

SHORT COMMUNICATION

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Molecular cloning and sequence analysis of the β -1,3-glucan synthase catalytic subunit gene from a medicinal fungus, *Cordyceps militaris*

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Abstract The entomopathogenic fungus *Cordyceps militaris* belongs to vegetable wasps and plant worms and is used as herbal medicine, but β -1,3-glucan biosynthesis has been poorly studied in *C. militaris*. The fungal *FKS1* gene encodes an integral membrane protein that is the catalytic subunit of β -1,3-glucan synthase. Here, we isolated cDNA clones encoding a full-length open reading frame of *C. militaris FKS1*. *Cordyceps militaris* Fks1 protein is a 1981 amino acid protein that shows significant similarity with other fungal Fks proteins. This study is the first report of molecular cloning of the β -1,3-glucan synthase catalytic subunit gene from vegetable wasps and plant worms.

Key words Catalytic subunit · cDNA cloning · *Cordyceps militaris* · β -1,3-Glucan synthase · Medicinal fungi

The medicinal mushroom *Cordyceps militaris* belongs to vegetable wasps and plant worms and is used as a tonic food and herbal medicine, but the molecular mechanisms of its pharmacological activities are not fully understood. A number of mushrooms contain biologically active β -glucans with antitumor and immunostimulating properties (Borchers et al. 1999; Wasser 2002). Mushroom β -glucans are widely sold as nutritional supplements and touted as beneficial for human health. It is known that lentinan, PSK (krestin), and schizophyllan show antitumor activity, which is beneficial in clinics when used in conjunction with chemotherapy. Moreover, mushroom β -glucans also seem to prevent oncogenesis and tumor metastasis. They do not attack tumor cells

directly but exert their antitumor effects by stimulating immune responses in the host.

The fungal cell wall is mainly constituted of polysaccharides such as β -glucan and chitin, and among them β -1,3-glucan is the most prevalent. β -1,3-Glucans are synthesized from uridine 5'-diphosphate (UDP)-glucose by a membrane protein complex, β -1,3-glucan synthase. It seems that β -1,3-glucan biosynthesis occurs on the cytoplasmic side of the plasma membrane and β -1,3-glucan chains are extruded toward the periplasmic space. The β -1,3-glucan synthase complex has been shown to be composed of the catalytic subunit Fksp, a large molecular size polypeptide with transmembrane domains, and the regulatory subunit Rho1p, a small molecular size GTPase (Douglas et al. 1994; Mazur and Baginsky 1996; Beauvais et al. 2001). The *Saccharomyces cerevisiae FKS1* (*ScFKS1*) gene encodes a 215-kDa integral membrane protein (ScFks1p), and *FKS2* (*ScFKS2*), a homologue of *FKS1* encoding a 217-kDa integral membrane protein (ScFks2p), has also been cloned (Mazur et al. 1995). Simultaneous disruption of *ScFKS1* and *ScFKS2* is lethal, suggesting that ScFks1p and ScFks2p are alternative subunits with overlapping function.

β -1,3-Glucan biosynthesis has been studied at the molecular level almost exclusively in yeast and filamentous fungi, but poorly in mushrooms, especially vegetable wasps and plant worms. Here we report the cloning and sequencing of the *C. militaris* gene homologue of *FKS1*. This study is the first report of molecular cloning of the β -1,3-glucan synthase catalytic subunit gene from vegetable wasps and plant worms.

Based on the nucleotide sequences of the conserved regions of fungal *FKS* genes (*Coccidioides immitis*, *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, and *S. cerevisiae*), two degenerate primers, cmfks-5 (5'-CCYGA RTAYACYCTSCGYACYCGYATYTGG) and cmfks-3 (5'-AARCCSAGRTCRCGRCCYTTRCCRCA YTG), were designed. Polymerase chain reaction (PCR) was done using these primers and *C. militaris* cDNA as a template, and a 950-bp DNA fragment was obtained. The nucleotide sequence of the cloned genes was analyzed by dideoxy nucleotide sequencing. Based on the obtained nucleotide

