Korean J. Environ. Biol. 23(4) : 335~342(2005)

Characterization and Phylogenetic Analysis of Chitin Synthase Genes from the Genera *Sporobolomyces* and *Bensingtonia subrorea*

Jin Sik Nam

Department of Food & Nutrition, Faculty of Food Science, Suwon Women's College
336-27 Sanggi-ri, Bongdam-eup, Hwasung-si, Kyonggi-do 445-895, Republic of Korea

Abstract - We cloned seven genes encoding chitin synthases (CHSs) by PCR amplification from genomic DNAs of four strains of the genus *Sporobolomyces* and of *Bensingtonia subrosea* using degenerated primers based on conserved regions of the CHS genes. Though amino acid sequences of these genes were shown similar as 176 to 189 amino acids except SgCHS2, DNA sequences were different in size, which was due to various introns present in seven fragments. Alignment and phylogenetic analysis of their deduced amino acid sequences together with the reported CHS genes of basidiomycetes separated the sequences into classes I, II and III. This analysis also permitted the classification of isolated *CHSs; SgCHS1* belongs to class I, *BsCHS1, SaCHS1, SgCHS2, SpgCHS1*, and *SsCHS1* belong to class II, and *BsCHS2* belongs to class III. The deduced amino acid sequences involving in class II that were discovered from five strains were also compared with those of other basidiomycetes by CLUSTAL X program. The bootstrap analysis and phylogenetic tree by neighbor-joining method revealed the taxonomic and evolutionary position for four strains of the genus *Sporobolomyces* and for *Bensingtonia subrosea* which agreed with the previous classification. The results clearly showed that CHS fragments could be used as a valuable key for the molecular taxonomic and phylogenetic studies of basidiomycetes.

Key words : *Sporobolomyces*, chitin synthase gene, phylogeny, PCR

INTRODUCTION

Chitin, the β-1,4-linked polymer of N-acetylglucosamine, is an important structural component in the cell wall and septum of many fungi (Cabib *et al.* 1988; Bowen *et al.* 1992). In *Saccharomyces cerevisiae*, chitin constitutes a small portion of the cell wall, but is indispensable for cell viability. And also, chitin is not found in plant or mammals, and therefore is one of the components that have been used as a taxonomic marker. Chitin synthase (CHS), which catalyzes the synthesis of a β-1,4 linked polymer of N-acetylglucosamine using UDP-N-acetylglucosamine as a substrate, performs several essential functions in fungal morphogenesis including vegetative growth, fruit-body formation, and sporogenesis (Cabib *et al.* 1988).

There are three kinds of chitin synthases, chitin synthase 1, 2, and 3 in *S. cerevisiae*, and they encoded by the three different genes designated chitin synthase 1 (*CHS1*), 2 (*CHS2*), and 3 (*CHS3*), respectively. CHS I is the enzyme that repairs the cell wall during cell separation, CHS II is an essential enzyme for primary septum formation and cell division, whereas CHS III is responsible for chitin ring formation at bud emergence and in the lateral cell wall and also for the formation of glucan-chitin linkage (Cabib *et al.* 1988; Shaw *et al.* 1991; Valdivieso *et al.* 1991; Cabib *et al.* 1992).

Bowen *et al.* (1992) have cloned 32 partial DNA fragments of chitin synthase genes (*CHSs*) from 14 fungal

* Corresponding author: Jin Sik Nam,
Tel. 031-290-8948, Fex. 031-290-8220,
E-mail. jsnam@suwon-c.ac.kr, jinsiknam@empal.com

species using degenerated PCR primers complementary to the conserved region of amino acid sequences of *S. cerevisiae* CHS1, CHS2 and *Candida albicans* CHS1, and showed the sequences fell into three distinct classes (I, II, III) which probably reflect the functional difference of chitin synthases by aligning the deduced amino acid sequences of the amplified fragments (Bowen *et al.* 1992). This strategy has been used to study various aspects of *CHS* homologues from a variety of fungi (Mehmann *et al.* 1994; Mellado *et al.* 1995; Thomsen and Beauvais 1995; Cho *et al.* 1997; Hong *et al.* 2002; Hirai *et al.* 2003). Therefore, these informations obtained from CHS gene analyses have also been used in phylogenetic and taxonomic studies of several fungi (Mehmann *et al.* 1994; Peng *et al.* 1995; Namgung *et al.* 1996; Nam *et al.* 1997; Nam *et al.* 1998).

The anamorphic basidiomycetous genus *Sporobolomyces* accommodates species that produce bilaterally symmetrical ballistoconidia. The genus is currently defined by ellipsoidal, subglobose, fusiform or cylindroidal cells, which are mostly polar, rarely lateral or multilateral budding, bilaterally symmetrical ballistoconidia, pink, orange, red, yellowish or pale colonies, and a presence of hyphae or pseudohyphae. Other physiological and chemical characteristics are as follows: no fermentation of sugars, Diazonium Blue B and urease positive, incapacity to utilize inositol and to produce amyloid compounds, absence of xylose from the whole-cell hydrolyzates, and CoQ 10 or CoQ 10 (H2) as their major ubiquinones (Hong *et al.* 2000; Valerio *et al.* 2002; Wang *et al.* 2004).

