on potato dextrose agar medium (PDA) including 100 mg/L ampicillin and kanamycin. The induced mycelia were cultured on Petri-dish plates (150 mm in diameter) to compare growth. Two different microbiological media [PDA and potato dextrose agar larch (PDAL)] were used to determine growth rate. Mycelia of the *S. latifolia* strains were cultured on medium PDA and PDAL. PDA was composed of 4 g potato starch, 20 g dextrose, 15 g agar, and 1 L distilled water. PDAL was prepared as PDA plus 1 L larch extract. The larch extract was prepared by extracting 100 g larch sawdust in 1 L distilled water for 2 h at 100 °C. After autoclaving the larch extract, the sawdust mixture was removed by filtering with a membrane. Petri dishes (150 mm in diameter) containing 100 mL of culture medium were sterilized at 121 °C for 20 min. The optimal pH for all isolates was 6.0 in PDA (data not shown). Mycelium that had been cultured for 4 weeks on PDA media was placed on medium surface. Small plugs of mycelium were used as inoculum to measure the growth rate of the isolate in each treatment and were placed on the center of the plate. The diameter of the growing mycelium was measured every 7 days to represent growth. The mycelium growth was measured in 150 mm Petri-dish plate at 28 days of inoculation (DOI). All replicates were grown together in a controlled temperature chamber at 25 °C in the dark during the whole period. All plates were sealed with double layers of wrap, which permitted gas exchange. Linear growth rate was estimated for each replicate by measuring the colony diameter weekly. Both PDA and PDAL were used to measure growth. All analyses were conducted in triplicate.

2.2. Determination of β-glucan content

Each strain of mycelia was cultivated in potato dextrose broth or potato dextrose larch broth for 30 days under the same culture conditions (25 °C in the darkness at 150 r/min). The cultured mycelium was harvested, rinsed to delete the growth medium, and frozen-dried with a speed vacuum. The β-glucan contents of lyophilized mycelium samples were analyzed using the Megazyme Mushroom and Yeast Beta-Glucan kit (K-YBGL, Megazyme, Wicklow, Ireland). All analyses were done in triplicate and reported on a dry matter basis.

2.3. Molecular techniques

Genomic DNAs were isolated from the mycelium. The materials were ground to a fine powder in liquid nitrogen. The DNA was extracted with GeneAll Exgene Plant SV mini kit (GeneAll, Seoul, Korea). *S. latifolia* sequence data were generated based on a previous study: (1) mitochondrial large subunit ribosomal DNA genes (mls rDNA), (2) nuclear small subunit of ribosomal DNA (nss rDNA), (3) ITS, and (4) RPB2 [5]. Wang *et al*. showed all of the primary primer sets we used in this study [5]. Another primer set was used for the *RPB2* genes (RPB2LP853; CTTAAATACTCCCTTGCCAC and RPB2RP2509; GTACGTGATACCGATAGTACC) to amplify a longer fragment (1656 bp) [5]. PCR reaction mixes contained 5 μL of 10 × buffer, 2.5 mmol/L deoxynucleotide triphosphates Mix, and 5 unit of *Taq* polymerase (Genotech, Daejion, Korea). The amplification program included 30–35 cycles of 95 °C for 20 s, 50 °C for 30 s, and 72 °C for 90 s. The PCR product was purified and sequenced using ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

For phylogenetic analyses, the nucleotide sequences generated in this study were submitted to GenBank (Table 1). To determine the genetic relationships of the four *Sparassis* isolates, we constructed phylogenetic trees using sequences of the ITS regions, *RPB2* gene, nss rDNA, and mls rDNA. Five datasets were analyzed, which were composed of mls rDNA, nss rDNA, ITS, *RPB2* genes, and partial RPB2 amino acid sequences. Each dataset was prepared using ClustalX (http://www.genome.jp/tools/clustalw/) with default settings. Multiple sequence alignments of the nucleotide sequences and the amino acid sequences were printed and shaded with the BOXSHADE 3.21 program (http://www.ch.embnet.org/software/BOX_form.html).