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Fig. 1. Nucleotide sequence of *Cordyceps militaris* *FKS1* and deduced amino acid sequence. The predicted transmembrane domains are *double underlined* and *numbered*. The region corresponding to the putative cytoplasmic domain is *underlined*. The CmFks1p domains 1 and 2 are indicated by *dashed underlines*. The termination codon and potential *N*-glycosylation sites are denoted by *asterisks*

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-21                                     GCTGACCTCCATAATAACAAT
1  ATGTCTGGTTACCAAGGTGGTCACCACGACCAGTACGACCAGGGCTACGGCCAGGCCGGA
1  M S G Y Q G G H H D Q Y D Q G Y G Q A G
61  CATGGCGATGGATATTACCAAGATGATCAGTACTACGACCAGGGCCACGGCGACCACGCT
21  H G D G Y Y Q D D Q Y Y D Q G H G D H A
121 GCCCAGGGCGATCACGCTGCCCAGGGCGACCACGGCGCCAGGGTACTCAAGGTGACGGC
41  A Q G D H A A Q G D H G A Q G T Q G D G
181 TACTATGATGAATCCGGCTACTATCACGCCGATGCCAACAACCCATATCACCAGGACGGA
61  Y Y D E S G Y Y H A D A N N P Y H Q D G
241 GGCTACTACGATGGGCATGACCAATACCAGGACGACTACTACAACAATAACCAGGGTTAC
81  G Y Y D G H D Q Y Q D D Y Y N N N Q G Y
301 TATGATGGCGAGTACAACCAAGGCTACGCTCAAGGTGGTCGCCACCCATCCGAAGAAGAA
101 Y D G E Y N Q G Y A Q G G R H P S E E E
361 TCCGAGACATTTCAGTGATTTTCACGATGAGGTCTGACATGGCTCGCGCCGCCGAAATGGAT
121 S E T F S D F T M R S D M A R A A E M D
421 TACTATGGCCGTGGCGATGAGCAATACAACGGATATGGAGAAGGTGGCCGTGGTTATCGC
141 Y Y G R G D E Q Y N G Y G E G G R G Y R
481 CCACCGTCTCGCAGCTGTCTTACGGGGTAATAGGTCGTCGGTGCCTCGACTCCCAAC
161 P P S S Q L S Y G G N R S S G A S T P N
541 TACGGCATGGAATACGGCAACGGCCTCGCAAGCCAGCGCTCCAAGGAACCATAACCTGCT
181 Y G M E Y G N G L A S Q R S K E P Y P A
601 TGGACATCTGACGCTCAAATCCCACCTTCAAAGGAAGAGATCGAGGATATTTTCTTGAT
201 W T S D A Q I P L S K E E I E D I F L D
661 CTAACGTCGAAATACGGATTCCAAAGAGATAGCATGCGCAACATGTACGACCACCTTATG
221 L T S K Y G F Q R D S M R N M Y D H L M
721 ACACTTCTGGACTCGAGAGCTTCTCGCATGACACCTAACCAGGCGCTCCTGTCCCTCCAT
241 T L L D S R A S R M T P N Q A L L S L H
781 GCCGACTACATTGGAGGTGACAATGCCAACTATCGCAAATGGTATTTTGGCTGCCACCTC
261 A D Y I G G D N A N Y R K W Y F A A H L
841 GACCTTGACGATGCTGTTGGCTTCGCAATGCCTCTACTCAATGAGATCAGAAAGCGCAAG
281 D L D D A V G F A M P L L N E I R K R K
901 GCCAAGAAGGGCAAAAAGAAGGGTGGTCGAGGCTGGAACAACGAGTCGGAGACCCTGCAA
301 A K K G K K K G G R G W N N E S E T L Q
961 GAACTCGAAGGTGATGATAGTCTTGAAGCCCGGAAGTATCGCTGGAAGACACGCATGAAC
321 E L E G D D S L E A R K Y R W K T R M N
1021 CGTATGTCTCAGTACGATCGCGTTCGCCAAATCGCACTTTACCTGCTGTGCTGGGGCGAA
341 R M S Q Y D R V R Q I A L Y L L C W G E
1081 GCCAATCAGGTCCGATTCATGCCAGAGTGCCTTTGCTTTATCTTCAAATGCGCAGACGAC
361 A N Q V R F M P E C L C F I F K C A D D
1141 TATCTCAATTCACCGGCTTGCCAGGCTCTTGTGGAGCCAGTTGAGGAGTTCACGTTCCCTC
381 Y L N S P A C Q A L V E P V E E F T F L
1201 AACACGTCATCACACCACTGTATCAATACTGTCGTGACCAGGGATATGAGATTCTCAAC
401 N N V I T P L Y Q Y C R D Q G Y E I L N

