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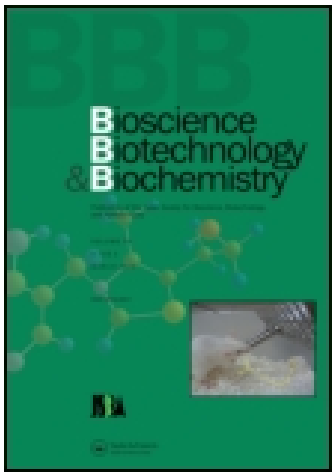

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
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High Level Expression of XMP Aminase in Escherichia coli and Its Application for the Industrial Production of 5′-Guanylic Acid

Tatsuro Fujio[a], Tatsunari Nishi[ac], Seiga Ito[ad] & Akihiko Maruyama[ab]

[a] Planning & Development Department/Food, Liquor & Food Division, 6-1-1 Ohtemachi, Chiyoda-ku, Tokyo 100, Japan

[b] Fine Chemicals/Biochemicals Dept., Medicals Research and Development Center, Kyowa Hakko Kogyo Co., Ltd., 6-1-1 Ohtemachi, Chiyoda-ku, Tokyo 100, Japan

[c] Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6 Asahi-machi, Machida-shi, Tokyo 194, Japan

[d] Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken, 411, Japan

Published online: 12 Jun 2014.

To cite this article: Tatsuro Fujio, Tatsunari Nishi, Seiga Ito & Akihiko Maruyama (1997) High Level Expression of XMP Aminase in Escherichia coli and Its Application for the Industrial Production of 5′-Guanylic Acid, Bioscience, Biotechnology, and Biochemistry, 61:5, 840-845, DOI: 10.1271/bbb.61.840

To link to this article: http://dx.doi.org/10.1271/bbb.61.840

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Biosci. Biotech. Biochem., **61** (5), 840–845, 1997

High Level Expression of XMP Aminase in *Escherichia coli* and Its Application for the Industrial Production of 5′-Guanylic Acid

Tatsuro FUJIO, Tatsunari NISHI,** Seiga ITO,*** and Akihiko MARUYAMA*,†

*Planning & Development Department/Food, Liquor & Food Division, and *Fine Chemicals/Biochemicals Dept., Medicals Research and Development Center, Kyowa Hakko Kogyo Co., Ltd., 6–1–1 Ohtemachi, Chiyoda-ku, Tokyo 100, Japan*
***Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3–6–6 Asahi-machi, Machida-shi, Tokyo 194, Japan*
****Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken, 411, Japan*
Received November 8, 1996

To improve the efficiency of the enzymatic conversion of 5′-xanthylic acid (XMP) to 5′-guanylic acid (GMP), we attempted to increase the activity of the conversion enzyme, XMP aminase (GMP synthetase) encoded by the *guaA* gene in *Escherichia coli*. By connecting the P_L promoter of λ phage, the SD sequence of *trpL* of *E. coli*, and ATG, at a suitable position upstream of the *guaA* gene, we obtained plasmid pPLA66. Sequencing of the nucleotides of the upstream region of the *guaA* gene on pPLA66 showed that the C-terminal region of the *guaB* gene, which encodes IMP dehydrogenase, was conserved and a short peptide consisted of 14 amino acids was coded. *E. coli* MP347/pPLA66 showed an increase in the activity of approximately 370 times when compared with that of the strain MM294, and the amount of the enzyme protein represented approx. 34% of the total cellular protein. Strain MP347/pPLA66 was cultivated in a 5-liter jar fermentor using a medium which contained mainly corn steep liquor. The culture broth had high XMP aminase activity. In the conversion reaction using mixed broths consisted of 600 ml of XMP-fermentation broth of *Corynebacterium ammoniagenes* KY13203 and 30 ml of cultured broth of *E. coli* MP347/pPLA66, a surfactant, Nymeen S-215 and xylene were added to the reaction mixture to make the cell membrane permeable to nucleotides. After 23 h of the reaction, 70 mg/ml (131 mM) of GMP·Na_2·$7H_2O$ was accumulated from 83 mg/ml (155 mM) of XMP·Na_3·$7H_2O$, without addition of ATP. The molar conversion yield was approx. 85%. The facts that the cell membrane was treated to allow nucleotides to permeate and that the conversion reaction proceeded well enough in spite of a small amount of *E. coli* cells indicate ATP was regenerated from AMP by *C. ammoniagenes* cells and supplied to *E. coli* cells. Therefore, it was considered that the coupling reaction between these two kind of strains was established.

