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Cloning and Expression of the *metE* gene coding for homocysteine methyltransferase from the basidiomycete *Ganoderma lucidum* in *E. coli*

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영지버섯으로부터 homocysteine methyltransferase를 암호화 하는 *metE* 유전자의 클로닝 및 *E. coli*에서의 발현

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ABSTRACT: The *metE* gene coding for N^5-methyl-H_4-folate; homocysteine methyltransferase from the basidiomycete *Ganoderma lucidum* was cloned by complementation of methionine-requiring mutants of *E. coli*. The size of a inserted DNA was about 1.54 kb and had 5 restriction enzyme sites. A physical map was constructed. Southern blot analysis confirmed the presence of a transforming DNA in the genome of *Ganoderma lucidum*, indicating the presence of a single copy.

KEYWORDS: ***Ganoderma lucidum*, *metE gene*, homocysteine methyltransferase**

Ganoderma lucidum is a kind of basidiomycete fungi, and has long been a popular folk or oriental medicine to cure various human diseases(Sone *et al*., 1985 and Chung *et al*., 1990).

The recent development of transformation systems for basidiomycete fungi makes many aspects of basidiomycete genetics accessible to molecular analysis(Dons *et al*., 1984; Munoz-Rivas *et al*., 1986; Binninger *et al*., 1987; Specht *et al*., 1988 and Binninger *et al*., 1991) There is much interest to learn about the general structure of the basidiomycete genes and sequences required for their expression.

The *metE* gene of *Ganoderma lucidum* encodes N^5-methyl-H_4-folate; homocysteine methyltransferase(non-B_{12}-methyltransferase, E.C.2.1.1.14), an important enzyme which catalyzes the conversion of homocysteine to methionine in the final step of methionine biosynthesis, requires as substrate N^5-methyl-H_4-folate with three or more glutamates, and is stimulated by phosphate ions(Brown *et al*., 1990 and Gonzalez *et al*., 1992). In bacteria, much has been reported on the studies of molecular genetics of metE gene(Schulte *et al*., 1984; Chu *et al*., 1985; Old *et al*., 1988 and Wu *et al*., 1992). However, there has been no attempt to research the molecular genetics of the *metE* gene from basidiomycete fungi, especially *Ganoderma lucidum*. Therefore, we were interested in genetic research on the *metE* gene from *Ganoderma lucidum*.

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In this paper, we describe the cloning and expression of the *metE* gene from the basidiomycete *Ganoderma lucidum* in *E. coli.*

Materials and Methods

Strains and plasmids

The strains and plasmids used in this experiment are listed in Table 1. A wild type strain of *Ganoderma lucidum* was used as a donor strain for the source of the DNA. *E.coli* strain ME5459 and ME6180 were used as a cloning host. *E. coli* JM109 was used for the propagation of plasmids. A pBluescriptII SK+ was used as a cloning vector.

Growth of cells

Ganoderma lucidum mycelia, for the isolation of DNA, were grown in a liquid complete medium which contained(in g/*l*) glucose, 30; sucrose, 20; peptone, 4; yeast extract, 10; casamino acid, 5; KH_2PO_4, 0.46; K_2HPO_4, 1 and $MgSO_4 \cdot 7H_2O$, 0.5. *E. coli* cells for the isolation of plasmid DNA and for transformation were grown in Luria-Broth (LB) containing antibiotics. For the selection of hybrid plasmids containing the *metE* gene, most of the minimal medium was supplemented with the appropriate requirements, antibiotics and glucose as a carbon source.

Isolation of DNA

Isolation of the chromosomal DNA from the mycelium of *Ganoderma lucidum* was performed by modification of a gentle extraction method previously described by Specht *et al*(1982). Plasmid DNA was isolated by the alkaline lysis method and purified with cesium chloride-ethidium bromide gradients centrifugation as described by Maniatis *et al*(1989).

Ligation of the chromosomal DNA in plasmid pBluescriptII SK+

Ganoderma lucidum DNA was partially digested with 10 units of *Bam*HI, and dephosphorylated with calf intestinal alkaline phosphatase. For ligation, dephosphorylated pBluescriptII SK+ DNA and digested *Ganoderma lucidum* DNA were mixed with a 1:5 ratio in the ligation buffer and T4 DNA ligase as described by Maniatis *et al* (1989). The DNA mixture was incubated to permit ligation for 16 hours at 14℃.

