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Bioinformatics analysis of metallothionein gene from *Agaricus bisporus*, *Ganoderma lucidum*, *Taiwanofungus camphoratus* and *Paxillus involutus*

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ABSTRACT

Metallothionein (MT) gene has an important role in the detoxification of toxic metals especially some heavy metals. Based on the published sequences of MT genes from *Agaricus bisporus*, *Garnoderma lucidum*, *Taiwanofungus camphoratus* and *Paxillus involutus* as a type of the edible or medical mushroom in NCBI database, their molecular structures, physiological functions and evolutionary relationship were analyzed using the bioinformatics methods. Results showed that *AbMT*, *GlMT*, *TcMT* and *PiMT* genes contained complete open reading frame (ORF) and their theoretical points of the last three encoding proteins were higher than 7.0. *AbMT*, *GlMT*, *TcMT* and *PiMT* were relatively rich in random coil and extended strand, but transmembrane helices and signal peptides were not found. 4 MTs were mainly localized in cell nucleus (over 60%) and their cellular functions might have some relation to central intermediary metabolism. Multiple sequence alignment indicated relatively high identity (more than 52%) and short genetic distances (lower than 0.900) among 4 MT nucleotide sequences. Abundant genetic diversity and strong codon bias were found based on the halotype diversity, average number of nucleotide differences, nucleotide diversity, effective number of codons, codon bias index and scaled chi-square. Simultaneously, we deduced that 4 MT genes during molecular evolution were under positive selection. The present study might provide basis for further investigation of MTs molecular mechanisms and genetic laws.

Keywords: Metallothionein Gene; *Agaricus bisporus*; *Ganoderma lucidum*; *Taiwanofungus camphoratus*; *Paxillus involutus*

1. INTRODUCTION

Metallothioneins (MTs) are a multigenic family of low-molecular weight (ranging from 6 to 7 kDa), cysteine-rich (ranging from 20% to 33%), metal-binding proteins (7 metals/MT) that are widespread in animals, higher plants, eukaryotic microorganisms and some prokaryotes [1-5]. They are divided into three different classes on the basis of their cysteine content and structure. The Cys-Cys, Cys-X-Cys and Cys-X-X-Cys motifs (in which X denotes any amino acid) are characteristic and invariant for metallothioneins [6]. Although the precise function of MTs remains elusive, many studies reveal that they play a variety of important biological roles in maintaining intracellular metal homeostasis, eliminating metal toxification and protecting against intracellular oxidative damages during the acute phase response [7-10]. In addition, the presence of MTs in the nuclear can protect the DNA from the damage induced by oxidative stress [11].

Edible mushrooms have occupied an increasingly important place in several parts of the world, because of their delicacy, abundance and also as a substitute for the seafoods [12,13]. In recent years, heavy metal pollution has become one of the serious public concerns and environmental problems [14,15]. However, certain mushrooms are known to accumulate heavy metals. Further studies showed that heavy metal concentrations in mush-

room are considerably higher than those in agricultural crop plants, vegetables and fruits [16]. This suggests that heavy metal pollution of the mushroom has been drawn more and more attention. To our delight, an important hallmark of MTs is their induction by multiple heavy metal species at the transcriptional level. Therefore the MT genes serve as a valuable model for investigating the mechanism of cellular response to heavy metals. The clarification of heavy metal-dependent gene regulation will in turn contribute to our understanding of the physiological roles of MTs [17].

As a type of edibel or medical mushroom, *Agaricus bisporus*, *Ganoderma lucidum*, *Taiwanofungus camphoratus*, *Paxillus involutus* are causing serious problems as heavy metal contamination. So in the present study, we completed the bioinformatics analysis of MT genes and their encoding proteins from these four mushrooms. The results will give some theoretical foundations for molecular mechanisms and genetic laws of MT genes in fungi.

2. MATERIALS AND METHODS

2.1. Sequence Retrieval

MT gene sequences of *A. bisporus*, *G. lucidum*, *T. camphoratus* and *P. involutus* were retrieved from the National Center for Biotechnology Information (NCBI) which can be accessed using the URL: http://www.ncbi.nlm.nih.gov/, then named *AbMT* (GenBank accession no. AJ271695.1), *GlMT* (EF489399.1), *TcMT* (DQ520933.1) and *PiMT* (AY525379.1), respectively. Their corresponding amino acid sequences designated as *AbMT* (CAB85689.1), *GlMT* (ABP02008.1), *TcMT* (ABF69031.1) and *PiMT* (AAS19463.1) were also obtained.