3. Results

3.1. Comparison of growth rate of four S. latifolia strains

Four *S. latifolia* strains (CLM1, CKM1, CKM2 and KJM1) were newly found in the southern region of the Korea Peninsula. The central stalk of the *S. latifolia* strain CLM1 was deeply rooted underground with a length of about 20 cm. The mushroom had attained a height and width of 50 cm and 40 cm, respectively (data not shown). Its basidome was azonate and white. The contorted and crinkled flabellae were clearly developed.

Table 1
List of *Sparassis* species used in this study and GenBank accession numbers.

Species	Isolate number	Locality	GenBank accession number			
			nss rDNA	mls rDNA	ITS	RPB2
S. brevipes	GER24	Germany	AY218381	AY218464		AY218543
S. crispa	AME27	USA/WA			AY218444	
S. crispa	FRA5	France	AY218376		AY218427	AY218534
S. crispa	GER25	Germany			AY218442	AY218544
S. crispa	AME9	USA/MA	AY218379	AY218462	AY218430	AY218537
S. crispa	KFRI 0642	Netherland			JX566465	
S. crispa	FIN4	Finland	AY218375			AY218533
S. crispa	FIN3	Finland				AY218532
S. crispa	HKAS15728					AY218545
S. crispa	AFTOL-ID 703					DQ408122
S. cystidiosa	THAI	Thailand			AY256891	
S. latifolia	KJM1	Korea	KF309253	KF309257	KF309261	KF309265
S. latifolia	CLM1	Korea	KF309254	KF309258	KF309262	KF309266
S. latifolia	CKM1	Korea	KF309255	KF309259	KF309263	KF309267
S. latifolia	CKM2	Korea	KF309256	KF309260	KF309264	KF309268
S. latifolia	CHN1	China	AY218373	AY218458	AY218423	AY218530
S. latifolia	CHN20	China			AY218437	AY218541
S. latifolia	KFRI 0640	Japan			JX566463	
S. latifolia	KFRI 0700	Korea			JQ586251	
S. latifolia	KFRI 0723	Korea			JQ586252	
S. latifolia	KFRI 0923	Korea			JQ586253	
S. latifolia	CHN2	China	AY218374	AY218459		AY218531
S. latifolia	CHN17	China		AY218463		AY218539
S. latifolia	CHN19	China				AY218540
S. latifolia	CHN21	China				AY218542
S. latifolia	HKAS59357	China				JN387125
S. miniensis	SPAIN	Spain			DQ270675	
Sparassis nemecii	CZ2	Czech Republic			JQ586254	
S. radicata	AME32	USA/TN			AY218449	AY218546
S. radicata	KFRI 0692	Canada			JX566471	
S. radicata	AME29	USA/CA				DQ270673
S. radicata	CAN26	Canada				DQ270672
S. radicata	TENN52558					AY218547
S. spathulata	AME7	USA/MA	AY218377	AY218460	AY218428	AY218535
S. spathulata	AME11	USA/MA	AY218380		AY218432	AY218538
S. spathulata	AME8	USA/NH	AY218378	AY218461		AY218536
S. spathulata	S. sp. AUS31	Australia	AY218382			
S. spathulata	S. sp. THAI	Thailand				AY256892
Sparassis sp.	DAI12549	China				JQ743096
Sparassis sp.	DAI10269	China				JQ743097
Sparassis sp.	DAI2441	China				JQ743095
Sparassis sp.	HMJAU5301	China				JQ743094
Sparassis sp.	HMJAU2955	Russia				JQ743093
Sparassis sp.	HMJAU2007	China				JQ743092
Sparassis sp.	HKAS59854	Japan				JQ743091
Sparassis sp.	HKAS59857	China				JQ743100
Sparassis sp.	HKAS59855	China				JQ743098
Sparassis sp.	HKAS59856	China				JQ743099

Despite similar mycelium morphology, strains incubated in PDA medium differed from one another in their growth habitat and rate of exponential growth (Figure 1A). It is interesting that CLM1 strain showed the greatest growth rate among 4 strains. But CKM1 strain showed the slowest growth in PDA medium. The CLM1 and KJM1 strains had the similar growth rate at 35 DOI. But, the CKM1 and the CKM2 strains still had the lowest growth rate at 42 DOI (data not shown) because CKM1 and CKM2 showed a long lag phase until 7 DOI. However, the growth rate in PDAL medium was very similar among the four strains (Figure 1B). The growth rate of all four strains improved in PDAL medium during the entire culture period because all strains showed a shorter lag phase than that in PDA medium, indicating that the larch extract promoted growth. The growth rate of the CKM1 strain was mostly improved in PDAL medium.

