RESEARCH NOTE

Mycobiology 40(3) : 208-209 (2012)
© The Korean Society of Mycology

http://dx.doi.org/10.5941/MYCO.2012.40.3.208
pISSN 1229-8093
eISSN 2092-9323

Isolation and Characterization of the *mheA* (Most Highly Expressed) Gene of *Aspergillus oryzae*

Pengcheng Liu[1], Ji-Young Lim[2], Hee-Seo Kim[2], Jong Hwa Kim[2] and Keon-Sang Chae[1]*

[1]*Department of Molecular Biology, Chonbuk National University, Jeonju 561-756, Korea*
[2]*Department of Pharmaceutical Engineering, Woosuk University, Samnye 565-701, Korea*

(Received August 21, 2012. Revised September 10, 2012. Accepted September 17, 2012)

The amino acid sequence of the *mheA* gene of *Aspergillus oryzae* encodes a putative metallothionein-like protein 1. The size of the *mheA* transcript was 497 nt and the *mheA* promoter was induced by glucose, consistent with results of analysis by Northern hybridization and with the *pdcA* promoter, respectively.

KEYWORDS : ***Aspergillus oryzae*, *mheA* promoter**

Various strong promoters in *Aspergillus oryzae* have been identified [1]. These strong promoters can be divided into two major groups: one group includes promoters of genes encoding various amylases, and the other group includes promoters of genes encoding enzymes involved in glycolysis. The promoter, which is not included in these two groups, is that of the *pdcA* gene coding for pyruvate decarboxylase. The *pdcA* gene appears to be one of the most highly expressed genes in *A. oryzae*, and induction of expression by the *pdcA* promoter has been suggested to occur in the presence of glucose [1, 2].

Sequence analysis of randomly selected clones in a 3'-directed expressed sequence tag (EST) library of a tissue or a cell type can identify highly expressed genes in a given tissue or a cell type [3]. Analysis of 345 randomly selected clones from a 3'-directed EST library of *A. oryzae* has been conducted; according to the results, the *pdc* gene and the *mheA* gene appear to show the highest level of expression [2]. The *pdcA* gene has been isolated previously [4]. For isolation of the *mheA* gene, the restriction map around a 3'-directed EST of the *mheA* gene (the EST No. AO07B12 corresponding to the nucleotide No. 4,966 to 5,165 in the sequence, whose GenBank accession No. is DQ004254) was constructed by Southern hybridization of chromosomal DNA, which demonstrated that the chromosomal DNA having the entire *mheA* gene can be obtained by combination of two genomic clones isolated from two genomic libraries (result not shown). One is a *Hin*dIII-digested genomic library and the other is a *Pst*I-digested genomic library. A 1.5 kb clone was isolated by colony hybridization, using the 3'-directed EST DNA as a probe, from a *Hin*dIII-digested library, which has been described previously [4]. The nucleotide sequence of the 1.5 kb fragment indicated that the 1.5 kb fragment may include a part of the *mheA* promoter therefore, another DNA fragment, a 4.8 kb *Pst*I-digested fragment, was isolated from a *Pst*I-digested genomic library using a 600 bp *Sac*I/*Pst*I-digested fragment of the 1.5 kb clone as a probe. The *Pst*I-digested genomic library was constructed by insertion of *Pst*I-digested chromosomal DNA fragments into pUC19. In total, a 5.5 kb genomic DNA fragment containing the *mheA* gene was isolated from two genomic libraries. According to the results of a GenBank Blast search, the nucleotide sequence and the amino acid sequence of the 5.5 kb DNA suggested that the *mheA* gene encodes a putative metallothionein-like protein 1 (MT1-1, GenBank accession No. AAS48540.1) of *Leptosphaeria maculans* (Fig. 1). Fourteen amino acids out of 21 in the MheA open reading frame (ORF) were exactly matched to those of the MT1-1.

For identification of the *mheA* promoter region, examination of the 5' end of the *mheA* transcript was performed using 5'-rapid amplification of cDNA end (5'-RACE) according to the procedure described previously [5]. The total RNA was isolated from 48 hr-cultured mycelia of a wild type strain, *A. oryzae* Fungal Genetics Stock Center (FGSC, Kansas, MO, USA) A815, in liquid yeast extract-peptone-dextrose (YEPD) medium. Isolation of

*Corresponding author <E-mail : chaeks@chonbuk.ac.kr>

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

```
           **** *  * * * * ** * **
mheA    1  MSPCSC-NC-CSGNCNSCSCSDC 21
MT1-1   1  MSPCNCASCKCAGDCTSCNCGDC 23
```

Fig. 1. Alignment of the total amino acids of the *MheA* ORF and the *MT1-1* ORF. *mheA*, the *MheA* open reading frame (ORF) of *Aspergillus oryzae*; *MT1-1*, metallothionein-like protein 1 of *Leptosphaeria maculans* (GenBank accession No. AAS48540.1). Asterisks indicate the identical amino acids between the two.