The phylogenetic study of the genus *Sporobolomyces* was revealed by sequence analysis of 18S rDNA and of the D1/D2 region of the 26S rDNA (Hong *et al.* 2000; Hong *et al.* 2002; Valerio *et al.* 2002; Wang *et al.* 2004). Fell *et al.* (1998) and Hong *et al.* (2000, 2002) conducted a phylogenetic study based on about 200~500 bp partial sequences of 26S rDNA and it is clear that the members of the genus *Sporobolomyces* are phylogenetically heterogeneous and polyphyletic. It is therefore essential to carry out further comprehensive phylogenetic study.

In this paper, we report the existence of genes for chitin synthase from four *Sporobolomyces* species and *Bensingtonia subrosea* (Golubev 1999), and describe the phylogenetic relationship between the genus *Sporobolomyces* and basidiomycetous yeasts through comparison with the deduced amino acid sequence data reported previously.

MATERIALS AND METHODS

1. Strain and culture

Bensingtonia subrosea KCTC 17308^T, *Sporobolomyces alborubescens* KCTC 7792^T, *S. gracilis* KCTC 17244^T, *S. griseoflavus* KCTC 7794^T, and *S. sasicola* KCTC 7795^T were obtained from the Korean Collection for Type Cultures (KCTC) and maintained on YM agar (dextrose 10.0 g, malt extract 3.0 g, peptone 5.0 g, yeast extract 3.0 g, agar 20.0 g per liter) for 2 days at 24°C.

2. Isolation of genomic DNA

For DNA isolation, yeast cells were inoculated in YM broth, incubated for 48 h at 24°C, and broken with glass beads and a TOMY micro tube mixer (TOMY, Seiko, Japan) (Sambrook *et al.* 1989). Total DNAs were extracted using a Genomic DNA Isolation Kit (Nucleogen, Korea) according to the supplier's guide.

3. Polymerase chain reaction

The degenerated primers complementary to the conserved regions of CHS described previously (Bowen *et al.* 1992) were synthesized on a model 392 DNA/RNA synthesizer (Applied Biosystems Inc., USA). Polymerase chain reaction (PCR) was performed in a reaction mixture containing 100 pmole of each primer, 50 ng of genomic DNA, 5 μL of 10x PCR reaction buffer (Promega, USA), 150 μM dNTP (USB, USA), 2.5 units of Taq polymerase (Promega, USA), and D.W. up to 50 μL. The sequences of the degenerate primers for CHS gene were based on the sequences reported in our previous study (Nam *et al.* 1997): primer 1, 5′-CTG AAG CTT ACN ATG TAY AAY GAR GAY-3′; primer 2, 5′-GTT CTC GAG YTT RTA YTC RAA RTT YTG-3′. After overlaying mineral oil (Sigma, USA), the reaction mixtures were preheated at 94°C for 2 min, and thereafter, 35 amplification cycles were carried out. Each cycle consisted of 1 min at 94°C, 1 min at 50°C, and 3 min at 72°C. Finally, an additional 10 min of extension reaction was performed at 72°C for complete extension. Amplification was performed in a PTC-100TM programmable thermal controller (MJ Research Inc., USA). The PCR products were electrophoresed on a 1.0% agarose gel, stained with ethidium bromide and visualized under UV

light (Sambrook *et al.* 1989).

4. Cloning and sequencing

The direct cloning strategy of PCR products described previously was used (Sambrook *et al.* 1989). The PCR products separated on agarose gels were excised and cleaned with QIAquick Gel Extraction Kit (Qiagen, Netherlands). Purified PCR products were cloned using a pGEM-T easy kit (Promega, USA). Transformation was carried out using *E. coli* strain XL1-blue (Sambrook *et al.* 1989). Plasmid DNA from transformants was isolated using QIAprep Spin Plasmid Miniprep Kit (Qiagen, Netherlands). The nucleotide sequences of the inserts were determined with BigDye terminator cycle sequencing kits (Applied Biosystems, USA) following the manufacturer's instructions using the T7 and SP6 primers. The gel electrophoresis and data collection were performed on an ABI 310 Genetic Analyzer (Applied Biosystems, USA).

5. Phylogenetic analysis

The nucleotide sequences of PCR clones were analyzed through NCBI data search using BlastN to verify which fragments were encoding the gene for CHS. The deduced amino acid sequences were compared with those from Basidiomycota using CLUSTAL X software (Thompson *et al.* 1997). The phylogenetic tree was constructed according to the neighbor joining method (Saitou and Nei 1987) using the PHYLIP version 3.5c program (Felsenstein 1993). The confidence level of the resultant tree topology was evaluated by performing 1,000 bootstrap trials.