3.2. Total β-glucan content

Figure 2 shows the amount of β-glucan contained in the strains. These data represented the content of (1–3), (1–6)-β-glucan in mycelium of *S. latifolia* as % (w/w) on a dry weight basis. Significant differences in total β-glucan content were observed among 4 strains, with average values from 21.3% to 29.5% (w/w) of the dry weight of the mycelium. *S. latifolia* CLM1 strain showed the highest β-glucan content among the four strains. In contrast, *S. latifolia* CKM1 strain, which had the slowest growth, contained the lowest β-glucan content, too. The CLM1 strain will be a good candidate for preparing a remedy because it not only grew faster but also produced more β-glucan than other strains.

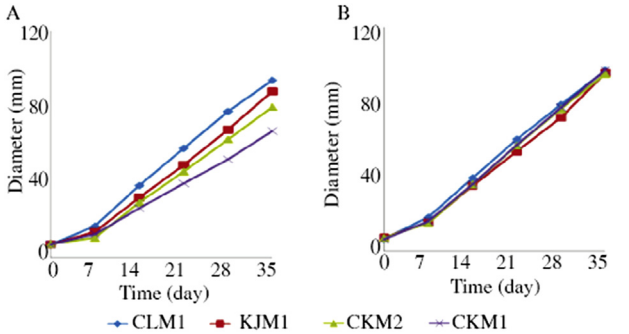

Figure 1. Measurement of the radial growth rate of CLM1, CKM1, CKM2, and KJM1.
A: Growth rate in PDA medium was measured every 7days for 35 days; B: Growth rate in PDAL medium was measured every 7days for 35 days. Mycelia were grown in Petridish plate (150 mm in diameter).

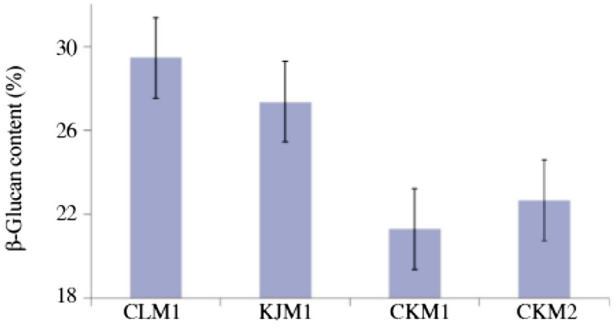

Figure 2. Comparison of β-glucan content in mycelia of *S. latifolia*.
The amount of β-glucan was calculated by Megazyme Mushroom and Yeast Beta-Glucan kit (K-YBGL, Megazyme, Ireland). This data is comparison of β-glucan content in different strains as % (w/w) on a dry weight basis.

3.3. Phylogenetic tree

Mushrooms are important directly as food and medicine for humans and different species of mushrooms have been cultivated for a long time. Thus, it is very important to accurately identify mushroom species. In this study we explored the kin relationships among *Sparassis* spp. that were isolated in the southern region of the Republic of Korea (Figures 3–5).

Genes that encoded the subunits of nuclear RNA polymerase (RPB1 and RPB2) are promising phylogenetic markers in fungal systematics [12]. The nucleotide sequences were aligned and phylogenetic trees were generated using multiple sequence alignment program (ClustalW 2.1, http://www.genome.jp/tools/clustalw/). Other nucleotide sequences from the other known *Sparassis* spp. were obtained from the NCBI GenBank to compare the phylogenetic relationships (Table 1).