2.2. Bioinformatics Analysis

Molecular structures and physicochemical properties were obtained by ProtParam tool (http://web.expasy.org/protparam/). The signal peptide was predicted using SignalP 4.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The potential protein subcellular localization, protein function, conserved domains(CDD), secondary structure and transmembrane domains were predicted using PSORT II Prediction (http://psort.hgc.jp/form2.html), ProFun 2.2 Server (http://www.cbs.dtu.dk/services/ProtFun/), Conserved Domain Database (CDD) (http://www.ncbi.nlm. nih.gov/Structure/cdd/cdd.shtml), a self-optimized method for protein secondary structure prediction (SOPM) (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopm.html) and TMHMM Server (version 2.0) (http://www.cbs.dtu.dk/services/TMHMM-2.0/), respectively. Multiple sequence alignment was carried out using ClustalX (version 1.83) software, respectively. Homology and genetic distances were calculated using DNAStar (version 5.01) and MEGA (version 3.1) software. The genetic diversity analysis was carried out using DnaSP software (version 4.0).

3. RESULTS AND DISCUSSION

3.1. Analysis of Molecular Structures and Physicochemical Properties

Structures and properties of nucleotide and corresponding amino acid sequences of MT genes from *A. bisporus*, *G. lucidum*, *T. camphoratus* and *P. involutus* were obtained by ProtParam tool (**Table 1**). *GlMT*, *TcMT* and *PiMT* genes contained complete ORF but lacing 5'-untranslated region (UTR) and 3' UTR. Then ORF and 5' UTR have been found in *AbMT* gene but lacing 3' UTR. So we found that amino acid residues were more than the other three proteins. Other than the *AbMT*, theoretical isoeletric points of the other three proteins in **Table 1** were higher than 7.0. The result showed that these proteins belonged to basic protein excepting *AbMT*. According to available reports, MT as acidic and basic protein tends to be in plant body and animal body, respectively. For example, MTs from *Camellia oleifer* [18], *Limonium sinense* [19] and *Tamarix androssow* [20], whereas MTs from *Musca domestica* [21], *Hyriopsis cumingii* [22], *Argopecten irradians* [23] and so forth. The reason is well worth further research. Hence, these physicochemical indices in **Table 1** may be pertinent to determine that they are a group gene with significant functional association and close genetic relation.

3.2. Analysis of Secondary Structure, Subcellular Localization and Some Physiological Functions

The secondary structures of *AbMT*, *GlMT*, *TcMT* and *PiMT* amino acid sequences were predicted using SOPM online server. Alpha helix, extended strand, beta turn and random coil were found only in *AbMT* and *GlMT*, while the first one was not predicted in *TcMT* and *PiMT* (**Table 2**). Thus, the current phenomenon was very worth regarding and further research. The secondary structure analysis showed 4 MT proteins were relatively rich in random coil and extended strand. The very high coil structure (over 50%) was attributed to create links in polypeptide chain and disrupting ordered secondary structure [24].

TMHMM Server online predicted that, there was no transmembrane helices in *AbMT*, *GlMT*, *TcMT* and *PiMT* proteins, implying that they may exert catalytic function

Table 1. Molecular structures and physicochemical properties of *AbMT*, *GlMT*, *TcMT* and *PiMT* amino acid sequences.

Index	*AbMT*	*GlMT*	*TcMT*	*PiMT*
Amino acid residues	71	33	45	34
Molecular weight (kDa)	7.31	3.29	4.47	3.42
Theoretical pI	6.85	7.52	8.07	7.52
Negatively charged residues	5	2	3	1
Positively charged residues	5	3	5	2
Formula	$C_{288}H_{469}N_{87}O_{103}S_{16}$	$C_{127}H_{210}N_{38}O_{48}S_8$	$C_{173}H_{286}N_{52}O_{64}S_{11}$	$C_{132}H_{215}N_{41}O_{49}S_8$
Extinction coefficients	875	1865	625	375
Estimated half-life (h)	4.4	30	1.1	30
Instability index	58.10	69.48	58.77	59.52
Aliphatic index	34.51	29.70	21.78	31.47
Grand average of hydropathicity	−0.203	−0.003	−0.109	−0.062
Charged aa.composition (%)	40.72	45.98	38.46	32.42
Acidic aa.composition (%)	8.45	7.42	3.67	3.78
Basic aa.composition (%)	8.77	11.68	18.24	7.50
Polar aa.composition (%)	49.27	53.60	39.02	56.50
Hydrophobic aa.composition (%)	15.39	14.66	20.75	15.51

Table 2. Composition of secondary structure of *AbMT*, *GlMT*, *TcMT* and *PiMT* amino acid sequences.

Protein	Composition of secondary structure (%)			
	Alpha helix	Extended strand	Beta turn	Random coil
AbMT	8.45	14.08	11.27	66.20
GlMT	3.03	33.33	12.12	51.52
TcMT	0.00	32.61	8.70	58.70
PiMT	0.00	38.24	5.88	55.88

directly in cytosol without transportation. Simultaneously, the signal peptide was not identified the four amino acid sequences using SignalP 4.0 online server. Server Determining subcellular localization is important as a first step towards studying its physiological function. With the help of PSORT II Prediction, sub-cellular localization analysis of 4 proteins demonstrated that MT was mainly localized in cell nucleus (over 60%), other were cytoplasm, mitochondrion, plasma membrane (other than *AbMT*) and so forth.