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Fig. 1. *Continued*

1261 GCGTCTACGTTTCGCCGAGAACGTGACCACAAGCACATTATCGGCTATGATGATTGCAAT
 421 G V Y V R R E R D H K H I I G Y D D C N

1321 CAGCTGTTTTGGTACCCTGAAGGTATCGAACGTATCGTTCTCGAAGACAAGAGTAAACTT
 441 Q L F W Y P E G I E R I V L E D K S K L

1381 GTCGATTTACCCCTGCTGAACGCTACCTTAAGTTGAAGGAGGTGAACTGGAAGAAGTGT
 461 V D L P P A E R Y L K L K E V N W K K C

1441 TTCTTCAAAACATACAAGGAATCACGCTCGTGGTTCCACTTGTACTCAATTTCAACCGT
 481 F F K T Y K E S R S W F H L L L N F N R

1501 ATCTGGGTCATACATCTGACCATGTTCTGGTTCTACACGTCACACAACGCTCCGTCGCTC
 501 I W V I H L T M F W F Y T S H N A P S L

1

1561 ATCACCTACCAATACGAGCAGCAGAAGGACAACCAACCACCAGCTCCAAGCAATTTTCG
 521 I T Y Q Y E Q Q K D N Q P P A S K Q F S

1621 ATTGTTGGCTTTGGTGGAGCTATTGCATCACTGATTCAAATTTTTCGACGCTGGCTGAG
 541 I V G F G G A I A S L I Q I F A T L A E

2

1681 TGGGTGTATGTTCCACGCCGTTGGGCCGGAGCGCAGCATCTCACGAAACGGCTACTGTTT
 561 W V Y V P R R W A G A Q H L T K R L L F

1741 TTAATTGTCATCCTCGTTCTCAACGTCGCACCAGGTGTCAAGTTTTTCATGTTTCACGGT
 581 L I V I L V L N V A P G V K V F M F H G

3

1801 AACAAAGATGGGAAAGACGCCGACCAGAAGAACAAGACACACCTATCGACAAGGCTATC
 601 N K D G K D A D Q K N K D T P I D K A I

1861 GGCATTGTCCACTTTGTCTATCGCAGTCTTTACGTTTTTGTCTTCTCGGTTATGCCTCTT
 621 G I V H F V I A V F T F L F F S V M P L

4

1921 GCGGCCCTCCTCGGCAGCTATCTGACAAAAAAGTCACGACGTTATGTGGCTAGCCAGACT
 641 G G L L G S Y L T K K S R R Y V A S Q T

1981 TTTACAGCCAGTTACCCTCGCCTGACTGGCAATGACATGGCCATGTCATTTGGTTTGTGG
 661 F T A S Y P R L T G N D M A M S F G L W

2041 CTGACCGTGTTCGGAGCGAAGTTTGGCGAATCTTATGTATACCTCACTCTTTCATTCCGT
 681 L T V F G A K F G E S Y V Y L T L S F R

2101 GATCCAATTCGATATCTGTCCATCATGAAGATTGACTGCTTGGGCGATGCGATGTTTCGGA
 701 D P I R Y L S I M K I D C L G D A M F G

2161 AGCACGGCGGCGACGCAGCAGATACTGTGCAAGCACCAGCCTACAATTGTTCTCATTCTC
 721 S T A A T Q Q I L C K H Q P T I V L I L

2221 ATGACGTTACCGATCTGATCTTCTTCTTCTTCTGACACCTATCTTTTCTATGTCATCTTG
 741 M T F T D L I F F F L D T Y L F Y V I L

5

2281 AATACCGTGTCTCCATTGCACGCTCTTTTTACATCGGCTCATCGATCTGGACGCCATGG
 761 N T V F S I A R S F Y I G S S I W T P W

6

2341 CGAAATATCTTTTCGAGACTGCCAAAGCGTATTTACTCCAAGGTGCTCGCGACGACTGAT