Key words: 5′-guanylic acid; XMP aminase (GMP synthetase); genetic engineering; enzymatic conversion; *Escherichia coli*

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XMP aminase (GMP synthetase; EC 6.3.4.1), which is encoded by the *guaA* gene in *Escherichia coli*, catalyzes the glutamine- or NH_3-dependent synthesis of 5′-guanylic acid (GMP) as shown by the following equations:

$$\text{XMP} + \text{ATP} + \text{glutamine} \rightarrow \text{GMP} + \text{AMP} + \text{PPi} + \text{glutamate}$$

$$\text{XMP} + \text{ATP} + \text{NH}_3 \rightarrow \text{GMP} + \text{AMP} + \text{PPi}$$

GMP, similar to 5′-inosinic acid (IMP), is widely used as a flavor improver, and has been produced on an industrial scale by the following methods: (1) enzymatic degradation of RNA extracted from yeast cells,[1] (2) chemical phosphorylation of guanosine, which is produced by fermentation,[2] and (3) enzymatic amination of 5′-xanthylic acid (XMP) produced by fermentation.[3] An RNA degradation method (1) was used widely on an industrial scale. However, at the present time, the method is scarcely ever used, and therefore the other two methods are mainly used for the industrial production of GMP.

We have investigated the improvement of the industrial production of GMP from XMP (method (3)). This method consists of a series of two separate processes: first, XMP is produced fermentatively by a mutant strain of *Corynebacterium ammoniagenes*[4] (formerly called *Brevibacterium ammoniagenes*[5]), and second, the XMP is converted to GMP with the culture broth of another mutant strain of *C. ammoniagenes* as the enzyme source.[3] The mutant strain used for conversion is resistant to decoyinine, which inhibits XMP aminase as an analog. Therefore, this second strain contains an increased amount of XMP aminase.[6]

In this process, XMP fermentation broth was heat-treated to sterilize the XMP-producing strain and was used as the XMP source in the conversion reaction. Then, the second strain was cultivated in the same fermentor and the conversion of XMP to GMP was performed only by the second strain. For the conversion reaction, efficient activities of XMP aminase and the ATP regenerating system were demanded. Therefore, a large volume (about one half of XMP fermentation broth) was needed for the cultivation of the second strain. In contrast to this process, a new process described here requires both an XMP-producing

† To whom correspondence should be addressed.

Abbreviations: PPi, pyrophosphate; Ap, ampicillin; Sm, streptomycin; P_{trp}, tryptophan promoter; OD, optical density; bp, base pair; kb, kilobase pair; SD, Shine–Dalgarno (sequence).

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strain (*C. ammoniagenes*) as a main donor of ATP regenerating activity, and an XMP aminase providing strain (*E. coli*). By increasing the activity of XMP aminase in *E. coli*, a reduction in the total amount of cells required for the conversion reaction is expected when whole cells are used as the enzyme source. The reduction of the cultivation volume of the conversion strain enables to increase the cultivation volume of XMP fermentation. Therefore, the total amount of the production of GMP per batch is expected to increase. So we attempted to obtain XMP aminase overproducing strains of *E. coli* using genetic engineering techniques.

In our previous paper,[7] we showed the conversion reaction of XMP to GMP using recombinant *E. coli* (MM294/pXAR33) cells. In the experiment, ATP was added to the reaction mixture.

In this report, we describe a more practical conversion process using recombinant *E. coli* cells as the source of XMP aminase and *C. ammoniagenes* cells that have accumulated XMP as the main source of ATP-regeneration enzymes.

Materials and Methods

Bacterial strains. Strain *C. ammoniagenes* KY13203, derived from *C. ammoniagenes* ATCC6872 through several mutational steps, was used as the XMP producer. This mutant is adenine-leaky requiring (growth is stimulated by adenine), guanine-requiring, manganese-insensitive, nucleotidase-weak, and temperature-sensitive for growth. *E. coli* MP347 (F^-, *lacZ*amY14, *trp*am8, *gal*, Sm^r, (λ *bio*252, *cI*857, ΔH1))[8] and *E. coli* MM294 (F^-, *sup*E44, *thi*1, *end*A1, *hsd*R17)[9] were used as host strains for the P_L promoter and trp promoter expression plasmids, respectively.