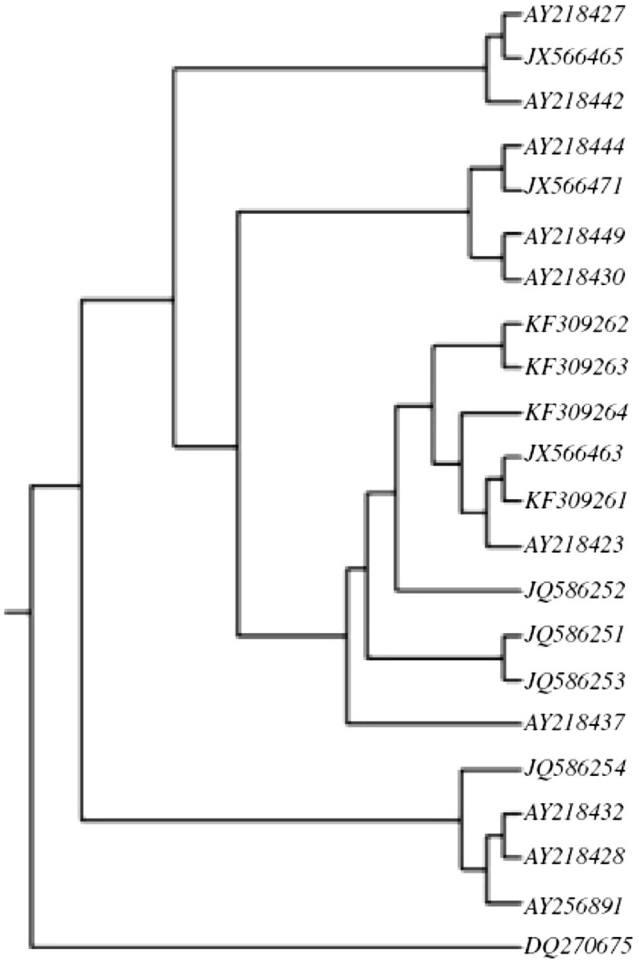

Figure 3. Phylogenetic tree for *Sparassis* species on the comparison of ITS regions (ITSI, 5.8 S rDNA, and ITS2).
This tree was created using multipe sequence alignment by ClustralW (http://www.genome.jp/tools/clustalw). AY218427, AY218430, AY218442, AY218444, and JX566465 belong to *S. crispa*. JX566463, JQ586251, JQ586252, JQ586253, AY218423, AY218437, KF309261, KF309262, KF309263, and KF309264 belong to *S. latifolia*, JX566471 and AY218449 belong to *S. radicata*. AY218428 and AY218432 belong to *S. spathulata*. Q586254 belongs to *Sparassis nemecii*. AY256891 belongs to *S. cystidiosa*. DQ270675 belongs to *S. miniensis*.

The phylogenetic analysis of the ITS regions showed that the four newly isolated mushrooms belonged to *S. latifolia* (Figure 3). Dai *et al*. insisted that the phylogenetic tree based on nuclear gene data supported *S. latifolia* as separate species from *S. radicata* and *S. crispa* [2]. The phylogenetic analyses of mls rDNA and nss rDNA regions also showed that our four isolated mushrooms also belonged to *S. latifolia* (data not shown). Partial nucleotide sequence analyses of ITS, mls rDNA and nss rDNA displayed a low degree of variability among our four *Sparassis* isolates (data not shown).

In previous studies, RNA polymerase II sequence data have been used to investigate molecular relationships among a broad array of eukaryotes. The nucleotide sequences of RPB2 appeared to be more variable than those of 3 other regions analyzed in this report (data not shown). The phylogenetic tree generated based on the *RPB2* gene sequences indicated that our four isolates were distinct and grouped into *S. latifolia*, which is separate from *S. crispa*, *S. radicata*, *S. spathulata*, *S. brevipes*, and *S. cystidiosa* (Figure 4A).

3.4. Identification

The nucleotide sequence variance (NSV) of ITS region, nss rDNA, and mls rDNA gene were very low (Figure 3). Thus, it is necessary to select other sequences, which have variable regions. Matheny *et al*. stated that the *RPB2* gene is more variable than the translation elongation factor 1-α, and it recovers well-supported clades at shallow and deep taxonomic levels in the phylogeny of Basidiomycota [13].

