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High Level Expression of XMP Aminase Gene in *Escherichia coli*

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*Escherichia coli*에서 XMP Aminase 유전자의 발현 증대

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ABSTRACT－In order to increase the expression of XMP aminase [EC 6.3.4.1], which catalizes the conversion of 5′-XMP to the DNA fragment containing *gua A* gene coding for XMP aminase from pLC 34-10 plasmid was subcloned into pBR 322, and 1.7 kb *gua A* gene fragment was recloned under the control of *trp* promoter of pDR 720, *E. coli* expression vector. XMP aminase activity had increased by about 17 times when compared with that of the strain carring pLC 34-10.

Keyword□Expression, *gua A* cloning, 5′-GMP, *E. coli*

Recombinant DNA technique is developed to isolate specific genes and to amplify the expression of a specific genes. Therefore gene amplification by cloning with multicopy vectors frequently causes increased production of the protein encoded by the cloned gene. According to this concepts, it seems reasonable to assume that amplification of genes involved in guanosine 5′-monophosphate (5′-GMP) biosynthetic pathway will improve the yield of end product, 5′-GMP. The history of nucleic acid-related compound research and industrial production is a period of less than 20 years. However, a large number of investigations on the industrial production of that compound, especially inosine 5′-monophosphate (5′-IMP) and 5′-GMP, and on the formation of nucleoside derivatives by microorganism have been done. Possible processes of 5′-GMP production are described below.

1) 5′-GMP can be produced by degradation of yeast ribonucleic acid.[10,14]

2) 5′-GMP can be synthesized by chemical methods from fermented intermediates.[7~9,12,17]

3) 5′-GMP can be synthesized by enzymatic conversion from xanthosine 5′-monophosphate (5′-XMP) by fermentative process.[1,4,5]

Several enzyme involved in the pathway of purine nucleotide biosynthesis have been shown to be regulated by the end products. Synthesis of these enzymes, especially PRPP amidotransferase, IMP dehydrogenase and XMP aminase, is regulated by guanine derivatives, and the reaction of these enzymes are markedly inhibited by 5′-GMP. Therefore the production of a large amount of specific enzyme in this way is extremely useful for the use of cell-free extract in the enzymatic production of useful compounds, since it is possible to reduce the formation of the by-product and catalysis by other enzymes contained in the cell-

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free extract.

XMP aminase [Xanthosine 5'-monophosphate: ammonia ligase (AMP)] catalyzes the glutamine or NH_3 dependent synthesis of 5'-GMP from 5'-XMP. The *gua A* gene coding for XMP aminase is located 54 min on the *E. coli* chromosomal map and forms one operon together with the *gua B* gene coding for IMP dehydrogenase.[16]

The genes are arranged in the order of *gua PO, gua B* and *gua A* and it is known that a second promoter for the *gua A* gene of low efficiency exists usptream of the *gua A* gene.[6] Recently the total DNA sequence of the *gua BA* operon was determined.[18,19] It was become apparent that the *gua B* and *gua A* gene products have 511 and 525 amino acid residues, respectively, and that between the two structural genes there lies a 68-bp intercistronic region. According to the nucleotide sequence of *gua A* gene and SDS-polyacrylamide gel electrophoresis, XMP aminase is a dimer of identical subunits of Mr about 58,000.

In the present work, the *gua A* gene fragment was subcloned into the pBR 322 and was recloned under the control of *trp* promoter of pDR 720, which was adopted for the high level expression of XMP aminase.

Matherials and Methods

Bacterial strains and plasmids—The bacterial strain used in this experiment was *E. coli* HB 101 (F^-, *hsd*20, r_B^-, m_B^-, *rec*A13, *ara*14, *pro*A2, *lac*Y1). *E. coli* strain JA 200 containing pLC 34-10[3,13] was obtained by courtesy of Dr. S.T.Kwon (KIST). Plasmids pDR 720 and pBR 322 used in this study. pDR 720 carries a *trp* promoter-operater fragment *Sma*I site of pKO-1. The strong *trp* promoter may be induced by 3-β-indolacrylic acid (IAA).