 781 R N I F S R L P K R I Y S K V L A T T D

2401 ATGGAGATCAAATACAAACCAAGGTCCTCATCTCCCAAGTCTGGAATGCCATCGTCATT
 801 M E I K Y K P K V L I S Q V W N A I V I

2461 TCAATGTATCGCGAGCATCTTCTCGCAATCGACCATGTGCAGAAGCTGTTGTATCACCAG
 821 S M Y R E H L L A I D H V Q K L L Y H Q

Fig. 1. *Continued*

2521 GTGCCCTCTGAGCAGGAAGGCAAACGGACATTACGCGCTCCGACCTTCTTCGTTTCCCAG
 841 V P S E Q E G K R T L R A P T F F V S O

2581 GAAGACCACTCGTTCAAAACAGAATTCTTTCCCAGCCACAGTGAAGCTGAGCGGCGCATC
 861 E D H S F K T E F F P S H S E A E R R I

2641 TCATTCTTTGCCAGTCTCTCTCCACACCTATTCCCGAACCCGTCCTGTGCATAACATG
 881 S F F A Q S L S T P I P E P V P V D N M

2701 CCTACGTTACAGTCATGATCCCCATTACAGTGAAAAGATTCTCCTCTCTCTGCGAGAA
 901 P T F T V M I P H Y S E K I L L S L R E

Domain 1

2761 ATCATTCGTGAAGACGAGCCCTACTCCCCTGTGACTCCTGGAATACCTGAAGCAGCTT
 921 I I R E D E P Y S R V T L L E Y L K O L

2821 CATCCGCATGAATGGGAATGCTTTGTCAAGGACACAAAGATTTTGGCAGATGAAACAGCT
 941 H P H E W E C F V K D T K I L A D E T A

2881 CAGATGAATGGCGAACCAGAGAAGAGCGAAAAAGACACAGCGAAGAGCAAATCGACGAT
 961 Q M N G E P E K S E K D T A K S K I D D

2941 TTGCCCTTTTACTGCATTGGTTTCAAGTCTTCTGCCCGGAGTATACTCTTCGCACACGT
 981 L P F Y C I G F K S S A P E Y T L R T R

3001 ATTTGGGCTCTTTGCGTTCGCAAACCTTTATATCGCACAGTGTCTGGCTTTATGAACTAC
 1001 I W A S L R S O T L Y R T V S G F M N Y

Domain 2

3061 AGTCGTGCCATCAAGCTCCTGTATCGTGTGAGAAATCCCAGGTTGTTTCAGATGTTTGGT
 1021 S R A I K L L Y R V E N P E V V O M F G

3121 GGAAACTCAGAAAAGTTGGAACGAGAAGTGGAGAGAATGGCTCGACGCAAGTTCAAGCTC
 1041 G N S E K L E R E L E R M A R R K F K L

3181 GTTGTGTCTATGCAACGCTATTCCAAATTCAAGAAGGAAGAGATGGAAAATGCCGAATTT
 1061 V V S M O R Y S K F K K E E M E N A E F

3241 TFACTGCGCGCTATCCGGATCTTCAAATTGCTTACCTGGATGAGGAGCCTCCTCTAGCA
 1081 L L R A Y P D L O I A Y L D E E P P L A

3301 GAAGCGAGGAACCTCGCCTGTACTCCGCACTTATCGACGGTCACTCAGAACTCATGGAG
 1101 E G E E P R L Y S A L I D G H S E L M E

3361 AATGGTATGAGGCGACCAAAGTTCCGTGTCCAGCTCTCTGGTAACCCGGTATTGGGTGAT
 1121 N G M R R P K F R V O L S G N P V L G D

3421 GGTAAATCCGACAACCAGAATCACGCCATCATCTTTTACCGGGTGAATACATTCAGCTC
 1141 G K S D N O N H A I I F Y R G E Y I O L

3481 ATCGACCCAACCAAGACAACCTATCTGGAAGAATGCCTGAAGATTCGCAGTGTCTCGCA