6. Nucleotide sequence accession number

The nucleotide and amino acid sequences obtained in this study are deposited in the GenBank database with the accession numbers DQ188261-DQ188267.

RESULTS AND DISCUSSION

1. PCR cloning and nucleotide sequence analysis

Agarose gel analysis of PCR products for CHS gene from five strains revealed the presence of one or two distinct bands, and their band sizes were about 600~1,000 bp. After cloning the PCR products, nucleotide sequencing and BlastN search of the inserts were performed. PCR products were identified as genes for CHS, and designated *BsCHS1* and *BsCHS2* for *B. subrosea* KCTC 17308^T, *SaCHS1* for *S. alborubescens* KCTC 7792^T, *SpgCHS1* for *S. gracilis* KCTC 17244^T, *SgCHS1* and *SgCHS2* for *S. griseoflavus* KCTC 7794^T, and *SsCHS1* for *S. sasicola* KCTC 7795^T. This result, however, can not rule out the existence of additional genes for CHS, because most of the yeasts, whose CHS genes has been investigated, showed the existence of more than two *CHSs* in one species. Complete sequencing of *BsCHS1*, *BsCHS2*, *SaCHS1*, *SpgCHS1*, *SgCHS1*, *SgCHS2*, and *SsCHS1* on both strands revealed that *SgCHS1* contained uninterrupted open reading frames of 567 bp, but *BsCHS1*, *BsCHS2*, *SaCHS1*, *SpgCHS1*, *SgCHS2*, and *SsCHS1* contained 819 bp, 936 bp, 963 bp, 792 bp, 527 bp, and 759 bp with any presumptive intron sequences when the nucleotide sequences of degenerated PCR primers were excluded (Fig. 1).