Media and cultivation. In the cultivation of *C. ammoniagenes* KY13203, the following media were used:

Seed medium A: 50 g/liter glucose, 10 g/liter polypeptone, 10 g/liter yeast extract, 5 g/liter urea, 5 g/liter $(NH_4)_2SO_4$, 1 g/liter KH_2PO_4, 3 g/liter K_2HPO_4, 1 g/liter $MgSO_4 \cdot 7H_2O$, 0.1 g/liter $CaCl_2 \cdot 2H_2O$, 10 mg/liter $FeSO_4 \cdot 7H_2O$, 10 mg/liter $ZnSO_4 \cdot 7H_2O$, 20 mg/liter $MnSO_4 \cdot 4\text{–}6H_2O$, 20 mg/liter L-cysteine·HCl, 10 mg/liter calcium D-pantothenate, 5 mg/liter vitamin B_1·HCl, 5 mg/liter nicotinic acid, 30 μg/liter biotin, 150 mg/liter adenine, and 150 mg/liter guanine, the pH adjusted to 7.2 with NaOH.

Seed medium B: 100 g/liter glucose, 12 g/liter polypeptone, 12 g/liter yeast extract, 1 g/liter KH_2PO_4, 1 g/liter K_2HPO_4, 1 g/liter $MgSO_4 \cdot 7H_2O$, 20 mg/liter $FeSO_4 \cdot 7H_2O$, 10 mg/liter $ZnSO_4 \cdot 7H_2O$, 4 mg/liter $MnSO_4 \cdot 4\text{–}6H_2O$, 0.8 mg/liter $CuSO_4 \cdot 5H_2O$, 15 mg/liter β-alanine, 20 mg/liter L-cysteine·HCl, 100 μg/liter biotin, 200 mg/liter adenine, 200 mg/liter guanine, and 5 mg/liter vitamin B_1·HCl (separately sterilized), adjusted to pH 7.2 with NaOH.

Fermentation medium: 154 g/liter glucose, 17 g/liter phosphoric acid, 13.3 g/liter KOH, 10 g/liter $MgSO_4 \cdot 7H_2O$, 100 mg/liter $CaCl_2 \cdot 2H_2O$, 20 mg/liter $FeSO_4 \cdot 7H_2O$, 10 mg/liter $ZnSO_4 \cdot 7H_2O$, 10 mg/liter $MnSO_4 \cdot 4\text{–}6H_2O$, 2 mg/liter $CuSO_4 \cdot 5H_2O$, 15 mg/liter β-alanine, 5 mg/liter nicotinic acid, 20 mg/liter L-cysteine·HCl, 20 mg/liter L-histidine·HCl, 150 μg/liter biotin, 150 mg/liter adenine, 80 mg/liter guanine, 5 mg/liter vitamin B_1·HCl (separately sterilized), and 4 mg/liter $ZnSO_4 \cdot 7H_2O$ (separately sterilized), and the pH was not adjusted (about pH 5.8–6.0).

A loopful of cells of *C. ammoniagenes* KY13203 was inoculated into test tubes (20 cm × 2 cm) containing 10 ml of the seed medium A and incubated at 28°C for 24 h on a reciprocal shaker (300 rpm). Twenty ml of the seed culture was transferred into a 2-liter Erlenmeyer flask with baffles, which contained 230 ml of the seed medium A, and was incubated at 28°C for 24 h on a rotary shaker (190 rpm). The culture (250 ml) was then inoculated 2.5 liter of the seed medium B in a 5-liter jar fermentor (KMJ-5, Mitsuwa Bio Systems Co., Ltd.). This seed cultivation was done at 32°C with aeration (1 vvm) and agitation (600 rpm). The pH of the medium was maintained at pH 7.3 with 28% NH_4OH. When the glucose in the supernatant of this seed culture was consumed, a 250-ml portion of the culture was transferred into 2.5 liters of the fermentation medium in a 5-liter jar fermentor. The cultivation was done at 32°C with aeration (1 vvm) and agitation (600 rpm). The pH of the medium was kept at pH 7.3 with 28% NH_4OH. When the glucose concentration of the supernatant of the culture reached 5%, 250 ml of a feed solution that contained 220 g of glucose, 21.1 g of KH_2PO_4, and 3.65 g of K_2HPO_4 was added to the medium and the cultivation was continued. The cultivation was stopped when the glucose in the supernatant of the culture was completely consumed.