The *RPB2* gene was partially amplified using the primer set (RPB2LP853; CTTAAATACTCCCTTGCCAC and RPB2RP2509; GTACGTGATACCGATAGTACC). The NSVs of the partial genomic *RPB2* gene were widely distributed among our four strains (Figure 4B), indicating that NSV density is high within the *RPB2* gene (22 bases per 1618 bases = 1.4%). The NSVs in RPB2 may be exploited as an identification marker as well as a diagnostic tool for *Sparassis* spp.

These partial RPB2 sequences contained a conserved intron (Figure 4B). Three major clades were inferred (Figure 4C). Three Korean isolates (KF309265, KF309267, and KF309268) were clustered together in Clade I, and one Korean isolate (KF309266) was separate. Four isolates of *S. subalpina* formed a monophyletic group in Clade II because their nucleotide sequences were completely the same. Three isolates of *S. cystidiosa* in Clade III were clustered but one sequence was slightly different. This intron sequence showed four divergent sites among 19 *S. latifolia* isolates, indicating that the NSV density of the intron was very high (4 bases per 49 bases = 8.2%) among 19 *S. latifolia* isolates (Figure 4B). Its nucleotide length (49 bases) was completely conserved between *S. latifolia* and *S. cystidiosa*. However, its nucleotide length was slightly longer (52 bases) in *S. subalpina*. These observations strongly suggest that this intron sequence can be used to classify *Sparassis* sp.

Significant divergence of the *RPB2* gene was observed among our four isolates (data not shown), and the variable region of the *RPB2* gene should be targeted to correctly identify them.

3.5. Comparison of amino acid sequence of the RPB2 gene

The putative encoded amino acid sequences of the *RPB2* gene (about 250 amino acids) in the standard one letter code were highly conserved among the 19 *S. latifolia* isolates

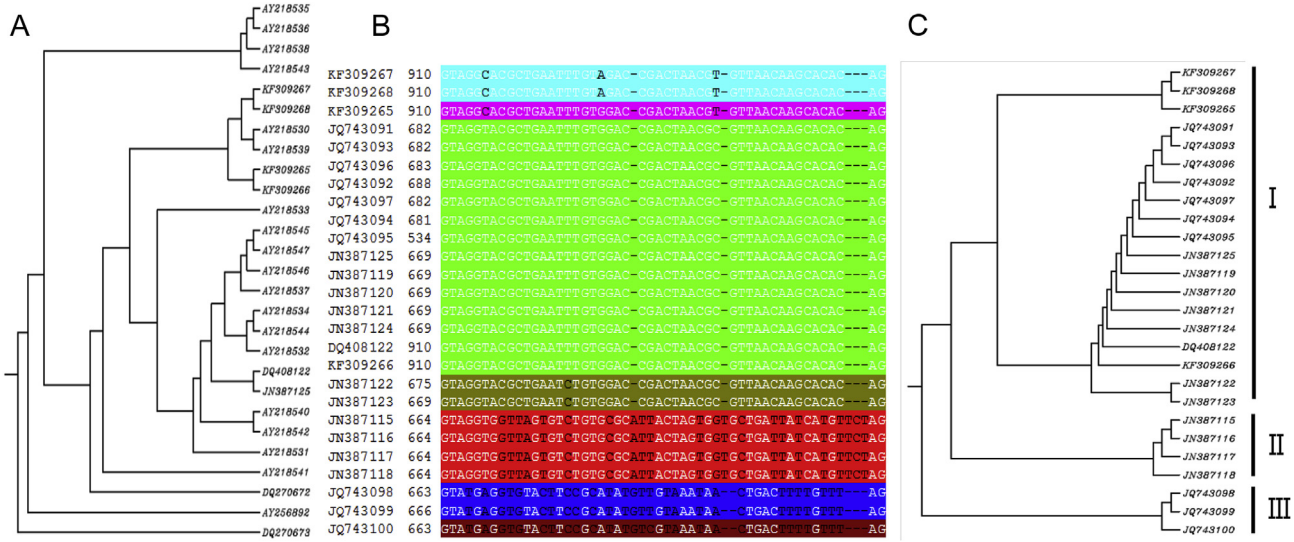

Figure 4. Analysis of nucleotide sequence based on the RPB2.