2. Analysis of deduced amino acid sequences

To identify which CHS the deduced amino acid sequences of isolated *CHSs* were most closely related to, the deduced amino acid sequences of *CHSs* were compared with the corresponding region of yeast *CHSs* registered in GenBank database using the BLASTP program. The deduced amino acid sequences of isolated *CHSs* were identified as group of urediniomycetous yeasts. Interestingly, the deduced amino acid sequences of isolated *CHSs* displayed the lower rate of identity (42~76%) and similarity (61~85%) with those of CHSs from basidimycetous yeasts through comparison with those of *CHSs* among ascomycetous fungi reported previously. And also, identity (38~74%) and similarity (59~88%) of the deduced amino acid sequences among four *Spolobolomyces* strains and *B. subrosea* strain were lower over against those of *CHSs* among strains of hymenomycetes or ustilaginomycetes of basidiomycetous yeasts (Fig. 2). These results are well consistent with the traditional classification in which basidiomycetous yeasts are clustered into three groups, Hymenomycetes, Ustilaginomycetes, and Urediniomycetes. Interestingly, the deduced amino acid sequences of the other genes which displayed higher rates of similarity with those of isolated *CHSs* were those from Hemiascomycota or Zygomycota than those

```
>BsCHS1
  1 GAGAAATTGTTTGATGATTCAATGTCTTCGATCGTCAAGAATATCCAACATTTGCAATCGAGAACTAAATCCAAGACTTGGGGTCCGAAA
    E K L F D D S M S S I V K N I Q H L Q S R T K S K T W G P K
 91 GCATGGGAAAAGATTGTTGTTTGCATTGTTGCTGATGGTAGAGgttcgtcagcggcgttctttagcttccttctagctgagaagcaaccg
    A W E K I V V C I V A D G R A
181 atttcgaacagCCAAATGTAATGCCAAAGTCTTGAAAATGCTTGGTTTATATGGTGTTTACCAAGAAGGCATCGCAAAAGACAGCGTCAT
                K C N A K V L K M L G L Y G V Y Q E G I A K D S V M
271 GGGTAAACCGGTGACTGCACACATCTTCGAAgtaagttgctgtctcatactgtcattcacgaacctaattcaaaaaaaatcagTATTCTT
      G K P V T A H I F E                                                                Y S S
361 CCCAAGTCGTAGTTGATCAAATGGGCAACGTAGCAGGAGGTATCGCACCAGTACAAATCATTTTTGTCCTCAAAGAACAAAATAAGAAGA
      Q V V V D Q M G N V A G G I A P V Q I I F V L K E Q N K K K
451 AGCTCAATTCCCATCGATGGTTCTTCGACGCTATTTGCGCTTCCCTGAATCCAAACGTTTGCATTCTCATCGATGTTGGAACAAGACCAT
      L N S H R W F F D A I C A S L N P N V C I L I D V G T R P S
541 CCGGTACTTCTCTTTACAAGCTTTGGAAAGAgtgcgtctgtcttgtatgctagatgatatgagctaatatatacccgtgcagATTCGACA
      G T S L Y K L W K E                                                                F D K
631 AGCATAGCAATgtaagtatacatatcttgaaagcttcaatgcactaagaaagcatctcgcagGTCGGGGGAGCTTGCGGCGAGgtatggc
      H S N                                                          V G G A C G E
721 caatttgccgatgcgatagcgcgatacttacatatttattagATCTGCGTCGATCTCGGCAGAGGCTGCGTGAACGTCTTCAATCCGTTG
                                                I C V D L G R G C V N V F N P L
811 GTCGCCGCA
    V A A

>BsCHS2
  1 AAAGGCAAGCTCGAAGATGGGACTAGAGTACCAGCTTGCCAAAGGATAGTTGTTTGTATTATCATGGATGGTATTGATCCTTGTGATAAA
    K G K L E D G T R V P A C Q R I V V C I I M D G I D P C D K
 91 CGCTCGCTGGACGTGCTGTCACTGCTAGGTGTCTTTCAGgtcggtctgctcggtttgacttgataagatagctcatatgcctacagGACA
    R S L D V L S L L G V F Q                                                          D N
181 ACATTgtacgctaattgctggctttgaaccatttctaggctaatatctgtatagATGAAGCGTCAAGTCAACGGAGAAGAAGTACAGGGA
      I                                                                M K R Q V N G E E E V Q G
