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Short communication

Sequence analysis and heterologous expression of lectin-like gene *CMLec3* from the medicinal fungus *Cordyceps militaris*

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ABSTRACT

Through data mining of the *Cordyceps militaris* genome, a lectin-like encoding gene, *CMLec3*, was identified. In this study, the *CMLec3* sequence was analyzed using bioinformatics approaches, and the gene was heterologously expressed in *Escherichia coli* BL21 cells. The biological activity of the product was examined. In addition, *CMLec3* gene expression levels were assessed. The results showed that the CMLec3 protein contained a lectin domain structure and was successfully expressed. The CMLec3 protein partly inhibited HeLa cell proliferation. *CMLec3* exhibited the highest gene expression in the primordium at a level 5.19 times that of the mycelium and 1.35 times that of the fruiting body. This suggests that the gene may be related to fruiting body development.

Lectins are known as carbohydrate-binding proteins and have been found in various organisms, including animals, plants, fungi, bacteria and viruses (Guillot and Konska, 1997). Lectins are able to induce cell agglutination and the precipitation of glycoconjugates (Lis and Sharon, 1986). They have attracted the attention of some researchers in view of their potentially exploitable activities including antiproliferative (Yang et al. 2005; Liu et al. 2006), immunoenhancing (Lam and Ng, 2002), antifungal (Ng et al. 2006) and antiviral (Sun et al. 2003) activities.

Ascomycete mushroom *Cordyceps militaris* has been used for a long time as a nutraceutical and in traditional Chinese medicines. There have been reports on the biological functions of its chemical constituents including cordycepin (Ng and Wang 2005) and polysaccharide (Yu et al. 2004). There have been two papers relate to lectins extracted from *C. militaris* and its biological function. However, the sequence of N-terminal amino acid are different between these two reports. There have been no reports about genetic analysis on lectins from *C. militaris* so far.

In this study, a lectin-like gene encoding lectin-ccm3 was find in the *C. militaris* genome data, GenBank accession number is XM_006666745. It was heterologously expressed in *Escherichia coli* BL21. The biological activity of product was examined.

1. Sequence analysis of CMLec3

The *CMLec3* nucleotide sequence was translated into amino acids using DNAMAN software. The nucleotide sequence was analyzed by the BLAST program (www.ncbi.nlm.nih.gov/BLAST). Physicochemical properties were analyzed using the Protparam tool on the Expasy server. A signal peptide search was performed with SignalP 4.1 (www.cbs.dtu.dk/services/SignalP). Conserved domains was analyzed using CD-search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). A multiple sequence alignment and phylogenetic tree were created using MEGA 6.0 (Tamura et al. 2013) with the maximum-likelihood method (bootstrap values performed on 1000 replicates). Species were selected for multiple sequence alignment from the non-redundant GenBank database (Karan et al. 2014).

The *CMLec3* gene sequence contains 1296 bp, encoding 432 amino acids. The molecular weight of the protein is about 48 kDa. It contains a lectin-like conserved domain from amino acids 21 to 236. The nature of this conserved domain can bind K^+ ion at the edge of a concave β-sheet. Binding of K^+ ion to this domain appears essential for transport of a subset of glycoproteins. There is a distinct signal peptide from amino acids 1 to 18. The *CMLec3* gene was found to be related to those of other ascomycetes (Fig. 1).

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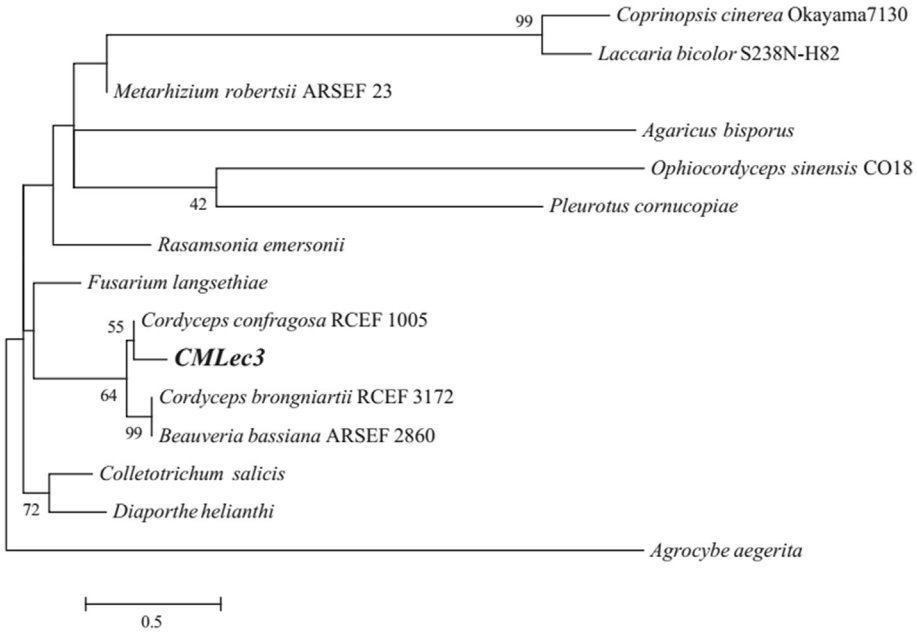