Enzymes and chemicals—Restriction enzymes, klenow fragmet of DNA polymerase I, T4 DNA polymerase and T4 DNA ligase were purchased from New England Biolabs (NEB) Inc. and International Biotechnologies (IBI) Inc.. IAA was purchased from SIGMA chemical company. TE, TBE, T4 DNA polymerase buffer and nick-translation buffer, LB and M9CA broth were prepard as described by Maniatis *et al.*

Manipulation of DNA and transformation—Alkaline lysis method by Birnboim and Doly[2] was used for preparation of plasmid DNA. In order to purify the double stranded DNA fragment. BRL nucleic acid chromatography system (NACS) was used. Transformation was performed as described by Cohen *et al* with some minor modification.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)—When A_{600} of the culture is 0.05, IAA was added to 50 μg per m*l*. The culture time after addition of IAA was varried 0, 2, 4 hr and 6 hr at 37℃. The 1 m*l* samples of culture grown in M9CA broth containing ampicillin (50 μg/m*l*) were harvested by centrifugation and the pellets were lysed in 100 μ*l* of sample buffer. After boiling for 3 min, samples were electrophoresed on 7.5% SDS-polyacrylamide gels.

Measurement of XMP aminase acivity—*E. coli* clones harboring recombinant plasmids which used in this study were grown in M9CA broth containing ampicillin (50 μg/m*l*). XMP aminase activity was measured by the method of Sakamoto *et al.*[15] The amount of 5'-GMP produced was estimated by measuring the increase in absorbtion of 290 nm of the reaction mixture deprotonized with perchloric acid. Protein concentrations were estimated spectrophotometrically by the method of Lowry *et al.*[11]

Results and Discussion

Subcloning of the *gua A* gene into pBR 322—The source of the *gua A* gene was used a hybrid plasmid consisting of the *gua* operon and col-E_1 DNA, pLC 34-10. The size of pLC 34-10 is approximately 15.2 kb as shown in Fig. 1, and according to Amelia A. Tiedeman *et al*,[18] it was assumed that the *gua A* gene is located on the approximately 1.7 kb long *Ban*II-*Cla*I fragment. This segment of pBR 322 was connected and recombinant plasmid pXA 12 was obtained. The plasmid pXA 12 was transferred into *E. coli* strain HB 101 and the transformant was selected according to its ampicillin resistance. After selection of transformants, the recombinant plasmid DNA was extra-

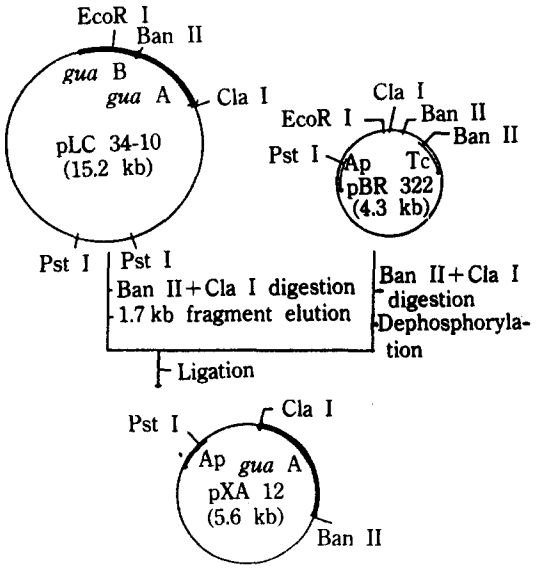

Fig. 1. Construction of recombinant plasmid pXA 12.