271 CATCTCTTCGAGgtagataaaacgatccatctaccactgcctaactcagaattgtctcttcagTATACTACGCAGCTTTCAGTCGAACCC
    H L F E                                                                Y T T Q L S V E P
361 AATCACAATGGGAAACCACAGCTGGTCGAGCCTTCGCgtgctggcaaagATATGCACACGAATCTAGTACCTGTACAGTACATGCTTCTC
    N H N G K P Q L V E P S H                                        M H T N L V P V Q Y M L L
451 ATCAAGCAAAAGAACGCAAAgtaaagcttgcctaccgctgtcaattgcttgcctgacaaatctgatcagAAAGATCAACTCGCACCGAgt
    I K Q K N A K                                                          K I N S H R
541 cggtttctatgacagccaatgacatgtaatgctgattcatgtcgtgatagTGGCTCTTCAATGGCTTTTGTAGACAGCTGAATCCGAATG
                                                        W L F N G F C R Q L N P N V
631 TCACTATTCTCATTGATGCTGGAACAAAACCGGGTCATAAAAGTCTCTATCATCTATGGAACGCCTTTCACAATGATAAGgtcagcattc
      T I L I D A G T K P G H K S L Y H L W N A F H N D K
721 gttaacttgtcatacgttttaactgacgcgcttcatcgccagaatctaggtatgatgtttgattgatcacaagtcacattgctgaccaga

811 gCTGATAGGCGGAGCTTGTGGTGAAgtaagacgactagctttgacgaatgaatcacgacttatctttgatagATCTTCGCTCAAACTAAC
      L I G G A C G E                                                          I F A Q T N
901 GGCGGCATCAAGCTAATCAATCCTTTGGTGGCTGCC
    G G I K L I N P L V A A

>SaCHS1
  1 GAGGAGCTGTTTTGCCGGACGCTTCACGGCGTCATGAgtatgtcgccgcccggctccatcgcgtcttagaagcgagaagaggtgggggct
    E E L F C R T L H G V M K
 91 gacctctcacccacgcacagAAAACATCTCTCAACTCTGCAAGCGGAAAAAGTCGGCGACGTGGGGCCCTGACGGATGGAAGAgtacgta
                        N I S Q L C K R K K S A T W G P D G W K K
181 tttctctctctattcgcaacaaggggcccgctcacactcgacgaactctgcagAGGTCGTCGTGTCGATCATCTCAGATGGACGGAAAGC
                                                          V V V S I I S D G R K A
271 GATCCATCCGCGCGTTCTCGATTGgtgcgttggaaatgattcacctctcgcaggcgcaggaaccgcgtgatgataqcggaccctcccgca
      I H P R V L D C
361 gCCTCTCTGCTCTGGGTGTCTACCAACCAAACGTGATGACCAACCAAATCGACGAGCAACCGGTCGCGTGCCACCTCTTCGAGAATACGG
      L S A L G V Y Q P N V M T N Q I D E Q P V A C H L F E N T V
451 TCCAGATGAGCATCGATCCCAACCTGACTTTTTCAGGCCTCGAGAAGGGCATCATGCCCTGTCAGATCATgtgcgctcacacgctccagc
      Q M S I D P N L T F S G L E K G I M P C Q I I
541 agtcgtgcgcactttactgacccgctccctctccccgccagCTTCTGCCTCAAGGAGAAGAAgtaggtcgggacgaggtggcaaaagact
                                                F C L K E K N
631 cagcctcaagcaaccctgacgccccttccctcccaacagCGCTAAAAAGATCCTAACTCGCATCATGGTGCTGCAAGCTTTTGCACCACA
                                                  A K K I L T R I M V L Q A F A P Q
721 ACTCGACCCGCGCGTCGTCGTCCTCATCGATGTCGGTACCAAGCCCGCCGCCGGCAGCTTGTACAGCCTGTGGAAGCAATTCGACgtgag
      L D P R V V V V L I D V G T K P A A G S L Y S L W K Q F D
811 ctcgctggggtcagcacctctgcgattcccggattccgctgatcgtgtttctcacgttcacagATGAACTCTAATGTCGGAGGAGCTTGC
                                                          M N S N V G G A C
901 GGCGAGATCGTCGCCATGAAGGGCAAGTACTGGCAAGGTCTGGTCAATCCGCTCATCGCGGCG
    G E I V A M K G K Y W Q G L V N P L I A A

>SgCHS1
  1 GATATTTTGCTTGCTAGAACCTTGAAGGGTATCTTCAAGAACATCAAGCATCTCGAATCGAGAACCAGGTCGCCGGTATGGGGAAAAGAT
    D I L L A R T L K G I F K N I K H L E S R T R S P V W G K D
 91 TCATGGAAGAAAATCGTCGTTTGCGTCGTCAGTGATGGTCGTTCGAAAATTAACGAGCGTGCTCAAGCGCTCTTGGCAGGTCTTGGAGTT
    S W K K I V V C V V S D G R S K I N E R A Q A L L A G L G V
181 TATCAGGAAGGTCTTGCCAAGAGTAGAGTCGACGACAAGAAGGTGCAAGCTCACATCTACGAATATACCACTCGAGTTGGTATTCTGAGT
```

