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Research Article

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A trehalose-6-phosphate synthase gene from *Phellinus igniarius*

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ABSTRACT

Trehalose, an important disaccharide in fungal spores, plays important role in protects against many environmental stresses, such as heat and salt. In this study, a gene (PiTPS) encoded trehalose-6-phosphate synthase was isolated from Phellinus igniarius (L.: Fr.) Quél, a basidiomycete fungus belonging to the family Polyporaceae. It contains many bioactive compounds that have been reported to possess antiviral, antibacterial, antitumor and antimutagenic activities. The PiTPS cDNA is composed of 2688 nucleotides, which encodes a protein of 517 amino acids and its molecular mass is about 100 kDa. The amino acid sequence has a relatively high homology with the TPS in several other filamentous fungi. The predicted protein contains a glycol_transf_20 domain and a trehalose_PPase domain. Genomic structure of PiTPS is composed of three exons with 607, 763 and 1,318bp and two introns with 73 and 52 bp. In this rearch, we clone and express the TPS gene in E. coli, constructed engineering strain, and laid the foundation for further study its structure and function.

Keywords: Trehalose-6-phosphate, *Phellinus igniarius*, expression

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INTRODUCTION

As a non-reducing disaccharide, Trehalose have two glucose units linked in α,α-1,1-glycosidic linkage. It exists widely in organisms as a primary energy source, including prokaryotes, fungi, invertebrates and plants[1,2]. Furthermore, trehalose play an important role in protein integrity and as stress protection metabolite for structure stabilization in stress tolerance[3-8]. In some plant and fungi, It also plays as signaling molecule. As we all know in *Escherichia coli* and *Saccharomyces cerevisiae*, the biosynthesis of trehalose has been described clearly. The formation of trehalose-6-phosphate from glucose-6-phosphateand UDP-glucose by trehalose-6-phosphate synthase (TPS) [9].

Phellinus igniarius is a large Rare Medicinal Mushroom with many medicinal value, for example anti-tumor, Cure vaginal bleeding, antibacterial, treat amenorrhea and so on[10-12], and is internationally recognized as the first of the medicinal mushroom in the biological anticancer efficiency, with a huge market potential. As a key enzyme of the important metabolite, it play an important role in the growth and development of *Phellinus igniarius*. therefore, this study provide a theoretical basis for the growth and metabolism of *Phellinus igniarius.*

TPS is a key enzyme, which catalyzes the first step in the biosynthesis of trehalose, In some fungis, TPS not only considered as a enzyme in trehalose biosynthesis but also as a regulator cope with stress, for example, it has important role in sugar-induced signaling and controlling sugar influx[13-18]. For its important biological role, many people have been cloned and expressed TPS gene from many organisms, in order to better understand its structure and function. Here, we selected *Phellinus igniarius* as the object, Cloned and expressed TPS gene, and further study its function.

EXPERIMENTAL SECTION

Strains, plasmids and culture conditions

Phellinus igniarius was presented from China University of Petroleum, Cultured on PDA medium, 28 □, 150rpm for 8 days. Plasmid pMD18-T was purchased from Takara (Dalian, Liaoning, China). pET22b was used as an expression vector and purchased from Invitrogen. All plasmids were amplified in *E.coli* DH5α from Takara (Dalian, Liaoning, China). For protein expression, plasmids were transformed in *E. coli* BL21(DE3)(pLysS) (hereafter called BL21) purchased from Invitrogen.

RNA extraction and cDNA synthesis

Phellinus igniarius was cultured at 28 ℃, 150rpm in PDA liquid medium for 8 days, Cells were collected by filters and soaked up all the water with absorbent paper, after ground in liquid nitrogen, extracted RNA using the Trizol method, detected of RNA purity. The first strand cDNA was prepared using the Prime ScriptII cDNA synthesis kit (Takara, Tokyo, Japan) following the manufacturer's instructions and stored at -20 ℃.