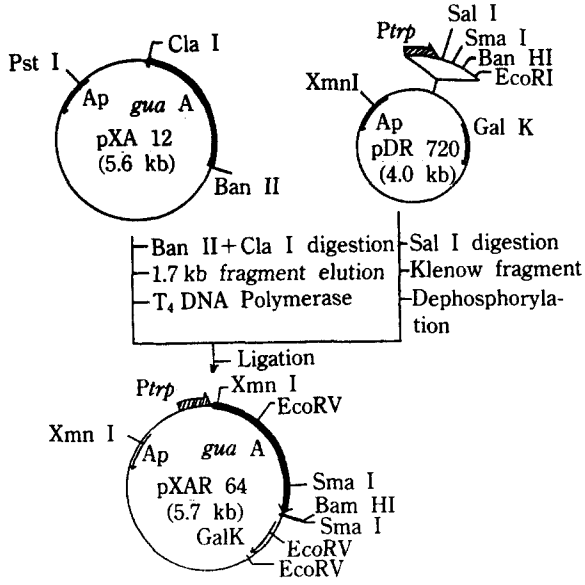

Fig. 2. Construction of recombinant plasmid pXAR 64.

cted by the method of small scale DNA preparation and then the restriction patterns of plasmid pXA 12 was examined.

Construction of recombinant pXAR 64—In order to overproduce the *gua A* gene, *E. coli* expression vector pDR 720 was adopted. Because the *Ban*II-*Cla*I fragment *gua A* gene does not contain its original promoter for *gua BA* operon, an apparent increase in XMP aminase activity was not seen in strains containing pXA 12 when compared with pLC 34-10. Therefore it was attempted to insert *gua A* gene under the control of the *trp* promoter with strong transcriptional activity. Protruding 5′ ends of the *Sal*I digested pDR 720 were filled in by the klenow fragment of DNA polymerase I and dephosphorylated by Calf Intestinal Phosphatase (CIP). The 3′ protruding ends of 1.7 kb *Ban*II-*Cla*I *gua A* gene fragment were digested by the 3′ to 5′ exonuclease activity of T4 DNA polymerase and 5′ protruding ends were filled in by the 5′ to 3′ polymerase activity of the same enzyme (Fig. 2). The resulting blunt-ended fragment of insert and pDR 720 were ligated and the recombinant plasmid pXAR 64 was obtained. Recombinant pXAR 64 was transformed into the *E. coli* strain HB 101 and ampicillin resistant colonies were analyzed. The resulting 5.7 kb plasmids were

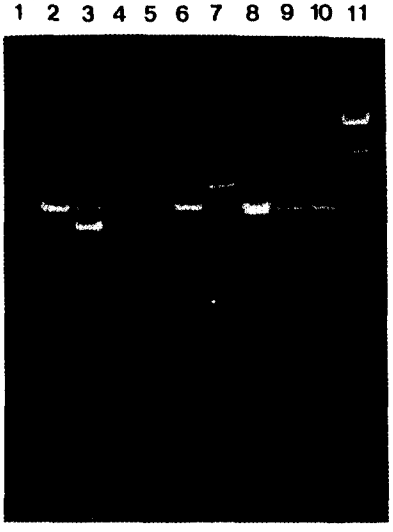

Fig. 3. Restriction pattern of plasmid pXAR 64.

1. pDR 720
2. pDR 720/*Xmn*I
3. pDR 720/*Xmn*I+*Sal*I
4. pXAR 64/*Xmn*I
5. pXAR 64
6. pDR 720/*Sma*I
7. pXAR 64/*Sma*I
8. pDR 720/*Eco*RV
9. pXAR 64/*Eco*RV+*Bam*HI
10. pXAR 61/*Eco*RV+*Bam*HI
11. λ*Hin*dIII