Transformation

E. coli ME5459 and ME6180 cells were grown in LB and made competent cells for transformation as described by the Hanahan method(1985). The ligation mixture was introduced to the competent cell suspensions for 30 min at 0℃, heated for 90 sec at 42℃, and then the transformed cells

Table 1. Strains and Plasmids.

Strains & Vectors	Genotypes & Properties	Source
Ganoderma lucidum	Wild type	J. S. Lee
E. coli JM109	recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, (lac-proAB) F[traD36, proA⁻, proB⁺, $lacI^q$, lacZ, M15]	
E. coli ME5459 (AT1243)	HfrH(pyrB→thr):pyrE, metE	National Institute of Genetics
E. coli ME6180	ilvA, metE, rbsK, gal	Gentic Stocks
(LS679[080Tna⁻, Bgl⁺])	(80hd-tna⁺, bgl⁻, 80h)	Research Center (Japan)
Plsmids		
pBluescriptII SK+	Ap^r	Stratagene Co.
pGLB4	Ap^r, $metE^-$	This work

were allowed to express with shaking for 1 hour at 37℃. 100 μl of the resuspended cells were spread on the surface of LB agar plates containing ampicillin(50 μg/ml). The plates were incubated for overnight at 37℃. 50 colonies were picked at random to determine the fraction of clones containing recombinant plasmids and to estimate the average size of the inserts.

Complementation of *E. coli metE*⁻ mutants

Plasmid DNA of transformants were extracted by the alkaline lysis method and analyzed by 0.75 % agarose gel using electrophoresis according to the standard method(Maniatis *et al.*, 1989). The recombinant plasmids were used for transformation into *E. coli metE* mutants(ME5459, ME6180) by the Hanhan method(1985). To select Met^+ cells, the transformed cells were centrifuged at 7,500 rpm for 10 min at 4℃ and resuspended in 1 ml of distilled water, and then 0.1 ml samples were spread on the surface of minimal agar plates with the appropriate growth factors except methionine. The plates were incubated at 37℃, and then the $metE^+$ colonies were identified on the selective medium.

Restriction analyses and Southern hybridization

Recombinant plasmid DNA containing the *metE* gene was isolated and identified in a 0.75% agarose gel using electrophoresis. Restriction maps and southern blots were done by standard methods(Maniatis *et al.*, 1989). Recombinat plasmid DNA containing the *metE* gene was completely digested with the restriction enzyme *Bam*HI, inserted fragments were separated by electrophoresis, and used as a probe DNA. The fragments were labeled with [$\alpha-^{32}P$]-dCTP using of a nick translation kit. Hybridization was carried out on Denhart's solution containing radiolabeled probe DNA for 30 hours at 42℃. The dried nitrocellulose paper was placed on X-ray film and exposed for 42 hours at −20℃.

Results and Discussion

Isolation of the *metE* gene from *Ganoderma lucidum*

Chromosomal DNA of *Ganoderma lucidum* was isolated and purified. The purified DNA was partially digested with various units of the restriction enzyme *Bam*HI(Fig. 1). Partially digested fragments of *Ganoderma lucidum* chromosomal DNA(lane C) were ligated into the plasmid vector pBluescriptII SK+, and *E. coli* ME5459 was used as a host for selection of the *metE* gene of *Ganoderma lucidum* by complementation.

Transformed cells were plated on a selective minimal medium containing ampicillin(25 μg/ml) but lacking methionine and incubated at 37℃. After 41 hours of incubation, 14 *metE* transformants were selected by ampicillin resistance and Met^+ phenotype.

The recombinant plasmid DNAs were isolated and analyzed by agarose gel electrophoresis. As shown in Fig. 2, the recombinant plasmid DNAs were proved to have three different patterns. Among the plasmids, about 4.5 kb plasmid DNA containing the smallest insert fragment(lane H) was chosen and analyzed further. The plasmid was named pGLB4.

In order to confirm the presence of the *metE* gene, pGLB4 was transformed into *E. coli* ME5459 and the other *metE* mutation *E. coli* ME6180. After 34 hours of incubation, 115 and 41 colonies were

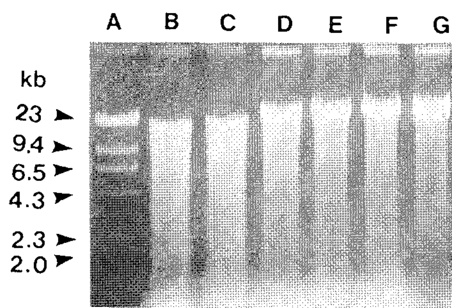

Fig. 1. Partial digestion of *Ganoderma lucidum* chromosomal DNA with *Bam*HI.
Lane A, λDNA/*Hind*III. Lane B-F, *Ganoderma lucidum* DNA treated with 20, 10, 5, 2.5 and 1.25 units of *Bam*HI, respectively. Lane G, *Ganoderma lucidum* DNA not treated with *Bam*HI. All samples were digested for 1 hour at 37℃.