Fig. 1. Rooted phylogenetic tree of the deduced amino acid sequence of CMLec3 and lectins from other species.

Lectins from *Cordyceps confragosa* RCEF 1005, *Beauveria bassiana* ARSEF 2860, and *Metarhizium robertsii* ARSEF 23 exhibited 87.96%, 85.19%, and 52.41% identity, respectively, with the CMLec3 complete amino acid sequence. However, CMLec3 exhibited lower similarity with lectins from basidiomycetes, such as *Laccaria bicolor* S238N-H82 (11.57%) and *Pleurotus cornucopiae* (12.50%).

2. Cloning and sequencing of full-length CMLec3 from C. militaris

Cordyceps militaris total RNA was extracted using the RNApure extraction kit (Bioteke, China). The primers used for amplifying the *CMLec3* gene sequence are listed in Table 1. The PCR product was purified and cloned into pMD19-T (TaKaRa, Dalin, China) for sequencing (Sangon Biotech, China).

3. Vector construction and prokaryotic expression

The target sequence was inserted into the prokaryotic expression vector pET-32a, and the correct open reading frame was confirmed by sequencing. The sequencing primers are S·Tag primer and T7 terminator primer (Table 1). The recombinant expression vector was transformed into *E. coli* BL21 (DE3) cells. Positive clones were inoculated into LB liquid medium including 100 μg/mL ampicillin. After overnight cultivation, the culture was expanded to 200 mL by 1% inoculum and incubated at 37 °C with shaking at 200 rpm until reaching an OD600 of 0.4–0.6. IPTG was added to a final concentration of 0.2 mM for inducing expression of the target protein, and the culture was incubated at 16 °C with shaking at 200 rpm for 12 h.

Table 1
List of primers used in this study.

Primer name	Primer sequence (5′–3′)
Lccm3-F	GAAGCATCGATGAATTCCAGTTTCTCATCAACGAGCT
Lccm3-R	CTGGGCCACGTGAATTCCAGATACTTCTTTGGGCTAG
S·Tag primer	GGTTCTGGTTCTGGCCATA
T7 terminator primer	CTAGTTATTGCTCAGCGGT
RT-L3F	GCTACACCTTTGGTATTACGG
RT-L3R	GTTGCTGTTCTGGTTGTTGT
Tubulin-F	CAACAACATCCAGAACGCTC
Tubulin-R	CAGTGAACTCCATCTCGTCC

4. Extraction and identification of recombinant protein

The bacterial solution was centrifuged at 4 °C and 7000×*g*. The supernatant was discarded, and bacteria were resuspended in 10 mL PBS. Cells were disrupted and protein extracted using a high-pressure disruptor. The suspension was centrifuged at 4 °C and 12,000 rpm, and the supernatant was collected and precipitated. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) using a 15% acrylamide slab gel in the presence of 2-mercaptoethanol. The gel was stained with Coomassie Brilliant Blue R-250. Molecular mass markers (10–200 kDa) were purchased from TaKaRa. Protein concentrations were determined according to the method of Bradford using bovine serum albumin as a standard (Bradford 1976). The protein concentration was 1.8 mg/mL. SDS-PAGE was performed to detect the target protein (Fig. 2). There was an obvious band at about 70 kDa (containing a Trx-tag) in the induced sample.

5. Growth inhibition of bacteria by CMLec3

LB medium was sterilized at 121 °C for 30 min. After cooling to 50 °C, a 1% volume ratio of overnight E. coli or Staphylococcus aureus culture was added, and plates were immediately poured. After plates had solidified, 2 μL CMLec3 protein extract was added to the surface of each plate. The expressed product of the empty vector was used as a negative control, and ampicillin was used as a positive control. Bacteria were cultivated for 10 h at 37 °C, and then growth was assessed by observing transparent growth rings.

According to the bacteriostatic test, CMLec3 protein had no effect on the growth of *E. coli*, but it has an inhibitory effect on the growth of *S. aureus*. We measured the diameter of the bacteriostatic ring against that of the positive control, ampicillin; the