Tps cDNA cloning

We designed two degenerate primers to amplify the fragment of TPS gene. A PCR product was separated for sequencing. After sequencing, the fragment was used for blast analysis (http://www.ncbi.nlm.nih.gov/BLAST), and then to design two special primers for amplifying a PiTPS cDNA fragment. The full-length PiTPS cDNA sequence was obtained by RACE PCR. The 3′-Full RACE kit (Takara, Dalian, Liaoning, China) was used to amplify the 3′-ends according to the manufacturer's manual. Each PCR products attained above were subcloned into the pMD18-T vector respectively, and transformed into *E.coli* DH5α for determination by BioAsia(Shanghai, China).

Phylogenetic analysis of PiTPS

We collected TPS amino acid sequences of different species from the NCBI database, a phylogenetic tree was constructed using Neighbor-Joining method, and the overall amino acid sequences of PiTPS were used[19].

Construction of expression plasmid

The cDNA encoding mature peptide of PiTPS was amplified by using the primers of PiTPS-orf1 (5′-CCGGAATTCATGGATTCGTTCAACGAACCACC -3′) with the *Eco*RI recognition site (underlined) and PiTPS-orf2 (5′- ATCTGCGGCCGCTTAGAGATTACTCTTTTCC-3′) with the *Not*I recognition site (underlined in front). The fragments ligated into pET32a with a His-tag vector to create pET32a-TPS. The valid recombinant plasmid was then transformed into expression strain *E. coli* BL21(DE3)(pLysS), transformants were incubated in Lysogeny broth (LB) medium (containing 0.1 mg/ml ampicillin), shaking with 150 rpm at 37℃. When the OD600 reached about 0.6, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 mM, Induced for about 4h, cells were collected by centrifugation, The expression of the empty vector was used as negative tests. His-tag was used to purified protein, His-tag had been reported not to affect secretion, folding or function of recombinant protein [20-21], And then SDS-PAGE analysis of the expression of TPS in *E. coli*.

RESULTS AND DISCUSSION

Sequence analysis of the TPS gene

Firstly, according to the NCBI database information of different organisms, design degenerate primers to amplify conserved regions of TPS gene, sequencing analysis of gene sequences, redesigned primer to amplify the complete sequence of the full-length gene TPS by RACE-PCR technology. *Phellinus igniarius* TPS gene total length is 2688bp (Fig. 1a), encode 895 amino acids (Fig. 1b), gene function forecasting domain contains two functional domains, no signal peptide structure. To further analyze the intron size and distribution of TPS gene sequence, sequence analysis, and comparison with the TPS gene analysis it contains 2 introns and 3 exons. (Fig. 2)

Phylogenetic analysis of PiTPS

We collected Amino acid sequences of TPS come from different species, the sequence informations download from NCBI database. according to these sequence information, a phylogenetic tree was constructed using Neighbor-Joining method. (Fig. 3)

SDS-PAGE assay of the recombinant PiTPS protein

After 4 h induction with 0.2 mM IPTG, the recombinantproteins were separated on the SDS-PAGE and one distinct band about 100 kDa was identified. No obvious expression of 100 kDa was observed in the control tests, and purified protein using HIS-tag. (Fig. 4)

Fig. 1 (a)Nucleotide sequences of PiTPS gene. The letters in shadow indicated the start codon (ATG) and asterisk indicated the stop codon (TAA). (b) Deduced amino acid total 895 amino acids, predicted isoelectric point 5.95

(a)