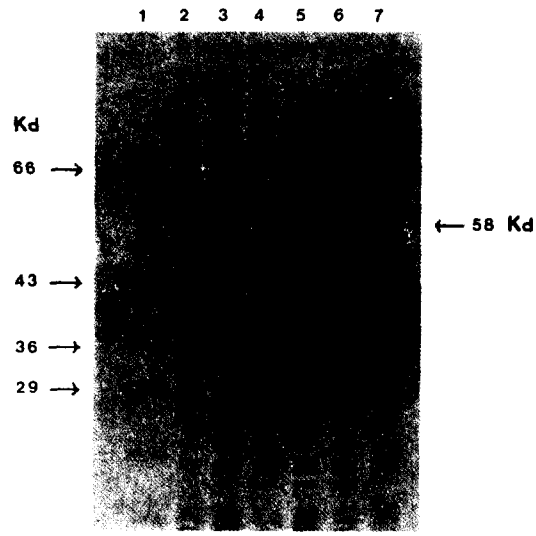

Fig. 4. SDS-Polyacrylamide gel electrophoresis of proteins synthesized by *E. coli* HB 101/pXAR 64.

1. Size marker
2. 6 hr-uninduced HB 101
3. 6 hr-uninduced HB 101/pXAR 64
4. 0 hr-induced HB 101/pXAR 64
5. 2 hr-induced HB 101/pXAR 64
6. 4 hr-induced HB 101/pXAR 64
7. 6 hr-induced HB 101/pXAR 64

Table 1. XMP aminase activity in strains carring various plasmids

Host	Plasmid	Specific activity (units/mg protein)	Ratio
E. coli HB 101		0.06	1.0
E. coli JA 200	pLC 34-10	0.95	14.1
E. coli HB 101	pXAR 12	0.13	2.0
E. coli HB 101	pXAR 64	15.71	235.5

Unit=5'-GMP 1 nmol produced/min

purified and digested with the endonuclease *Sma* I (Fig. 3) and the recombinant plasmid carring *gua A* gene in the sense orientation at the downstream of the *trp* promoter was confirmed.

Expression of the recombinant plasmid—To express the *gua A* gene, *E. coli* HB 101 was used as the host strain because this strain is commonly used as a recipient in transformation and is a good host for large scale growth and purification of plasmid. When A_{600} of the culture is 0.05, IAA was added to 50 μg/*ml*. The culture time after the addition of IAA was varried 0, 2, 4 hr and 6 hr at 37℃. And SDS-polyacryamide gel electrophoresis (7.5%) was used to analyze XMP aminase synthesized by *E. coli* HB 101 harboring pXAR 64 incubated under induced and non-induced condition.

In the strain carring pXAR 64 induced with IAA, a dark band with an apparent molecular weight of around 58 kd was obserbed but in uninduced culture the band could not be distinguishable from host protein bands (Fig. 4). Because the molecular weight of expected protein could be calculated from the DNA sequence to be 58,604 daltons, it was thought that this band represented XMP aminase. The XMP aminase activities of the strains obtained in this study are showed in Table 1. In the strain containing pLC 34-10, a 14 fold increase in activity compared with the host strain was observed. pXAR 64, in which a *trp* promoter was inserted upstream of the *gua A* gene, the XMP aminase activity had increased by about 17 fold when compared with that of the strain carring pLC 34-10.

국문요약

5'-XMP를 5'-GMP로 전환하는 효소인 XMP aminase[EC 6.3.4.1]의 활성을 증가시키기 위하여 XMP aminase의 유전자를 함유한 1.7 kb *gua A* gene fragment를 pLC 34－10으로부터 분리하여 pBR 322에 subcloning한 뒤 *trp* promoter를 가지고 있는 대장균 발현 벡터 pDR 720에 도입하였다. 재조합된 pXAR 64에 존재하는 *gua A* 유전자는 *trp* promoter에 의하여 발현이 증대되었으며 3-β-indoleacrylic acid에 의하여 XMP aminase의 생성이 유도되었다. XMP aminase의 비활성은 pLC 34－10을 함유한 균주에 비하여 약 17배 증가되었다.

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