ProtFun 2.2 Server online analysis showed that cellular function of 4 proteins might have some relation to central intermediary metabolism and this laid some evidence to subcellular localization prediction, because central intermediary metabolite occurred usually within the cell [24]. Non N-glycosylated site was recognized, but some putative O-glycosylated sites were predicted in *AbMT* (8 sites), *GlMT* (2 sites), *TcMT* (2 sites) and *PiMT* (3sites), playing the important role in recognizing and binding some heavy metal ions. And then CDD recognized the absence of conserved domains in these proteins. Furthermore, protein may contain a total of 8 motifs, whose corresponding amino acid regions carry out specific biochemical functions and respective genetic evolutional information [24].

3.3. Analysis of Multiple Alignment and Evolutionary Relationship

We aligned *MT* gene sequences among *A. bisporus*, *G. lucidum*, *T. camphoratus* and *P. involutus* using DNAStar (version 5.01) and MEGA (version 3.1) software. The alignments displayed a relatively high degree of homology (more than 52% identity in all the matches), and a relatively short genetic distances (lower than 0.900), especially C-terminal fragment of the cDNA sequences (**Table 3**). To investigate the evolutionary relationship of these *MT* genes, a phylogenetic tree was generated by MEGA (version 3.1) software using the Neighbor-Joining method with 1500 bootstrap replicates (**Figure 1**). The result showed that the genetic relationship coincided

Table 3. Percent identity and genetic distances of *AbMT*, *GlMT*, *TcMT* and *PiMT* sequences using DNAStar (version 5.01) and MEGA (version 3.1) software.

	AbMT	*GlMT*	*TcMT*	*PiMT*
AbMT	****	69.9	67.0	52.8
GlMT	0.394	****	73.9	52.8
TcMT	0.499	0.395	****	52.9
PiMT	0.896	0.893	0.852	****

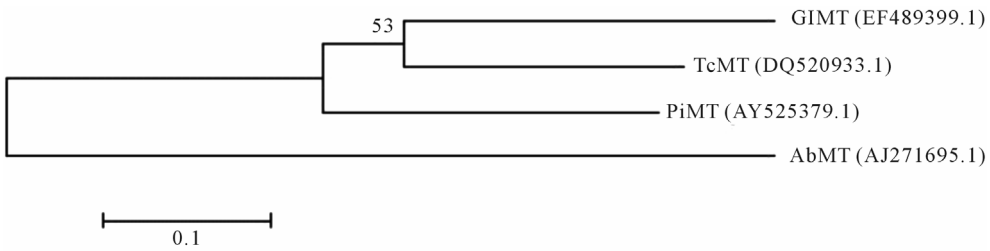

Figure 1. The phylogenetic tree of *AbMT*, *GlMT*, *TcMT* and *PiMT* was generated from the cDNA sequences using MEGA (version 3.1) software. The reliability of the tree was measured by bootstrap analysis with 1500 replicates.

with the morphological classification and Xie *et al*. (2007) report [25]. For example, *G. lucidum* and *T. camphoratus* belonging to the same order in the morphology were come together in a group. Thus it can be seen that *MT* gene is one of the most efficient class of molecular marker that has been used widely to detect the deleterious effects of heavy metals [26].

8 monomorphic sites and 94 polymorphic sites were detected from 4 *MT* gene sequences by the genetic diversity analysis of DnaSP (version 4.0) software. Singleton variable sites and parsimony informative sites was 74 (amounting to 72.55%) and 20 (amounting to 19.61%), respectively. At the same time, 4 haplotypes were also sorted. Haplotype diversity, average number of nucleotide differences and nucleotide diversity was 1.000, 61.00. and 0.59804, respectively. According to calculation using the total number of mutations, there was no significance ($P > 0.10$) (Fu and Li's D* test statistic: −0.36481, Fu and Li's F* test statistic: −0.55017). Codon usage analysis showed that effective number of codons, codon bias index and scaled chi-square was 35.406, 0.794 and 1.730, respectively. Then a strong codon bias was found among 4 *MT* gene sequences. Multiple nucleotide sequence alignment indicated that number of synonymous sites, nonsynonymous sites, and synonymous sites and non-coding positions was 23.29, 75.71 and 26.29, respectively. Furthermore, we also found that nonsynonymous sites were over three times as much as synonymous sites. Thus, we deduced that 4 MT gene during molecular evolution were under positive selection according to Guo (1993) and Qiang *et al*. (2010) report [27,28].

In conclusion, as maintaining intracellular metal homeostasis, eliminating metal toxification and protecting against intracellular，MTs may be inducted by mutiple heavy metals at the transcriptional level and need to pay more attention. In the present study, molecular characteristics and physiological functions of MT genes and their corresponding proteins from *A. bisporus*, *G. lucidum*, *T. camphoratus* and *P. involutus* were analyzed using some bioinformatics methods to provide basis for further investigation.

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