CATTATCTACTTATTGCTTAAAGCATTCAACCAAACAAGGGACTTTGTGAAAGCAATCTCATCATACCATG
GATTCGTTCAACGAACCACCGAGCTCTCGTTCTCGCCAAGAGTTCTCGTTGAGTCTTGAGGACGTCAAC
AAGCAGATCACAGCCCTCAAGAACGAGTACGCAGCAAAGGGCATCGAGCTCTCCGGGCGGATCATCCA
CGTCACCCATTATCTCCCGCTCACAGTCCACCTCCTGCCCAGCAAGAACGGCGTCCTCTCCCCACCGCT
CACCCCGCCCGTGAAACCCGCGGATGTAGCGCTCTCGCCGACAGAGGAGGAGAGGCCGCTCCCGAAGA
TCGTCGAGTCGGCTGCCGGGAGCGAGTCGGACGGTCCGCGCTGGACTATCGGCCCTCGTTGGGGCCAC
TCGGCGATGGTCAGCGGCATTGAAAGCTTGAGCAACGCGCACGAGCAGATCATCGTTGGCTGGACGGG
CGATATCGAGCGCGGAGCGGCCTCGACGGGCGACAAAGTCAAGATCCCGCTGAGTTCGGTCTCAGACG
AGGACCGTGCCACGCTCACCGCGGCTATCGAGTCTCACAAAACTGAAGACGGCCCATCCAAGTACGTT
CCGGTCTGGCTGGACGATCTTGTTGCACACGGACATTATGATGGTTATTGCAAGACAACGCTTTGGCCGC
TATTCCATTACCTACTATGGCAGGACGTAGCGAACGAAGCTGCAAGCGCCGATGAACACTGGGGCGAGT
ACAAAGAAGCGAACTACGTCTACGCGAAGCGCGTGGCAGAAGTATACAAACCAGGAGACCTCGTCTGG
GTGCACGACTACCACCTTCTGCTTGTCCCGCAGATTCTGCGCCAGCTCATCCCCGACGCAGTCACGGGC
CTCTTCGTGCACACGCCATTCCCGAGCTCCGAGATCTTCAGATGCCTGCCGAGGAGGAAGGAGATCCTC
GACGGCATGCTCGGCGCGAACCTCGTGTGCTTCCAGACGTACTCCTACTCCCGCCACTTCACATCGTCC
TGCGTCCGCGTGTGCGGGTACGAGTCTACCGGGAAGGGGATTGATAACCAGGGACATGTGACGACTGT
TGCGCACTGCCCCGTCGGCGTCGACGCCGAGCGTATCGCGCAGAACGTGGTCAGACCGGGTGTCAAAC
CCAAATTCGATGCGCTTCGATCGTTATACGAGGGAAAGAAGATCATCGTTGCGCGCGATAAGCTTGATGT
CGTCAAGGGTGTCGTGCAGAAACTCCGCGCGTTTGAGCGACTTCTGCAGGACTATCCGGAATGGATTGG
CAACGCTGTCCTAATTCAGGTCACATCTCCTGCCTTGACGGACTCGCCGAAACTCGAGCGCCAGGTCTC
TGAGCTTGTCGCACATATCAACGGCGAGTTTGGTGCACTGGACTTTGTTCCTGTTCACCATTACCACCAG
ATGATCAAGCGCGATGAATTTTACGCTCTTCTTTCTGTTGCTGATCTTGCTGTGATTACACCCCTGCGCGA
TGGTATGAACACTACGTCGATGGAGTTTGTGATCGCGCAGCACTCAACGAAGAAAAGCCCGCTCGTCCT
GTCCGAGTTCATGGGCATCTCGAGTACTCTGGAAGAAGCTCTCCAGGTCAACCCCTGGGATCTCGGGGG
TGTTGCTGCTGCGATTCATCACGGTTTGCTTATGCGGGATAATGAGAAATTCCAACGGCACTCGAAGTTG
TACGAGAGTGTTACGACGCATACAAGCCATACGTGGGCGGCGATACTCGTTCGCATGCTCTTACATCAAT
TTGGTAAAGAACACACTGCGCATAGCACGCCGTTCCTCCAGCGAGAGCTCTTGCAGGCTGCGTACAAG
AAGGCGAAACGGCGCCTGTTCCTGTTTGATTACGACGGTACGCTGACTCCGATTGTGAAGACGCCGAGT
ATGGCGGTCCCGTCGGGCGAGACGCTGGAAGCGCTTGCGAAGCTCACCGAAGACCCTGCAAACGTCGT
GTACATCATCTCGGGGCGGGATAGCGAGTTCCTCGAGACGCACCTCGGGCATCTGAAGAAGCTCGGCAT
GTCCGCCGAGCACGGGTCCTTCGTCCGGGAGCGCGAAGAGTCGGAATGGACGAACTTGACGGAGAAG
CTCGACATGAGCTGGATGAGCGAGGTTGAGGAGATATTCAAGTACTACACCGAGCGCACGACGGGGAG
CAACATCGAGATCAAGAAGAGCTCCATCACCTGGCACTACCGCTCCGCCGACCCGGAGTGGGGCCAGT
TCCAGTGCCGGCAGTGCCAGGACCTGCTCGAGAACAACCTCGCGCGCAAGCGGCCGATCGAGGTGCTC
GTGGGGAAGAAGAACCTCGAGGTGCGGCCCCTGGCGGTGAACAAGGGCGAGATCGTGAAGCGCATAT
TGTACGCGCACCCCGAGGCGGAGTTCGTGTTCTGCGCGGGGGACGACAAGACGGATGAGGATATGTTC
CGCGCGCTTGCACCGTTTACGACTAGTTCGAACTCGGCTGTTGCTTCTATGATGGACGCGCCGGTTGGC
GTGAGGGACGAGGAGAACGGATCGAGGACGCCGCCGAAGGAGCTCAGGCTCAGCCCTGAGGGCGTGT
TCTCGACGGCGGTGGGCTCGAGCAGTAAGAAGACGCTCGCGAGCTGGCATGTGACTAGCCCGCATGAG
GTCGTTGAGCATATGTTGGACCTCGTTTCTTCTGCTGAGTCTGAGGGTGCAAGGAAGGAGGAAAAGAGT
AATCTCTAATTGATTCCACATTCCTCTTTCTCTCCCTCTCTCACCCCCTCTTTTTTATCGGTTTCTTTTCGC
GGGTTTTTATTGTCCACCGGCTTTTATGAACCGCGTCTCCACGGACTACG