Fig. 2. Patterns of recombinant plasmids on 0.75% agarose gel.
Lane A, λDNA treated with *Hind*III, lane B-O, recombinant plasmids DNA.

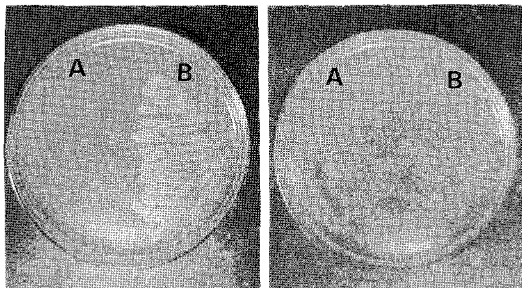

Fig. 3. Identification of transformants acquired Met⁺ phenotype in *E. coli* ME5459 and ME6180; The strains cultivated in a selective medium for 34 h at 37℃. Left A, *E. coli* ME5459 cells transformed with pBluescriptII SK+, B, *E. coli* ME5459 cells transformed with pGLB4 containing *metE* gene. Right A, *E. coli* ME 6180 cells transformed with pBluescriptII SK+, B, *E. coli* ME6180 cells transformed with pGLB4 containing *metE* gene.

acquired, respectively. As shown in Fig. 3, the cells containing pGLB4 showed normal growth as compared with cells transformed with pBluescriptII SK+ which was not grown. Therefore, it suggests that pGLB4 plasmid DNA has the *metE* gene.

Physical map of pGLB4 plamsid DNA

Single and double digestions of the pGLB4 plasmid DNA with several restriction enzymes allowed us to construct a physical map. As shown in Fig. 4, the inserted DNA of pGLB4 was about 1.54 kb and had 5 restriction enzyme sites. The inserts contained a single site for *Bam*HI, *Hind*III, *Sal*I and double restriction sites for *Hinc*II and *Xho*I. A physical map of pGLB4 is shown in Fig. 5.

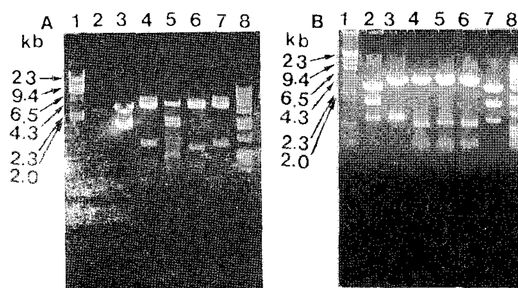

Fig. 4. Analysis of restriction patterns of the pGLB4 plasmid. pGLB4 plasmids were completely digested with restriction enzymes and showed the restriction sites within the inserted DNA by electrophoresis. Each lane revealed restriction fragments as follows: (A) Lane 1, λDNA/*Hind*III; lane 2, pBluescriptII SK+/*Bam*HI; lane 3, pGLB4/*Bam*HI; lane 4, pGLB4/*Hind*III; lane 5, pGLB4/*Hinc*II; lane 6, pGLB4/*Xho*I; lane 7, pGLB4/*Sal*I and lane 8, 1 kb ladder DNA. (B) Lane 1. λDNA/*Hind*III; lane 2, pGLB4/*Bam*HI/*Hind*III; lane 3, pGLB4/*Hind*III/*Sal*I; lane 4, pGLB4/*Sal*I/*Xho*I; lane 5, pGLB4/*Bam*HI/*Xho*I; lane 6, pGLB4/*Hind*III/*Xho*I; lane 7, pGLB4/*Bam*HI/*Sal*I and lane 8, 1 kb ladder DNA.

Southern hybridization

In order to determine the copy number of the *metE* gene in the genome of *Ganoderma lucidum*, and whether the cloned *metE* gene originated from the chromosomal DNA of *Ganoderma lucidum* or not, southern hybridization was carried out with genomic DNA from *Ganoderma lucidum* after digestion with *Bam*HI.