A: Phylogenetic trees of the *RPB2* gene for *Sparassis* species, which matched base pairs (about 600 bases). AY218543 belonged to *S. brevipes*. AY218532, AY218533, AY218534, AY218537, AY218544, AY218545, and DQ408122 belonged to *S. crispa*. KF309265, KF309266, KF309267, KF309268, AY218530, AY218531, AY218539, AY218540, AY218541, AY218542, and JN387125 belonged to *S. latifolia*. AY218530, AY218531, and AY218539 were isolated from the northern region of China. AY218546, AY218547, DQ270672, and DQ270673 belonged to *S. radicata*. AY218535, AY218536, AY218538, and AY256892 belonged to *S. spathulata*; B: Sequence alignment based on intron sequences of the *RPB2* gene. Background color represents sequence similarity. The white letter indicates the highly conserved nucleotide sequence, whereas the black letter indicates low conservation; C: Phylogenetic trees based on intron sequences of the *RPB2* gene. KF309265, KF309266, KF309267, KF309268, DQ408122, JN387119, JN387120, JN387121, JN387122, JN387123, JN387124, JN387125, JQ743091, JQ743092, JQ743093, JQ743094, JQ743095, JQ743096, and JQ743097 belonged to *S. latifolia*, JQ743098, JQ743099, and JQ743100 belonged to *S. cystidiosa*. JN387115, JN387116, JN387117, and JN387118 belonged to *S. subalpina*.

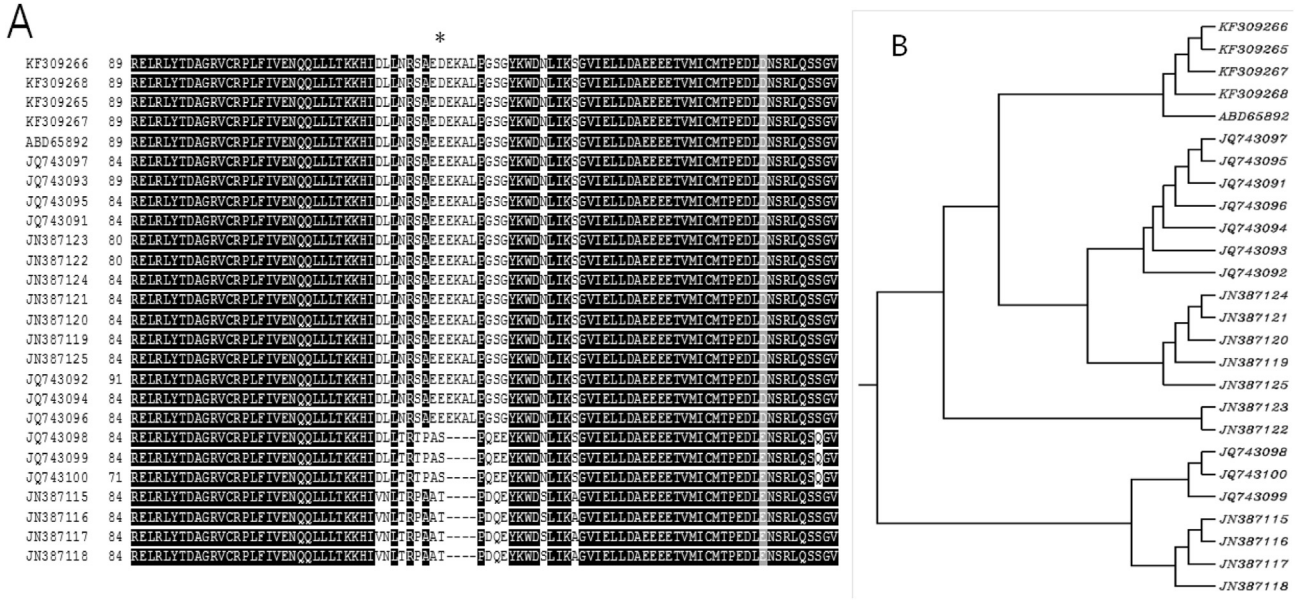

Figure 5. Sequence alignment and phylogenetic tree based on the putative amino acid sequence of *RPB2* gene, which matched amino acid (about 250 amino acids).