LB medium (1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl, adjusted to pH 7.2 with NaOH) was used for the cultivation of *E. coli* strains. For the small scale cultivation of recombinant *E. coli* strains that expressed the *guaA* gene under the control of P_L promoter, cultivation was done as follows. A loopful of cells of *E. coli* was inoculated into 10 ml of LB medium containing 100 mg/liter ampicillin in a test tube, and then it was cultured at 28°C for 18 h on a reciprocal shaker (300 rpm). One hundred μl of the seed culture was inoculated into 10 ml of LB medium containing 100 mg/liter ampicillin in a test tube, and cultured at 30°C for about 4–5 h. When the OD_{550} reached around 1.0, the cultivation temperature was elevated to 40°C, and the cultivation was continued for 4 h. The XMP aminase activity of this temperature-shifted culture broth was measured as described in our previous paper.[7]

The cultivation of *E. coli* was scaled up to a 5-liter jar fermentor as follows. A loopful cells of *E. coli* MP347/pPLA66 was inoculated into 10 ml of LB medium containing 100 mg/liter ampicillin in a test tube, and then culture with reciprocal shaking (300 rpm) at 28°C for 24 h. Ten ml of the resulting culture was inoculated into 250 ml of LB medium containing 100 mg/liter ampicillin in a 2-liter Erlenmeyer flask with baffles and then incubated at 28°C for 24 h on a rotary shaker (190 rpm). This culture (250 ml) was transferred to a 5-liter jar fermentor that contained 2.5 liters of a liquid medium comprising 120 g/liter corn steep liquor, 10 g/liter glutamic acid, 2 g/liter lactic acid, 0.5 g/liter tryptophan, and 100 μg/liter biotin, and adjusted to pH 7.0 with NaOH. The cultivation was done at 30°C for 5 h, with aeration (1 vvm) and agitation (600 rpm). After 5 h of cultivation, the temperature was shifted to 40°C and cultivation was continued for 5 h. The pH of the medium was kept at 7.6 with 6 N H_2SO_4 throughout the culture.

Enzyme assay. The XMP aminase activity of the *E. coli* strain was measured as described in our previous paper.[7] The culture broth of *E. coli* was treated with 20 ml/liter of xylene for 20 min at 37°C, and this treated broth was directly used in the enzyme assay. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol of GMP per minute under these assay conditions.

Genetic techniques. DNA manipulations were done generally according to the protocols.[10] The nucleotide sequence of N-terminal region of the *guaA* gene on pPLA66 was analyzed by the method of Maxam and Gilbert.[11]

Measurement of the constituents. In the conversion reaction, each constituent was analyzed as follows: XMP, GMP, and xanthosine were measured by paper chromatography[3] and high performance liquid chromatography (HPLC). The conditions for HPLC were: column, Partisil 10 SAX 4.6 mm × 250 mm (Whatman); detection, 254 nm; mobile phase, 0.05 M KH_2PO_4 (pH 4.0); column temperature, 50°C; and flow rate, 2.0 ml/min. Glucose, inorganic phosphate, and magnesium ion were measured using assay kits, GlucoseB-Test Wako, PhosphorB-Test Wako, and MagnesiumB-Test Wako respectively (Wako Pure Chemical Ind., Ltd. (Osaka)).

Conversion reaction. In the conversion reaction, the cultured broths of *C. ammoniagenes* KY13203 and *E. coli* MP347/pPLA66 in 5-liter jar fermentors were used as XMP and enzyme sources. The XMP fermentation broth (600 ml) of *C. ammoniagenes* KY13203 and the cultured broth (30 ml) of *E. coli* MP347/pPLA66 were mixed in a 2-liter jar fermentor. Then the following ingredients were added as the final concentration: 50 g/liter glucose, 2 g/liter phytic acid, 4.23 g/liter NaH_2PO_4, 5 g/liter $MgSO_4 \cdot 7H_2O$, 4 g/liter Nymeen S-215, and 10 ml/liter xylene. The mixture was incubated at 42°C with stirring (900 rpm) and aeration (1.2 vvm). During the reaction, the pH was kept at 7.3 with 28% NH_4OH. Glucose (20 g/liter as the final concentration) was fed at 10 h of the reaction.

Chemicals. The surfactant, Nymeen S-215 (polyoxyethylene stearylamine), was purchased from Nippon Oil and Fats Co., Ltd., (Tokyo). All other chemicals were commercially available and of analytical grade.