 1161 I D A N Q D N Y L E E C L K I R S V L A

3541 GAGTTCGGGGAAATGAAGCCAGACAACCATTTCCCCTTACACACCCGGAGTCAAGAACGAT
 1181 E F G E M K P D N H S P Y T P G V K N D

3601 GTGCACACTCCAGTTGCTATTCTTGGTGCTCGCGAGTACATTTTCTCGGAGAATATTGGT
 1201 V H T P V A I L G A R E Y I F S E N I G

3661 ATTCTCGGTGATGTTGCTGCTGGAAAAGAACAACATTTGGTACCCTCTTTGCCCGTACT
 1221 I L G D V A A G K E O T F G T L F A R T

3721 ATGGCACAGGTTGGTGGCAAACCTTCACTACGGCCATCCTGATTTCTAAACGGTATCTTC
 1241 M A Q V G G K L H Y G H P D F L N G I F

Fig. 1. Continued

3781 ATGACAACACGAGGGGTGTATCGAAGGCTCAAAGGGACTGCATCTCAACGAAGATATT
 1261 M T T R G G V S K A O K G L H L N E D I

3841 TTCGCAGGTATGAATGCACTTGTCCGCGGTGGACGCATTAAGCACTGCGAATACTACCAG
 1281 F A G M N A L V R G G R I K H C E Y Y O

3901 TGTGGCAAGGTCGTGATCTTGGATTTGGTTCTATTCTCAATTTACGACCAAGATTGGC
 1301 C G K G R D L G F G S I L N F T T K I G

*

3961 ACCGGCATGGGAGAACAATGGCTCTCTCGGAATACTACTACCTCGGCACTCAACTTCT
 1321 T G M G E Q W L S R E Y Y Y L G T O L P

4021 CTTGACAGATTTCTGTCCTTCTACTATGCACACGCGGGCTTCCACGTCAACAACATGTT
 1341 L D R F L S F Y Y A H A G F H V N N M F

4081 ATTATGCTGTCTGTCCAGTCATTTATGCTGACGCTGATGTCCATTGGCGCACTGCGACAC
 1361 I M L S V Q S F M L T L M S I G A L R H

7

4141 GAAACAATCCGCTGCGACTACAATCCACAGAAACCAATCACGGATCCTCTGTATCCTACG
 1381 E T I R C D Y N P Q K P I T D P L Y P T

4201 AAATGCTCCAACACGGACGAGCTGATGGGCTGGGTCTACCGTTGCATTATCTCTATTTTC
 1401 K C S N T D E L M G W V Y R C I I S I F

4261 TTCGTCTTCTTCATCTCTTTTCGTGCCGCTCATTGTCCAAGAATTGACCGAACGAGGTGTG
 1421 F V F F I S F V P L I V Q E L T E R G V

8

4321 TGGCGTGCAGCTTTGCGCTTCATTAACAGTTTGTCTCTATCTCCGTTCTTCGAAGTG
 1441 W R A A L R F I K Q F C S L S P F F E V

9

4381 TTCGTCTGTGAGATTTATGCCAACTCAGTCCAGTCGGATCTTGCATTTGGTGCGCTCGT
 1461 F V C Q I Y A N S V Q S D L A F G G A R

4441 TATATTGGTACTGGTCGTGGTTTTCGCAACGGCTCGTATTCCGTTTGGCGTTTTGTATT
 1481 Y I G T G R G F A T A R I P F G V L Y S

4501 CGATTTGCCGGACAGTCGATTTATTTCCGGTGCAAGATTACTGATGATGCTCCTGTTCCGCT
 1501 R F A G Q S I Y F G A R L L M M L L F A

10

4561 ACTTCCACTGCCTGGCAACCTGCTCTGACCTACTTCTGGATCGTTCTGCTTGGTCTCATC
 1521 T S T A W Q P A L T Y F W I V L L G L I

11

4621 ATCTCACCCTCCTGTACAACCTCATCAGTTTGCCTGGACCGATTTCTTCATAGATTAC