As shown in Fig. 6, the inserted DNA of pGLB4 as a probe was not hybridized with *E. coli* chromosomal DNA, but was hybridized with *Ganoderma lucidum* chromosomes. It suggests that cloned fragments containing the *metE* gene derived from the *Ganoderma lucidum* genome. And it indicated that a single copy of the gene existed in the genome.

Molecular studies of gene expression in basidiomycete has been hampered by the absence of methods for isolating specific genes and by the lack of a DNA mediated transformation system in this organism. The ability to isolating specific genes from basidiomycete is important for the further development of genetic molecular analyses.

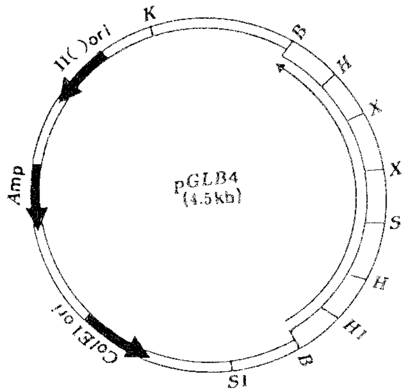

Fig. 5. Physical map of pGLB4.
The pGLB4 was diagrammed with the restriction sites after the calculation of the size of fragments by single and double digestions. The thin line represents the sequence derived from pBluescriptII SK+, the thick line indicates *Ganoderma lucidum* DNA containing the *metE* gene(1.54 kb). The arrow shows the coding region and direction transcription. Only relevent restriction sites are shown. Abbreviations are as follows: B, *Bam*HI; H, *Hi-nc*II; H1, *Hind*III; K, *Kpn*I; S, *Sal*I; S1, *Sac*I; X, *Xho*I.

For several yeasts and other filamentous fungi, however, a few genes have been cloned from these organisms by complementation of *E. coli* mutants(Munoz-Rivas *et al.*, 1986; Froliger *et al.*, 1987; Mellon *et al.*, 1987; Byun *et al.*, 1989 and Burrow *et al.*, 1990). The data presented in this paper show that *metE* gene can be cloned from *Ganoderma lucidum* by complementation of *E. coli* mutants.

摘 要

담자균류인 영지버섯으로부터 homocysteine methyltransferase를 code하는 *metE* 유전자를 methionine 요구성 균주인 대장균에 complementation시켜 cloning하였다. 그 결과 삽입된 DNA의 크기는 약 1.54 kb이었고, 5개의 제한효소 부위가 존재하였다. 이 clone체의 제한지도를 작성하였고, southern blot 분석으로 *metE* 유전자는 영지버섯의 genome으로부터 유래하였으며, 단일 복제수로 존재함을 확인하였다.

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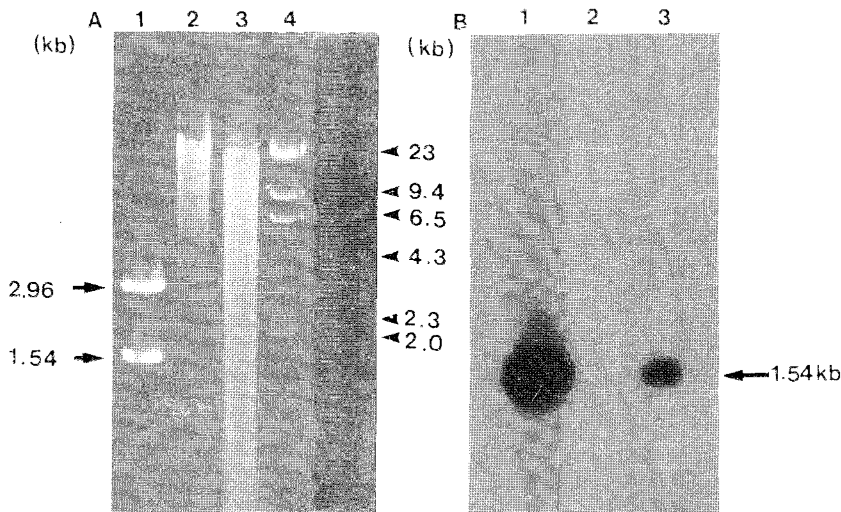

Fig. 6. Identification of the pGLB4 gene with southern blot hybridization. A: Photograph of agarose gel electrophoresis. B: Autoradiogram of southern blot hybridization. Lane 1, pGLB4/*Bam*HI; lane 2, *E. coli* DNA/*Bam*HI; lane 3, *Ganoderma lucidum* DNA/*Bam*HI and lane 4, λDNA/*Hind*III.

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