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Results

Expression of the guaA gene using trp promoter

In the previous paper,[7] we constructed the plasmid pXAR33, which overproduced XMP aminase up to about 10% of the total cellular protein. On this plasmid, the *guaA* gene is expressed under the control of the *E. coli* tryptophan (trp) promoter. We further attempted to select plasmids that had higher activity than pXAR33 using the same expression system, but we could not obtain such a plasmid. During this experiment, we found some transformants that could not grow on M9 minimal medium. These strains could grow and express *guaA* at very low levels when excess tryptophan was added to the medium. Only when a limited amount of tryptophan was added to the medium, though growth was limited, these strains expressed *guaA* at a sufficient level (data not shown). From these results, we considered that overexpression of XMP aminase is lethal for *E. coli*.

Therefore, we tried to use the P_L promoter of λ phage, which can control the expression of a downstream gene by temperature,[8] to obtain a plasmid that has higher activity than pXAR33.

Expression of the guaA gene using P_L promoter

We examined two approaches to obtain plasmids that overproduce XMP aminase using the P_L promoter. One, the *guaA* gene was connected with a P_L-ATG vector.[12] The other, a plasmid that directly expressed the *guaA* gene was constructed using synthesized DNA fragments. The P_L-ATG vector was constructed from the P_{trp}-ATG vector, pTrS3.[12] A 0.37-kb trp promoter (*Eco*RI–*Cla*I fragment) of pTrS10, a derivative of pTrS3, was changed to a 0.22-kb P_L promoter fragment (*Eco*RI–*Cla*I fragment) derived from λ phage.[13] The plasmid thus obtained (the P_L-ATG vector) was named pPA1 (Fig. 1).

Construction of pPLA66

The plasmid pXAR33[7] was cleaved with *Hpa*I, followed by digestion with BAL31 exonuclease. Then, these BAL31-treated DNA fragments were cleaved with *Pst*I and the larger DNA fragments (about 3.0 kb) were purified using a low-gelling-temperature agarose gel, as described by Wieslander.[14] On the other hand, the P_L-ATG vector, pPA1, was cleaved with *Sph*I and followed by converting the 3′-protruding ends of *Sph*I into blunt ends with T4 DNA polymerase (see Figs. 1 and 2). This blunt ended DNA