 1541 I S P F L Y N P H Q F A W T D F F I D Y

4681 CGTGAATCCTTCGATGGCTGTCTCGAGGCAATTCGCGCGCACATGCTTCTTCTGGATC
 1561 R D F L R W L S R G N S R A H A S S W I

4741 ATGTTTTGCCGGCTCTCCCGTACTCGCATCACGGGATACAAGCGCAAAGTCATGGGCGAT
 1581 M F C R L S R T R I T G Y K R K V M G D

4801 GCTTCTGCCAAGATGTCTGCTGACGTTCCCCGCGCGGCTGTGGCCAACATCTTCTTGACC
 1601 A S A K M S A D V P R A A V A N I F L T

4861 GAGATCCTCAGCCATTGCTACTTGGCTGCCACCACCACGGTTGCTTACCTATTTCGTC
 1621 E I L T P L L L A A T T V A Y L F V N

12

4921 GCACAGACCGGTGTTACAGACAATGACAAGAACTCGAGTTCCTCGCCTGGCTTTAAGATT
 1641 A Q T G V T D N D K N S S S S P G F K I

*

4981 GGGCCTATCGGCGCATTGATTCGATTGGCTGTCGTGGCTTTTGGCCTATCGGCATCAAC
 1661 G P I G A L I R L A V V A F A P I G I N

Fig. 1. Continued

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5041 GCCGGCGTTCTTGCAGCCATGTTTGGAAATGGCTTGTGTATGGGACCAGTTCTGAACATG
1681 A G V L A A M F G M A C C M G P V L N M
                                     13
5101 TGTTGCAAGAAGTTCGGGCCTGTTCTCGCTGGTATCGCCCACGGCGCGGCTGCAGTCTTC
1701 C C K K F G P V L A G I A H G A A A V F

5161 ATGATTATCTTCTTCGAAGTTATGTATGTTTTGGAAGGATTCAACTTCGCCAGAGCTCTT
1721 M I I F F E V M Y V L E G F N F A R A L
                                     14
5221 GCGGGTATCATCGCCGCCATGTGCATCCAGCGTTTCATTTTTAAGCTCATTGTGTCACTG
1741 A G I I A A M C I Q R F I F K L I V S L

5281 GCGTTGACCAGAGAGTTCAAGACTGATCAGTCCAACATTGCCTTCTGGAACGGAAAATGG
1761 A L T R E F K T D Q S N I A F W N G K W

5341 TACTCAATGGGCTGGCACTCAGTATCCCAACCGGCCGAGAGTTTTTATGCAAGATTACG
1781 Y S M G W H S V S Q P A R E F L C K I T

5401 GAACTCAGCATGTTCTCTGCTGATTTTCAATTCTGGGACATTGGATTTTTATTCATGATGGCT
1801 E L S M F S A D F I L G H W I L F M M A
                                     15
5461 CCGTTGATTTTGATCCCGCAGATTGATAAGATCCATTCATGATGCTCTTCTGGTTGCTG
1821 P L I L I P Q I D K I H S M M L F W L L

5521 CCTAGTCGTCAGATTGCCCCACCCATCTACTCCATGAAGCAGTCAAAGCTTCGGCGTAGA
1841 P S R Q I R P P I Y S M K Q S K L R R R

5581 CGTGTATTTCGATTTGCCATTCTTTACTTTGTTCTTTCATCATTTTTCTGGCGCTCGTT
1861 R V I R F A I L Y F V L F I I F L A L V
                                     16
5641 GTTGCTCCAGGCGTCATTGGCAAGAAGTTCCCTTGGCGATAACAATCTTCAAAGCTCTGGAT
1881 V A P G V I G K K F L G D T I F K A L D