(b)

MDSFNEPPSSRSRQEFSLSLEDVNKQITALKNEYAAKGIELSGRIIHVTHYLPLTVHLLPSKNGVLSPPLTPPV
KPADVALSPTEEERPLPKIVESAAGSESDGPRWTIGPRWGHSAMVSGIESLSNAHEQIIVGWTGDIERGAAST
GDKVKIPLSSVSDEDRATLTAAIESHKTEDGPSKYVPVWLDDLVAHGHYDGYCKTTLWPLFHYLLWQDVA
NEAASADEHWGEYKEANYVYAKRVAEVYKPGDLVWVHDYHLLLVPQILRQLIPDAVTGLFVHTPFPSSEIF
RCLPRRKEILDGMLGANLVCFQTYSYSRHFTSSCVRVCGYESTGKGIDNQGHVTTVAHCPVGVDAERIAQN
VVRPGVKPKFDALRSLYEGKKIIVARDKLDVVKGVVQKLRAFERLLQDYPEWIGNAVLIQVTSPALTDSPKL
ERQVSELVAHINGEFGALDFVPVHHYHQMIKRDEFYALLSVADLAVITPLRDGMNTTSMEFVIAQHSTKKSP
LVLSEFMGISSTLEEALQVNPWDLGGVAAAIHHGLLMRDNEKFQRHSKLYESVTTHTSHTWAAILVRMLLH
QFGKEHTAHSTPFLQRELLQAAYKKAKRRLFLFDYDGTLTPIVKTPSMAVPSGETLEALAKLTEDPANVVYII
SGRDSEFLETHLGHLKKLGMSAEHGSFVRREREESEWTNLTEKLDMSWMSEVEEIFKYYTERTTGSNIEIKKS
SITWHYRSADPEWGQFQCRQCQDLLENNLARKRPIEVLVGKKNLEVRPLAVNKGEIVKRILYAHPEAEFVFC
AGDDKTDEDMFRALAPFTTSSNSAVASMMDAPVGVRDEENGSRTPPKELRLSPEGVFSTAVGSSSKKTLAS
WHVTSPHEVVEHMLDLVSSAESEGARKEEKSNL

Fig. 2 Schematic representation of PiTPS protein(a) and PiTPS gene(b) structure, drawn to corresponding scales as in the fig

(a)