Fig. 1. The nucleotide and deduced amino acid sequences of the PCR-amplified segments for CHS genes of yeast strains. The primer sequences are excluded. Intron sequences are underlined and arranged as small letters.

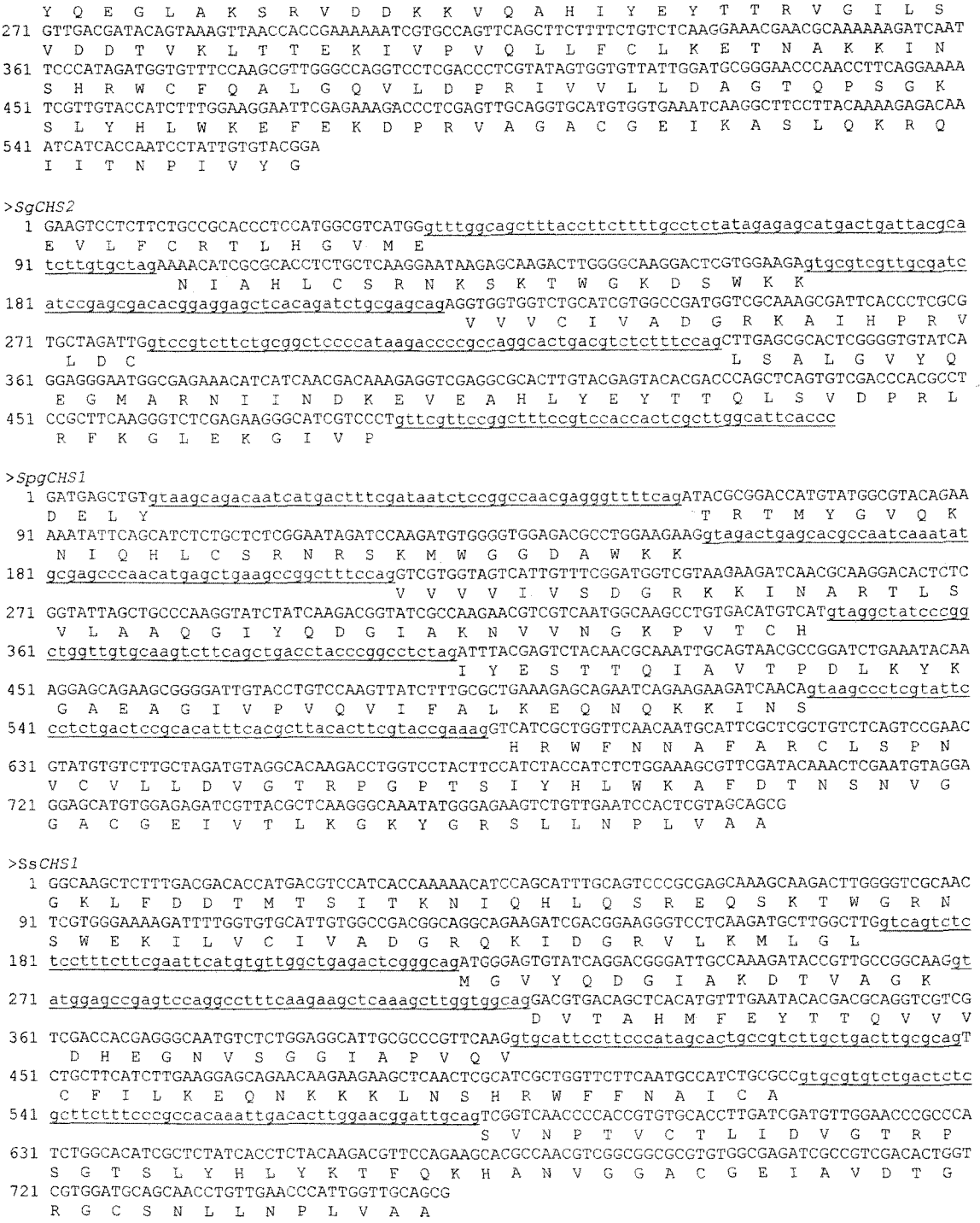

Fig. 1. Continued.

from Ascomycota. These results may reflect some evolutionary relationships of Basidiomycota.

This analysis also permitted the classification of isolated *CHSs; SgCHS1* belongs to class I, *BsCHS1, SaCHS1, SgCHS2, SpgCHS1*, and *SsCHS1* belong to class II, and *BsCHS2* belongs to class III because all the *CHSs* shown in this study belong to either class I, II, or III CHS defined by phylogenetic trees. Multialignment of the deduced amino acid sequences of isolated *CHSs* with those of *CHSs* from Basidiomycota confirmed that *CHSs* belonged to I, II, or III

```
SaCHS1    EELFCRTLHGVMKNISQLCKRKKSATWGPDGWKKVVVSIISDGRKAIHPRVLDCLSALGV
SgCHS2    EVLFCRTLHGVMENIAHLCSRNKSKTWGKDSWKKVVVCIVADGRKAIHPRVLDCLSALGV
SpgCHS1   DELYTRTMYGVQKNIQHLCSRNRSKMWGGDAWKKVVVVIVSDGRKKINARTLSVLAAQGI
SsCHS1    GKLFDDTMTSITKNIQHLQSREQSKTWGRNSWEKILVCIVADGRQKIDGRVLKMLGLMGV
BsCHS1    EKLFDDSMSSIVKNIQHLQSRTKSKTWGPKAWEKIVVCIVADGRAKCNAKVLKMLGLYGV
            *:  :: .: :** :* .* :*  ** ..*:*::* *::***   . :.*. *.  *:

SaCHS1    YQPNVMTNQIDEQPVACHLFENTVQMSIDPNLTFSGLEKGIMPCQIIFCLKEKNAKKILT
SgCHS2    YQEGMARNIINDKEVEAHLYEYTTQLSVDPRLRFKGLEKGIVP----------------
SpgCHS1   YQDGIAKNVVNGKPVTCHIYESTTQIAVTPDLKYKGAEAGIVPVQVIFALKEQNQKKINS
SsCHS1    YQDGIAKDTVAGKDVTAHMFEYTTQVVVDHEGNVSG---GIAPVQVCFILKEQNKKKLNS
BsCHS1    YQEGIAKDSVMGKPVTAHIFEYSSQVVVDQMGNVAG---GIAPVQIIFVLKEQNKKKLNS
          ** .:  : :  : * .*::* : *: :       *   ** * *  * *** * **

SaCHS1    RIMVLQAFAPQLDPRVVVLIDVGTKPAAGSLYSLWKQFDMNSNVGGACGEIVAMKGKYWQ
SgCHS2    ------------------------------------------------------------
SpgCHS1   HRWFNNAFARCLSPNVCVLLDVGTRPGPTSIYHLWKAFDTNSNVGGACGEIVTLKGKYGR
SsCHS1    HRWFFNAICASVNPTVCTLIDVGTRPSGTSLYHLYKTFQKHANVGGACGEIAVDTGRGCS
BsCHS1    HRWFFDAICASLNPNVCILIDVGTRPSGTSLYKLWKEFDKHSNVGGACGEICVDLGRGCV
                *      * *  * **** *   *** * * *    *********    *

SaCHS1    GLVNPLIAA
SgCHS2    ---------
SpgCHS1   SLLNPLVAA
SsCHS1    NLLNPLVAA
BsCHS1    NVFNPLVAA
             *** **
```

Fig. 2. Multialignment of the deduced amino acid sequences of the class II chitin synthase-encoding gene fragments from 7 yeast strains. The alignment was derived by CLUSTAL X. Bs: *Bensingtonia subrosea*; Sa: *S. alborubescens*; Sg: *S. gracilis*; Spg: *S. griseoflavus*; Ss: *S. sasicola*. Asterisks indicate complete identities; Dots indicate conservative substitutions.

(data not shown). However, it is of interest that the isolated *BsCHS1* and *SsCHS1* encode three less amino acids than other class II *CHSs* which encode 189 amino acids. In contrast, *SgCHS2* encodes eighty six less amino acids (Fig. 2). The biological meaning of this unique feature of the isolated *CHSs* may require further investigation.