5701 AATGGCAACGGCGGCCCGCCAATTTGCACCTTCTGCAGCCGTGGGGACTGGACAACAAC
1901 N G N G G P A N L H L L Q P W G L D N N
                                     *
5761 AACACTGAAGGCAAGACTGAAACTGGCACCAAGGCTGGTGGTGCTGATGCATCTGCCACA
1921 N T E G K T E T G T K A G G A D A S A T

5821 GATGCCTCGAAGCTGCGTCTTATTCTAAGCTTCAAACGCCACACGCATCGATTTGAAAAG
1941 D A S K L R L I L S L K R H T H R F E K

5881 CCACCATGTTCTGGGAAATCACAAACCATTGTACCGATTATTAGATTGTTTCTGTTATTA
1961 P P C S G K S Q T I V P I I R L F L L L

5941 TTTTGATCCATTTTGTCTCGGATAGGGTTAATACAGAAGTTTTGGTTGG
1981 F *

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sequences, several primers were designed for 5'/3' rapid amplification of cDNA ends (RACE). RACE was done using a 5'/3' RACE kit (Takara, Otsu, Japan). Sequence comparisons to databases were performed using the BLAST program (National Center for Biotechnology Information). Sequence analysis was performed with the Genetyx program (Genetyx, Tokyo, Japan). Hydrophathy analysis was performed by the method of Sipos and von Heijne (1993).

Here, we isolated a single *FKS* homologue that we named *CmFKS1*. An open reading frame of 5943bp was identified in the complete sequence (Fig. 1). The deduced sequence of 1981 amino acids shows 72% identity to *P.*

brasiliensis Fks1p, 71% to FksAp from *Aspergillus nidulans*, 70% to Fksp from *Aspergillus fumigatus*, 63% to *Yarrowia lipolytica* Fks1p, 62% to Fks1p and Fks2p from *S. cerevisiae*, 61% to *Candida albicans* Fks1p, and 60% to *C. neoformans* Fks1p. Sequence analysis showed that *C. militaris* Fks1p (*CmFks1p*) is an integral membrane protein very similar to other Fksp in yeast and filamentous fungi. Similar to *S. cerevisiae* Fks1p (*ScFks1p*), *CmFks1p* possesses 16 potential transmembrane domains with a relatively large hydrophilic domain in the middle of the protein. The region of *CmFks1p* most homologous to *ScFks1p* is the large hydrophilic domain of 578 amino acids that is predicted to be a cytoplasmic domain. This domain is 84%

Fig. 2. Domains 1 and 2 are conserved regions between CmFks1p and BcsAp. Identical amino acids are denoted by *asterisks*; + indicates similar amino acids. CmFks1p, *C. militaris* Fks1p; BcsAp, catalytic subunit of cellulose synthase from *Acetobacter xylinum*

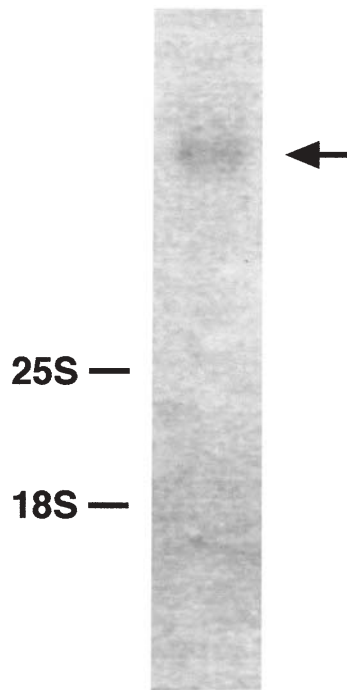
Domain 1

CmFks1p	890	PIPEPVPVDNMPTFTVMIPHYSEKI	914
		*** * **+ ** + ** * **+	
BcsAp	138	PLPLPDNVDDWPTVDIFIPTYDEQL	162

Domain 2

CmFks1p	997	LRTRIWASLRQSQTLYRTVSGFMNYSRAIKLLYRVENP	1033
		** + + * ++ * + * +++ + +++++**	
BcsAp	350	LRIPVASGLATERLTTHIGQRMWARGMIQIFRVDNP	386