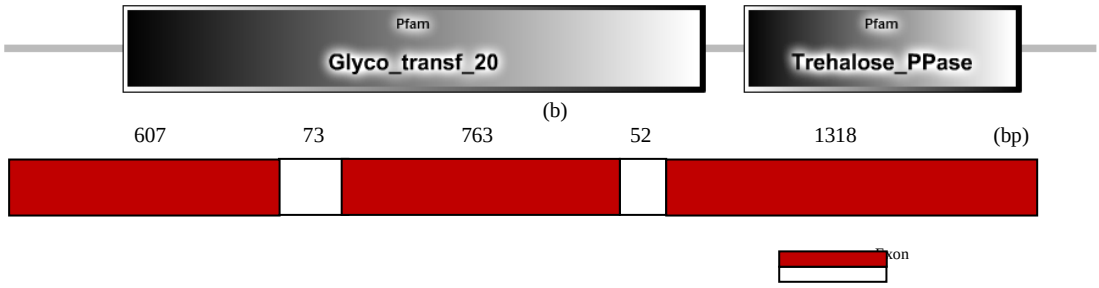


Fig. 3 Phylogenetic analysis of PiTPS showing relationship with other known TPS. Alignment of amino acid sequences with CLUSTALW, consensus Neighbor-Joining tree with MEGA4.0

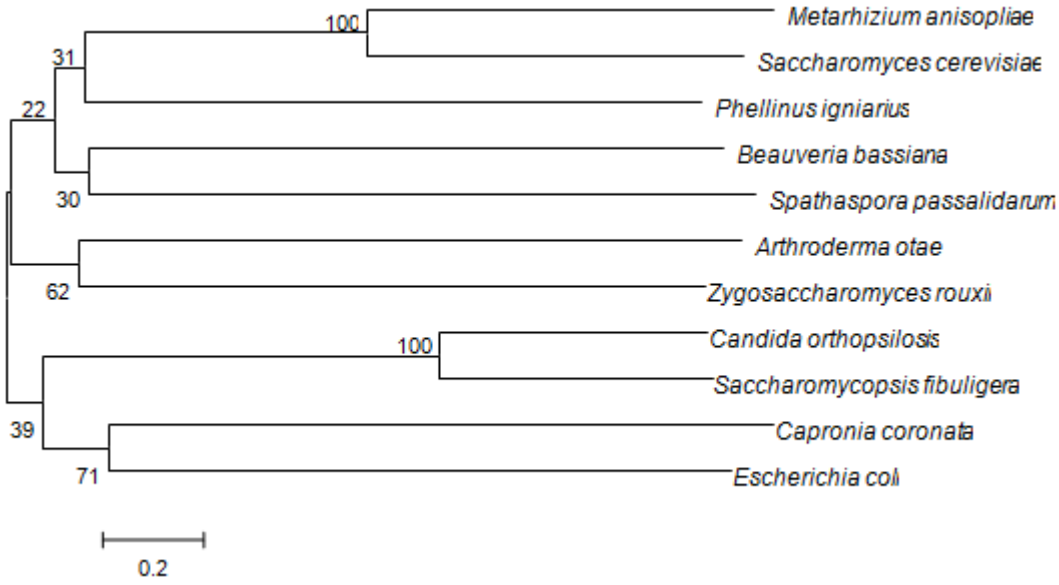
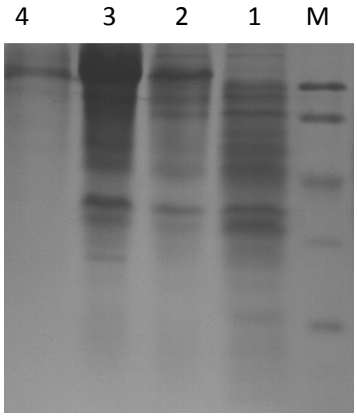


Fig. 4 Analysis of TPS with SDS-PAGE (10% polyacrylamide gel)



M :standard protein molecular weight; 1:crude enzyme from BL21 at 16 ℃for 10h; 2:crude enzyme from BL21 (DE3)/ pET32a-TPS induced by IPTG (0.2mMol) at 28 ℃ for 4 h; 3: Supernatant of crude enzyme;4:Purified protein of Nickel column

DISCUSSION

In this study, we obtained the conserved sequence of the trehalose-6-phosphate synthase (TPS) using PCR firstly, and then Using RACE approaches we obtained the full-length cDNA and genomic DNA , it is the first time clone and expression about TPS from *Phellinus igniarius.*

By comparing genomic and cDNA, we also analyzed the intron position and size, and analyzed the domains and whether contains the signal peptide, a phylogenetic tree was constructed based on total amino acid sequence, all these

provides a basis for the analysis and comparison of gene and amino acid composition of TPS.