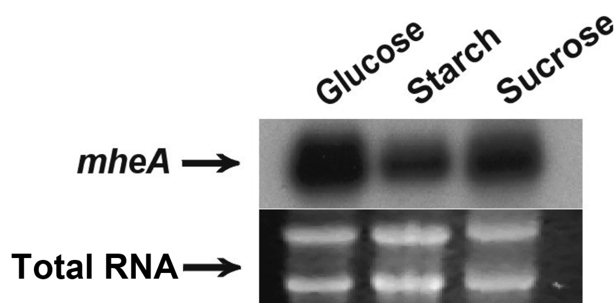

Fig. 2. Comparison of the promoter activity of the *mheA* promoter in the presence of different carbon sources. Total RNA was isolated from 48 hr-cultured mycelia of a wild type strain of *Aspergillus oryzae* Fungal Genetics Stock Center (FGSC, Kansas, MO, USA) A815 in liquid yeast extract-peptone-dextrose (YEPD) medium supplemented with 2% glucose, starch, or sucrose, as shown on each lane, and hybridized with a *mheA*-specific probe.

mRNA from total RNA was performed using an oligo-dT cellulose column. The 5' part of the *mheA* transcript was synthesized using a primer, 5'-AAG CCC TCA TCA TCA CCA-3' (5,119~5,102), followed by inverse PCR using a pair of primers whose sequences were 5'-TCA TCC CAT CCT CAC TGG-3' (5,004~5,021) and 5'-TAC TTA TCT TCC TCG GGG-3' (5,051~5,068). Results of 5'-RACE showed that transcription of the *mheA* gene starts at the 4,668th nucleotide "T" and that the full-length of the *mheA* transcript is 497 nt without poly(A). This result was consistent with that of analysis by Northern hybridization, showing that the size of the *mheA* transcript was approximately 500 nt (result not shown).

Because the principles for isolation of the *pdcA* gene and the *mheA* gene were identical, the question of whether expression of the *mheA* gene is induced in the presence of glucose was examined. Total RNA was isolated from 48 hr-cultured mycelia of the wild type strain *A. oryzae* A815 in liquid YEPD medium and hybridized using a *mheA*-specific probe. When another carbon source was supplemented, 2% starch or sucrose was added instead of glucose. The *mheA*-specific probe was the AO07B12 EST DNA, which was labeled with [α-P^{32}]-dCTP using the Prime-a-Gene Labeling System (Promega, Medison, WI, USA). As shown in Fig. 2, the *mheA* transcript level was higher in the presence of glucose than in the presence of starch or sucrose, indicating that expression by the *mheA* promoter is induced by glucose.

Acknowledgements

This work was supported by a research grant from the Korea Forest Research Institute (KFRI) in 2010. Pengcheng Liu was supported by a full scholarship from Chonbuk National University from 2009 to 2011.

References

1. Nakajima K, Sano M, Machida M. Current progress in the analysis of transcriptional regulation in the industrially valuable microorganism *Aspergillus oryzae*. Biotechnol Bioprocess Eng 2000;5:253-62.
2. Hwang HA, Lee DW, Kim JH, Lee TK, Yang MS, Chae KS. Quantitative analysis of expressed genes in *Aspergillus oryzae* by sequencing 3'-directed cDNA clones. J Microbiol 1998;36:111-7.
3. Okubo K, Hori N, Matoba R, Niiyama T, Fukushima A, Kojima Y, Matsubara K. Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression. Nat Genet 1992;2:173-9.
4. Lee DW, Koh JS, Kim JH, Chae KS. Cloning and nucleotide sequence of one of the most highly expressed genes, a *pdcA* homolog of *Aspergillus nidulans*, in *Aspergillus oryzae*. Biotechnol Lett 1999;21:139-42.
5. Maruyama IN, Rakow TL, Maruyama HI. cRACE: a simple method for identification of the 5' end of mRNAs. Nucleic Acids Res 1995;23:3796-7.