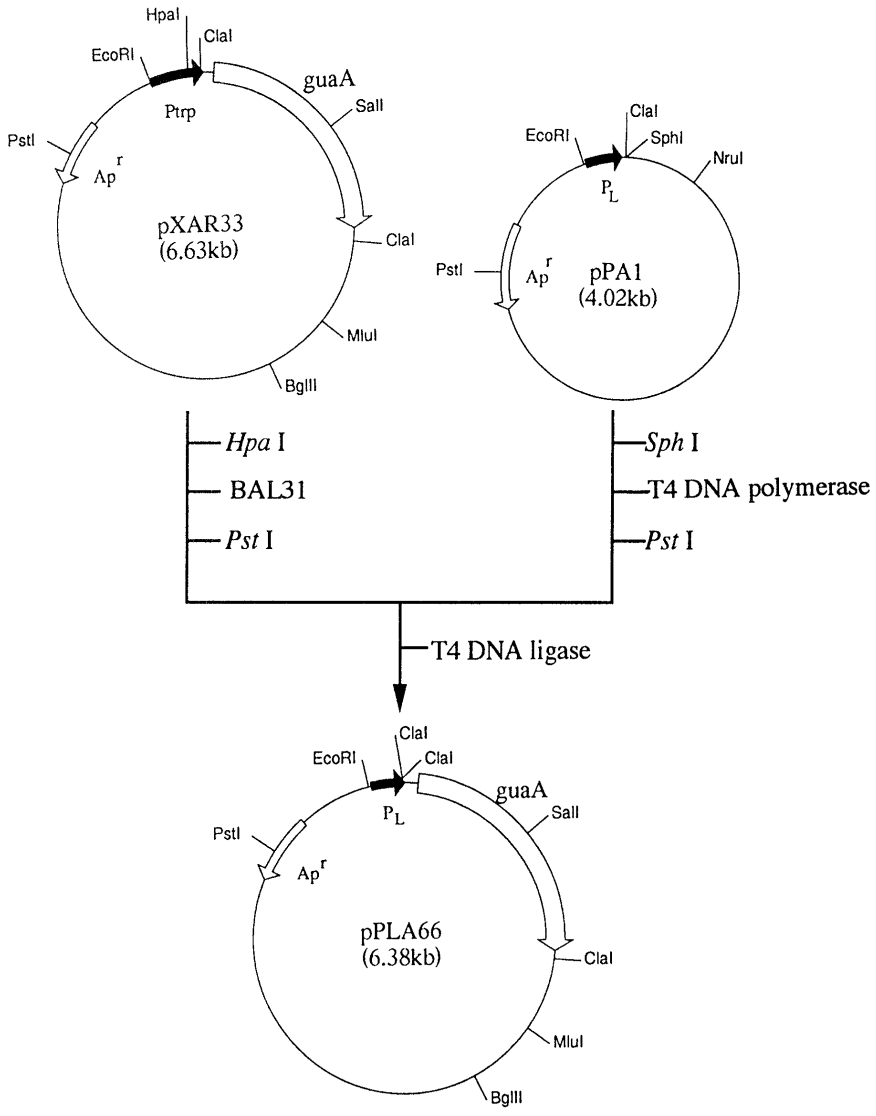

Fig. 1. Construction of Plasmid pPLA66.

guaA genes are shown by the thick open arrows. The ampicillin resistance genes (Ap^r) are indicated by the thin open arrows. Thin black arrows represent the P_{trp} or P_L promoter.

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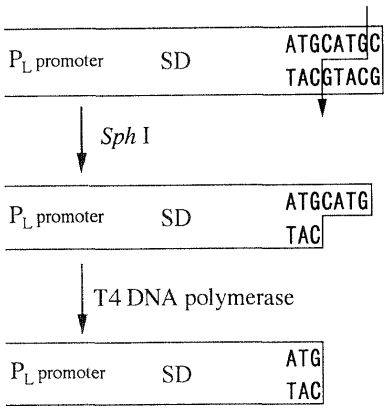

Fig. 2. Structure of a P_L-ATG Vector and Method to Create a Blunt End Just Downstream from an ATG Coding for an Aminoterminal Methionine.

"SD" designates the Shine Dalgarno sequence

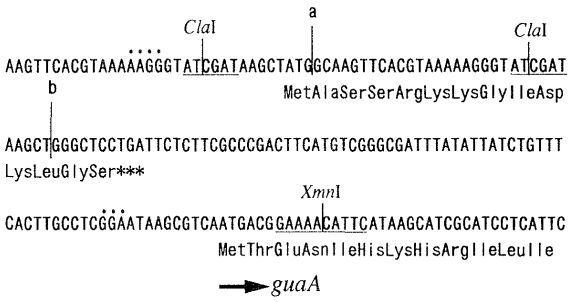

Fig. 3. Nucleotide Sequence of Upstream Region of *guaA* on pPLA66.

The upstream sequence from "a" is derived from pPA1. The sequence between "a" and "b" is derived from pGBY1.[7] The downstream sequence from "b" is derived from pLC34-10.[7] Dots above the sequence indicate the Shine–Dalgarno sequence of P_L promoter (AAGG), and *guaA* gene (GGA) respectively. *Cal*I and *Xmn*I cleavage sites are also represented.

fragments were digested with *Pst*I and the smaller DNA fragments (about 1.0 kb) were purified. The purified two kinds of DNA fragments were ligated with T4 DNA ligase and transformed *E. coli* MP347. From among the Ap^r transformants, 202 transformants were cultured as described in Materials and Methods, and their XMP aminase activities were measured. Among them, 143 transformants had the same or higher activities of XMP aminase as the strain MM294/pXAR33. The remaining strains had almost the same level as the host strain MM294. The strain that showed the highest activity was named MP347/pPLA66.

Nucleotide sequence of the upstream region of the guaA gene on pPLA66

We sequenced the nucleotides of the upstream region of the *guaA* gene on pPLA66 by the method of Maxam and Gilbert.[11] As shown in Fig. 3, the 68-bp intercistronic region between *guaB* and *guaA* was conserved.[15] The 10-bp of C-terminal region of the *guaB* gene including the stop codon (TGA) was also conserved. Interestingly, a short peptide consisted of 14 amino acids was encoded upstream of the *guaA* gene. This short peptide started at the ATG codon of the ATG vector, pPA1, and ended at the TGA stop codon of the *guaB* gene. The role of the existence of this short peptide is discussed afterwards.

Construction of pPLC14

We attempted to construct a plasmid that directly

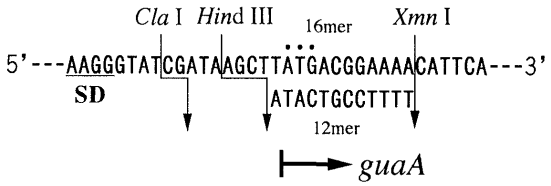

Fig. 4. Nucleotide Sequence of N-terminal Region of *guaA* on pPLC14.