According to the analysis of the deduced PiTPS amino acid sequence, There are two functional domains (a Glyco_transf_20 domain and a Trehalose_Ppase domain) which comprises. The difference is that, in *Escherichia coli* or *Saccharomyces cerevisiae* there is only one domain. It is reported that the TPS gene in Filaments of higher fungi appears to bea fused gene, which is a homolog of Ost A and Ost B in *E. coli*.

Phellinus igniarius are important medicinal fungi, and TPS is key enzyme of the important metabolites, it plays a key role in the growth and development of *Phellinus igniarius*, so the study of the structure and enzymatic properties of TPS genes is Meaningful for the research *Phellinus igniarius* itself.

Prokaryotic expression system was selected to express TPS genes, and pET32a as the expression vector. Because pET32a has the HIS-tag, so it can facilitate purification of proteins, purified protein provides a basis for further study of enzymatic properties.

REFERENCES

[1] Elbein AD (**1974**) *Adv Carbohyd Chem Bi* 30:227–256
[2] Chung JS (**2008**) *Saline Syst* 4:18
[3] Goddijn OJM, van Dun K (**1999**) *Trends Plant Sci* 4:315–319
[4] Thevelein JM, Hohmann S (**1995**) *Trends Biochem Sci* 20:3–10
[5] Bonini BM, Van Vaeck C, Larsson C, Gustafsson L, Ma P,Winderickx J, Van Dijck P, Thevelein JM (**2000**) *Biochem J* 350:261–268
[6] Noubhani A, Bunoust O, Rigoulet M, Thevelein JM (**2000**) *Eur J Biochem* 267:4566–45576
[7] Eastmond PJ, van Dijken AJ, Spielman M, Kerr A, Tissier AF, Dickinson HG, Jones JD, Smeekens SC, Graham IA (**2002**) *Plant J* 29:225–235
[8] Elbein AD, Pan YT, Pastuszak I, Carroll D (**2003**) *Glycobiology* 13(4):17R–27R
[9] Cabib E, Leloir LF (**1958**) *J Biol Chem* 231:259–275
[10] Lung, M.Y., Tsai, J.C., Huang, P.C., **2010**. *J. Food Sci.* 75, E18–24
[11] Song, T.Y., Lin, H.C., Yang, N.C., Hu, M.L., **2008**. *J. Ethnopharmacol*. 115, 50–56
[12] Liu, Y.F., Yang, Y., Jia, W., Zhang, J.S., Tang, Q.J., Tang, C.H., **2006**. *Acta Edulis Fungi* 13, 49–52
[13] Tang B, Chen J, Yao Q, Pan Z, Xu W, Wang S, Zhang W (**2010**) *J Insect Physiol* 56:813–821
[14] Zhang N, Wang F, Meng X, Luo S, Li Q, Dong H, Xu Z, Song R(**2011**) *Mol Biol Rep* 38:2241–2248.
[15] Wang G, Zhao G, Feng Y, Xuan J, Sun J, Guo B, Jiang G, Weng M, Yao J, Wang B et al (**2010**) *Mar Drugs* 8:2065–2079
[16] Vogel G, Fiehn O, Jean-Richard-dit-Bressel L, Boller T, Wiemken A, Aeschbacher RA, Wingler A (**2001**) *J Exp Bot* 52:1817–1826
[17] Cai Z, Peng G, Cao Y, Liu Y, Jin K, Xia Y (**2009***) J Biosci Bioeng* 107:499–505
[18] Kwon HB, Yeo ET, Hahn SE, Bae SC, Kim DY, Byun MO(**2003**) *FEMS Yeast Res* 3:433–440
[19] Kumar S, Tamura K, Nei M (**2004**) *Brief Bioinform* 5:150–163
[20] Su, W., Mertens, J. A., Kanamaru, K., Campbell, W. H., and Crawford, N. M.: *Plant Physiol*., 115, 1135–1143 (**1997**).
[21] Zhou, R., Kroczyńska, B., and Miernyk, J. A.: *Protein Expr, Purif*., 19, 253–258 (**2000**).
[22] Hwang, J. S., Yamada, K., Honda, A., Nakade, K., and Ishihama, A.: *J. Virol.*, 74, 4074–4084 (**2000**)