A: Sequence alignment; B: Phylogenetic tree. The asterisk indicates the distinct amino acid residue (Asp), which is only unique in four Korean isolates. The black background represents the highly conserved sequence. KF309265, KF309266, KF309267, KF309268, ABD65892, JN387119, JN387120, JN387121, JN387122, JN387123, JN387124, JN387125, JQ743091, JQ743092, JQ743093, JQ743094, JQ743095, JQ743096, and JQ743097 belong to *S. latifolia*. JQ743098, JQ743099 and JQ743100 belong to *S. cystidiosa*. JN387115, JN387116, JN387117, and JN387118 belong to *S. subalpina*.

(Figure 5A). However one region was highly diverse among *Sparassis* sp. (*S. latifolia*, *S. cystidiosa*, and *S. subalpina*) as well as other organisms containing fungi such as *Trametes pubescens*, *Amyloporia carbonica*, *Dentocorticium sulphurellum*, *Fomitopsis rosea*, *Antrodia albida*, and *Laetiporus sulphureus*. The site of diverse region coincided with that of the diverse region of the RPB2 nucleotide sequences (data not shown). It was located between RNA polymerase RPB2 domain 4 and RNA polymerase RPB2 domain 5. Among the amino acid differences, only one amino acid sequence was very distinct in all Korean isolates: Glu (E) was changed to Asp (D), which was marked with asterisk (Figure 5A). This Asp residue can be used as a marker to distinguish the Korean isolates from the Chinese isolates. Although the intron nucleotide sequence of KF309266 was more similar to those of the Chinese isolates (Figure 4B), its putative amino acid sequence was more similar to those of other Korean isolates than those of the China isolates (Figure 5B). In addition, the variability in the protein sequence was less than the nucleotide divergence in *S. latifolia* (Figure 5A) indicating that several of the nucleotide changes are silent mutations.

4. Discussion

Zhao *et al.* reported that *S. latifolia* isolates are easily distinguishable species with a wide distribution throughout Eastern Asia [6]. The analyses of nucleotide sequences help us to decrease the misidentification rate of cauliflower mushrooms due to simple morphological characters. A few sequence divergences were detected in the ITS sequence, the mls rDNA sequence, and the nss rDNA sequence among our cauliflower mushrooms. However, a partial nucleotide sequence of the *RPB2* gene would be most effectively used to identify individual *Sparassis* specimen. The Asp residue, which is marked with an asterisk (Figure 5A), can also be used as a marker to distinguish the Korean isolates from the Chinese isolates, indicating that the Korean isolates were independently evolved from the Chinese isolates.

We found that PDAL was a more suitable medium for mycelial growth of *Sparassis* sp., because the larch extract promoted growth by shortening the lag phase (Figure 1). Thus, it will be important to elucidate which elements of the larch tree extract promote mycelial growth.

β-Glucan molecules are biological response modifiers because of their ability to activate the immune system. In particular, the dietary β-glucan of *Sparassis* sp. is useful for treating cancer and for its immunomodulatory effect [9,10]. Lee *et al*. showed that β-glucan content was about 10.64% (w/w) in the mycelium of *S. crispa* Wulf. Ex Fr [14]. The content of β-glucan in CLM strain is 2.8 times higher than that of *S. crispa* Wulf [14]. Kim *et al*. reported that they generated 2 mutants, S7 and B4, that produced high β-glucan content [23.6% and 25.4% (w/w), respectively] [15]. CLM1 strain may be best for developing a remedy to prevent or treat cancer and other chronic diseases due to its higher β-glucan content and its better growth than other strains. We will further investigate why or how CLM1 strain produced higher β-glucan content, compared to other strains. The β-1,3-glucan synthase genes for β-glucan biosynthesis in CLM1 strain may apparently be of constitutive expression. The observed differences of β-glucan production may therefore be linked to the promoter region, which is sequenced and studied to understand why strains of the same species produce different amounts of β-glucan.

Conflict of interest statement

We declare that we have no conflict of interest.

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