The synthetic DNAs are represented between *Hind*III and *Xmn*I cleavage sites. The Shine–Dalgarno sequence is indicated by an underline. Dots above the sequence show the initiation codon of *guaA*.

Table Comparison of XMP Aminase Activities

Host/Plasmid	Growth[a] [g/liter]	Activity [U/ml][b]	Activity [U/g][c]	Activity [fold]
MM294	6.48	0.0057	0.88	1
MM294/pXAR33	7.22	0.531	73.5	83.5
MP347/pPLA66	6.94	2.27	327	372
MP347/pPLC14	5.37	0.118	22	25

[a] Wet cell weight of collected cells.
[b] U = μmol of GMP formed per minute; U/ml = U/ml of cultured broth.
[c] U/g = U/gram of wet cells.

expressed *guaA* using synthetic DNAs. As shown in Fig. 4, we designed two synthetic DNAs (16-mer and 12-mer) which could connect the *Hind*III site downstream from the P_L promoter and the *Xmn*I site in the N-terminal region of *guaA*. As the source of the P_L promoter, a plasmid, pPLGM1, which is one of the derivatives of the plasmid pPA1 and has a *Hin*III site downstream from the P_L promoter, was used (precise construction methods omitted). The nucleotide sequence of the N-terminal portion of *guaA* was conserved and the distance between SD and ATG was 14 bp (see Fig. 4). Thus the constructed plasmid was named pPLC14.

Comparison of XMP aminase activities

Table shows the XMP aminase activities of the obtained recombinant *E. coli* strains. The XMP aminase activities of the host strains, MM294 and MP347, were at almost the same level (data not shown). Among them, MP347/pPLA66 showed the highest activity, about 370 times higher than the host strain, MM294. This strain expressed XMP aminase as about 34% of total cellular protein measured by SDS–polyacrylamide gel electrophoresis (data not shown). On the other hand, MP347/pPLC14 showed only about 25 times higher activity than MM294. Therefore, we decided to use the MP347/pPLA66 strain as the enzyme source for the conversion reaction.

Cultivation of MP347/pPLA66 in a 5-liter jar fermentor

We cultivated strain MP347/pPLA66 in a 5-liter jar fermentor using the medium which contained mainly corn steep liquor as described in Materials and Methods (at the part of scaled up cultivation of *E. coli*). Figure 5 shows the time course of cultivation of this strain. After heat induction (30→40°C, after 5 h of cultivation), the XMP aminase activity appeared and increased gradually, and finally (after 5 h of cultivation at 40°C) reached 36 U/ml. After heat induction, the OD increased more rapidly, on the other

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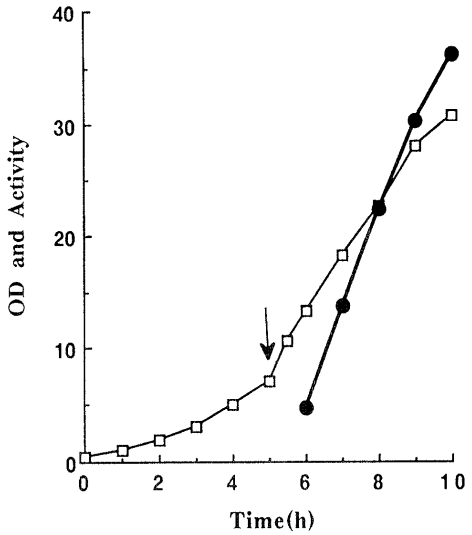

Fig. 5. Growth Pattern of MP347/pPLA66 in a 5-Liter Jar Fermentor.
Symbols: □, OD_{550}; ●, XMP aminase activity (U/ml). The heat induction time (30→40°C) is indicated by a closed arrow.

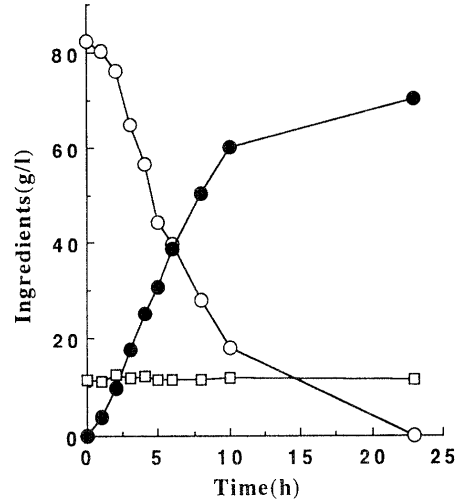

Fig. 6. Conversion Reaction from XMP to GMP in a 2-Liter Jar Fermentor.
Symbols: ○, XMP·Na_2·$7H_2O$, ●, GMP·Na_2·$7H_2O$, □, xanthosine.