Fig. 3. Northern blot analysis of *C. militaris* *FKSI* transcript. Northern blot of total RNA isolated from *C. militaris* and probed with the 1.7-kb *CmFKSI* cDNA fragment. *Arrow* indicates the position of the *CmFKSI* mRNA band. Positions of the 25S and 18S rRNA markers are indicated



identical to the corresponding region of ScFks1p and is a candidate for the location of the catalytic site. As reported with other Fksp, CmFks1p does not contain the proposed UDP-glucose binding motif QXXRW, but it has regions with homology to BcsAp, the catalytic subunit of cellulose synthase from *Acetobacter xylinum* (Kelly et al. 1996). These specific domains (domains 1 and 2, Fig. 2) are located in the hydrophilic central region, as indicated in Fig. 1. Because cellulose synthase catalyzes the formation of β -1,4-glucan using UDP-glucose as a substrate, these conserved domains may be involved in UDP-glucose binding. Similar to ScFks1p, CmFks1p has six potential N-glycosylation sites. *CmFKSI*, the gene encoding a catalytic subunit Fks1p of β -1,3-glucan synthase in *C. militaris*, was studied for the first time in vegetable wasps and plant worms. The complete nucleotide sequence of *CmFKSI* has been submitted to DDBJ/EMBL/GenBank under accession number AB219141.

To analyze the *CmFKSI* gene expression, Northern blot analysis was done as described previously (Ujita et al. 1995). *Cordyceps militaris* was grown in a potato dextrose broth medium (Difco) at 25°C. At the late logarithmic phase of the growth, the hyphae were harvested by centrifugation. For total RNA isolation, an RNeasy kit (Qiagen) was employed. Thirty micrograms of total RNA from *C. militaris* was electrophoresed in a denaturing 2.2M formaldehyde-1.0% agarose gel and transferred as described previously (Ujita et al. 1995). A 1.7-kb cDNA fragment corresponding to nucleotide positions 2998–4668 in Fig. 1 was labeled using DIG DNA labeling and detection kit (Roche) and used as a probe. Detection of the hybridized probe was done according to the manufacturer's instructions. Northern blot analysis revealed that *C. militaris* *FKSI* mRNA of approximately 9kb was expressed in hyphae (Fig. 3). A single transcript was detected as observed with *S. cerevisiae* (Mazur et al. 1995), *A. nidulans* (Kelly et al. 1996), *C. albicans* (Mio et al. 1997), *C. neoformans* (Thompson et al. 1999), and *Y. lipolytica* (Leon et al. 2002) (Fig. 3).

The cell wall β -glucan of yeast, filamentous fungi, and mushrooms is essential for their shape and viability, and some fungal β -glucans show antitumor activity by activating immune systems. It has been shown that the maitake mushroom (*Grifola frondosa*) β -glucan exhibits strong anticancer activity by increasing immunocompetent cell activity (Kodama et al. 2002). *Grifola frondosa* β -glucan has been reported to exert its antitumor effect in tumor-bearing mice by enhancing the immune system through activation of macrophages, T cells, and natural killer (NK) cells, which are cytotoxic for tumor cells and play an important role in regulating immune responses. *Grifola frondosa* β -glucan also appears to repress cancer progression (Kodama et al. 2003). Furthermore, the biosynthetic pathways of β -glucans are possible targets for the design of novel chemotherapeutics against human pathogens such as fungi, because mammals are not thought to possess β -glucan synthases. Although β -1,3-glucan synthesis has been intensively studied, the biosynthetic pathway is not fully understood. Molecular cloning of the *CmFKSI* gene might lead to the elucidation of the biosynthetic pathway of *C. militaris* β -glucans that are used as a tonic food and herbal medicine and integrate the pharmacological activities of vegetable wasps and plant worms with basic science.

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