3. Phylogenetic analysis

For the taxonomic and phylogenetic study, bootstrap analysis of the deduced amino acid sequences of the isolated *CHSs* was carried out with those of the class II *CHS* fragments of Basidiomycota registered in GenBank by the neighbor joining method (Saitou and Nei 1987) using the PHYLIP version 3.5c program (Felsenstein 1993). Also, to gain a more detailed picture of possible phylogenetic relationships and evolutionary distances, a phylogentic tree was constructed using the neighbor joining method in PHYLIP (Fig. 3). As shown in Fig. 3, the class II *CHSs* analyzed were clearly separated into three clusters. These results were well consistent with the traditional classification based on morphological features; cluster 1 of the taxa belonging to Urediniomycetes (Ur) that includes the *CHSs* isolated in this study, cluster 2 of those belonging to Ustilaginomycetes (Us), and cluster 3 of those belonging to Hymenomycetes (Hy). These analyses, therefore, may reflect a close evolutionary relationship between the genus *Sporobolomyces* and the Urediniomycetes of Basidiomycota. The bootstrap analysis and phylogenetic tree also revealed that the Urediniomycetes are more closely related to the Ustilaginomycetes than to the Hymenomycetes of Basidiomycota.

Phylogenetic analysis of 18S rDNA and of the D1/D2 region of the 26S rDNA for Urediniomycetous yeasts deposited in the GenBank database (Hong *et al.* 2000; Hong *et al.* 2002) was well consistent with results obtained in this study. However, because sequence data of 18S rDNA for ustilaginomycetous and hymenomycetous yeasts were not searched in the GenBank database, a comparison of consistency in taxonomical level was not accomplished between

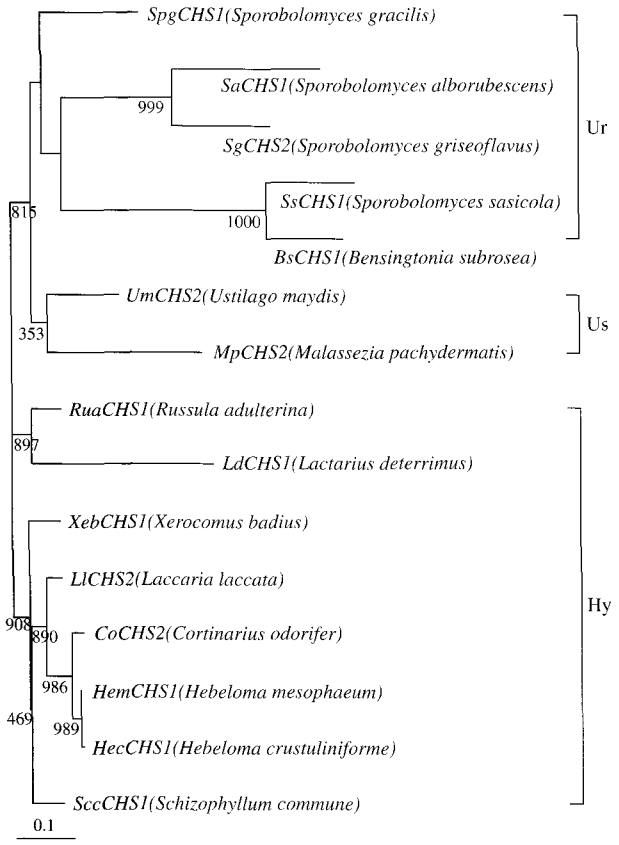

Fig. 3. Phylogenetic analysis of the deduced amino acid sequences of class II-chitin synthase fragments for the Basidiomycota and those of isolated *CHSs*. The deduced amino acid sequences were compared by neighbor joining method using the PHYLIP version 3.5c. Bootstrap values for 1,000 replicates are reported above internal branches. Hy: Hymenomycetes, Us: Ustilaginomycetes, Ur: Urediniomycetes.

CHSs and 18S rDNA sequences. It is therefore essential to carry out further comprehensive phylogenetic study.

In conclusion, the present results clearly showed that the PCR amplification of *CHS* fragments and subsequent analysis of their amino acid sequences could be used as a valuable key for the molecular taxonomic and phylogenetic studies of yeast.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Basic Research Program of Suwon Women's College (No. 2005-2007)

REFERENCES

Bowen AR, JL Chen-Wu, M Momany, R Young, PJ Szaniszlo and PW Robbins. 1992. Classification of fungal chitin synthases. Proc. Natl. Acad. Sci. USA 89:519-523.

Cabib E, A Sburlati, B Bowers and SJ Silverman. 1989. Chitin synthase 1, an auxiliary enzyme for chitin synthesis in *Saccharomyces cerevisiae*. J. Cell Biol. 108:1665-1672.