hand viable cell count was increased first 0.5 h (from 4.6×10^9 to 7.2×10^9 cells/ml), and then gradually decreased to the level of about 1.5×10^9 cells/ml at 3 h and this level was maintained to 5 h of the culture at 40°C. The ratio of plasmid-harboring strains (approximately 100% as ampicillin resistant colony at 0 h of heat induction) decreased rapidly and at the end of cultivation (5 h), all living cells lost the plasmid (all of them were ampicillin sensitive).

Conversion reaction of XMP to GMP

The XMP fermentation broth (600 ml) of *C. ammoniagenes* KY13203 and the cultured broth (30 ml) of *E. coli* MP347/pPLA66 were mixed in a 2-liter jar fermentor, and the conversion reaction of XMP to GMP was done as described in Materials and Methods. As shown in Fig. 6, 70 mg/ml (131 mM) of GMP·Na_2·$7H_2O$ was produced from 83 mg/ml (155 mM) of XMP·Na_2·$7H_2O$ in 23 h of reaction without addition of ATP. The molar conversion yield from XMP to GMP was about 85% considering the slightly increased final volume. At the end of the reaction, small amounts of guanosine and guanine were also produced (data not shown).

Discussion

We have obtained the XMP aminase-overproducing strain MP347/pPLA66 using the P_L-ATG vector. The strain showed 370 times higher activity than the host strain and the content of XMP aminase was as high as 34% of the total cellular protein. On the other hand, the direct expression strain, MP347/pPLC14, using synthetic DNAs, had only 25 times higher activity than the host strain. This was an unexpected result for us, because we considered that the expression of the *guaA* gene should be optimized using synthetic DNAs in the construction of pPLC14. These results indicate that the conservation of the intercistronic region between *guaB* and *guaA*, and the existence of a short peptide that terminates at the stop codon of *guaB* are indispensable and play an important role in overexpression of XMP aminase. The conserved SD sequence of *guaA* may also be important. After all, this two-cistronic system may be needed for the efficient expression of *guaA*.

In the cultivation of MP347/pPLA66, *guaA* expression was switched on at an appropriate time by temperature shift. The fact that the reduction of viable cell count and the disappearance of the plasmid holding cell during the heat induction indicates that overexpression of XMP aminase is lethal for *E. coli*. This is the reason why we could not obtain a strain with very high activity using the trp promoter system. The P_L promoter system is strictly regulated by temperature, therefore we could obtain a higher activity strain than MM294/pXAR33.

In the conversion reaction, in spite of the small amount of *E. coli* broth (5%), GMP formation proceeded efficiently without addition of ATP. This fact is attractive and advantageous from the industrial point of view, because the reduction of *E. coli* broth added to the conversion reaction enables us to increase the amount and thus the ratio of XMP fermentation broth in the conversion reaction, which leads to the improvement of the efficiency of GMP production in the process.

ATP is needed for the conversion reaction of XMP to GMP. Considering the ratio of the volume of the cultured broths of *E. coli* to *C. ammoniagenes* in the conversion reaction, it is presumed that ATP is regenerated mainly by *C. ammoniagenes* cells, because *E. coli* cells present in the conversion reaction were much fewer than *C. ammoniagenes* cells. That is to say, consumed ATP in *E. coli* cells must permeate out to the medium as AMP, and the AMP is regenerated to ATP in the intracellular region of *C. ammoniagenes* cells using glucose as an energy source.[16] The treatment of cells with the surfactant, Nymeen S-215 and xylene makes the cell membranes permeable and enables ATP to be exchanged between *E. coli* and *C. ammoniagenes* cells.[16] Other nucleotides such as XMP and GMP can also permeate across the cell membrane.

In this report, we indicated the possibility of the coupling reaction where ATP was efficiently exchanged between two kinds of strains. This coupling reaction is useful for the production of many useful substances formed in the ATP-requiring biosynthetic reaction, such as 5′-inosinic acid (IMP), CDP-choline, and glutathione.

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Acknowledgments. The authors wish to thank Mrs. Eriko Morishita and Mr. Satoru Mizutani for their helpful technical assistance. We also wish to thank Dr. Moriyuki Sato for the synthesis of DNA fragments.

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