Cabib E, B Bower, A Sburlati and SJ Silverman. 1988. Fungal cell wall synthesis: the construction of a biological structure. Microbiol. Sci. 5:370-375.

Cabib E, SJ Silverman and JA Shaw. 1992. Chitinase and chitin synthase 1: counterbalancing activities in cell separation of *Saccharomyces cerevisiae*. J. Gen. Microbiol. 138:97-102.

Cho SP, SK Lee, DH Lee, KS Bae, HM Park and PJ Maeng. 1997. Cloning of two chitin synthase gene fragments from *Penicillium diversum*. J. Microbiol. 25:167-175.

Fell JW, T Boekhout, A Fonseca, G Scorzetti and A Statzell-Tallman. 1998. Validation of the basidiomycetous yeast, *Sporidiobolus microsporus* sp. nov., based on phenotypic and molecular analyses. Antonie Leeuwenhoek 74:265-270.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle.

Golubev WI. 1999. *Mastigobasidium*, a new telemorphic genus for the perfect state of ballistosporous yeast *Bensingtonia intermedia*. Int. J. Sys. Bacteriol. 49:1301-1305.

Hirai A, R Kano, Y Nakamura, S Watanabe and A Hasegawa. 2003. Molecular taxonomy of dermatophytes and related fungi by chitin synthase 1 (*CHS1*) gene sequences. Antonie Leeuwenhoek 83:11-20.

Hong SG, J Chun, JS Nam, YD Park and KS Bae. 2000. Phylogenetic analysis of genus *Sporobolomyces* based on partial sequences of 26S rDNA. J. Microbiol. Biotechnol. 10:363-366.

Hong SG, KH Lee and KS Bae. 2002. Diversity of yeasts associated with natural environments in korea. J. Microbiol. Biotechnol. 40:55-62.

Lanfranco L, L Garnero, M Delpero and P Bonfante. 1995. Chitin synthase homologs in three ectomycorrhizal truffles. FEMS Microbiol. Lett. 134:109-114.

Mehmann B, I Brunner and GH Braus. 1994. Nucleotide sequence variation of chitin synthase genes among ectomycorrhizal fungi and its potential use in taxonomy. Appl. Environ. Microbiol. 60:3105-3111.

Mellado E, A Aufauvre-Brown, CA Specht, PW Robbins and DW Holden. 1995. A multigene family related to chitin synthase genes of yeast in the opportunistic pathogen

Aspergillus fumigatus. Mol. Gen. Genet. 246:353-359.

Nam JS, DH Lee, HY Park and KS Bae. 1997. Cloning and phylogenetic analysis of chitin synthase gene from entomopathogenic fungus, *Beauveria brongniartii*. J. Microbiol. 35:222-227.

Nam JS, DH Lee, KH Lee, HM Park and KS Bae. 1998. Cloning and phylogenetic analysis of chitin synthase genes from the insect pathogenic fungus, *Metarhizium anisopliae* var. *anisopliae*. FEMS Microbiol. Lett. 159:77-84.

Namgung J, BC Park, DH Lee, KS Bae and HM Park. 1996. Cloning and characterization of chitin synthase gene fragments from *Penicillium chrysogenum*. FEMS Microbiol. Lett. 145:71-76.

Peng M, SM Karuppayil, L Mendoza, TA Levins and PJ Szaniszlo. 1995. Use of the polymerase chain reaction to identify coding sequences for chitin synthase isozymes in *Phialophora verrucosa*. Curr. Genet. 27:517-523.

Saitou N and M Nei. 1987. The neighbor-joining method: A new method for reconstruction of phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Sambrook J, EF Fritsch and T Maniatis. 1989. Molecular cloning: a laboratory Manual (2nd. ed.). Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Shaw JA, PC Mol, B Bowers, SJ Silverman, MH Valdivieso, A. Duran and E. Cabib. 1991. The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J. Cell. Biol. 114:111-123.

Thompson JD, TJ Gibson, F Plewniak, F Jeanmougin and DG Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids. Res. 24:4876-4882.

Thomsen L and A Beauvais. 1995. Cloning of two chitin synthase gene fragments from a protoplastic entomophthorale. FEMS Microbiol. Lett. 129:115-120.

Valdivieso MH, PC Mol, JA Shaw, E Cabib and A Duran. 1991. *CAL1*, a gene required for activity of chitin synthase 3 in *Saccharomyces cerevisiae*. J. Cell Biol. 114:101-109.

Valerio E, M Gadanho and JP Sampaio. 2002. *Sporobolomyces odoratus* sp. nov., a new species in the *Sporidiobolus ruineniae* clade. FEMS Yeast Res. 2:9-16.

Wang QM and FY Bai. 2004. Four new yeast species of the genus *Sporobolomyces* from plant leaves. FEMS Yeast Res. 4:579-586.

Manuscript Received: September 20, 2005
Revision Accepted: October 10, 2005
Responsible Editorial Member: Seung Bum Kim
(Chungnam Univ.)