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# Identification of Potential *Corynebacterium ammoniagenes* Purine Gene Regulators Using the *pur-lacZ* Reporter in *Escherichia coli*

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**Abstract** This study has developed *Corynebacterium ammoniagenes* (*C. ammoniagenes*) purine gene transcriptional reporters (*purF-lacZ* and *purE-lacZ*) that function in *Escherichia coli* (*E. coli*) DH5a. After transformation of a *C. ammoniagenes* gDNA library into *E. coli* cells harboring either *purF-lacZ* or *purE-lacZ*, *C. ammoniagenes* clones were obtained that repress *purF-lacZ* and *purE-lacZ* gene expression. The potential *purE* and *purF* regulatory genes are homologous to the genes encoding transcription regulators, the regulatory subunit of RNA polymerase, and genes for purine nucleotide biosynthesis of various bacteria. The *C. ammoniagenes purE-lacZ* and *purF-lacZ* reporters were repressed by adenine and guanine within *E. coli*, indicating similarity in the regulatory mechanism of purine biosynthesis in *C. ammoniagenes* and *E. coli*. Gene regulation of *pur-lacZ* by adenine and guanine was partly abolished in cells expressing potential purine regulatory genes, indicating functionality of the purine gene regulators in repression of *purE-lacZ* and *purF-lacZ*. The *purE-lacZ* and *purF-lacZ* reporters can be used for the screening of genes involved in the regulation of the *de novo* synthesis of the purine nucleotides.

**Key words:** *Corynebacterium ammoniagenes*, *de novo* purine nucleotide biosynthesis, reporter system, *purE*, *purF*, reporter, repressor

Purine nucleotides together with pyrimidine nucleotides perform vital roles in cellular processes. These compounds are indispensable precursors for the synthesis of DNA and RNA. They are components of major coenzymes, such as NAD+, NADP+, and coenzyme A, and they provide energy for cellular processes in the forms of ATP and GTP. These nucleotides also serve as critical elements in metabolism and as carriers of activated metabolites for biosynthesis. Several of the purine nucleotides, including inosine-5-monophosphate (IMP) and guanosin-5-monophosphate (GMP), have been used as flavor-enhancing food additives. *Corynebacterium ammoniagenes* (*C. ammoniagenes*) is a Gram-positive coryneform bacterium used in the industrial production of metabolic compounds, including IMP and GMP purine nucleotides [7, 13]. The *C. ammoniagenes* strains used for the production of IMP and GMP are primarily obtained by either chemical or spontaneous mutagenesis. However, these processes result in cumulative genetic alterations that limit the generation of improved bacterial strains for the production of purine nucleotides. Therefore, development of a method for the target-specific knock-down of a gene involved in the global regulation of purine nucleotide biosynthesis would be useful for the generation of bacteria that produce high levels of useful purine nucleotides.

The *de novo* biosynthesis of purine nucleotides requires 10 enzymatic steps to convert 5-phosphoribosyl-1-pyrophosphate (PRPP) to IMP [37, 39]. The formation of both AMP and GMP requires two subsequent steps after IMP [37, 39]. Although the *de novo* pathways for purine nucleotides in both AMP and GMP formation are identical, the patterns of gene organization and enzymes vary between the different organisms [38, 39]. In *E. coli*, genes encoding enzymes for *de novo* purine biosynthesis are scattered through the chromosome as small polycistronic operons and single cistrons [14, 37]. The global regulatory protein PurR is involved in regulation of the series of genes for the synthesis of purine nucleotides [14, 37, 39]. In *B. subtilis*, the genes for purine nucleotide biosynthesis consist of three clusters of overlapping genes separated by

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intercistronic spaces: *purEKB-purC*(*orf*)*QLF-purMNH*(*J*)-*purD* [9, 38]. The *B. subtilis* PurR has also been isolated by characterization of the repression of the *purF-lacZ* fusion gene integrated into the chromosome of *B. subtilis* [27, 34]. The *B. subtilis* PurR also regulates the expression of an array of genes involved in purine metabolism [30, 32, 34, 38]. In *Corynebacteria*, several genes involved in *de novo* synthesis of purine nucleotides, including *purF* encoding glutamine PRPP-amidotransferase and *purEK* encoding 5-phosphoribosyl-5-aminoimidazol (AIR) carboxylase, have been characterized [7, 13]. Currently, a *Corynebacteria* regulatory factor involved in the regulation of purine biosynthesis has not been characterized.

A search was made for *C. ammoniagenes* regulatory factors involved in the regulation of the genes that control *de novo* purine nucleotide biosynthesis using the *C. ammoniagenes purF-lacZ* and *purE-lacZ* reporters. The *C. ammoniagenes pur-lacZ* reporters expressed in *E. coli* cells produced β-galactosidase activity [16]. That allowed the use of *E. coli* as a host for screening the *C. ammoniagenes* gDNA genes involved in transcriptional regulation of the *C. ammoniagenes purF* and *purE* genes [17]. Genes for potential regulatory proteins of *C. ammoniagenes* were obtained by screening clones involved in the repression of the *pur-lacZ* reporters. The *C. ammoniagenes* genes repressing *pur-lacZ* reporters were sequenced and compared against the National Center for Biotechnology Information (NCBI) database (Bethesda, MD, U.S.A.). The genes repressing the *pur-lacZ* reporter were similar to transcription factors and purine biosynthesis genes in their deduced amino acid sequences. Further characterization was made for *pur-lacZ* reporter gene regulation by purines under both overexpression and non-overexpression conditions. Purine regulation of the *Corynebacteria purF-lacZ* and *purE-lacZ* reporters within *E. coli* indicates a similarity between the regulatory mechanisms of the genes involved in the production of purine nucleotides in *E. coli* and those of *C. ammoniagenes*.

## MATERIALS AND METHODS

### *pur-lacZ* Reporter Construction

To isolate a *Corynebacteria amoniagenes purE* promoter DNA fragment, a polymerase chain reaction (PCR) was performed with the 5'-GTTCCCGCGACCAGGATCCA-TCTCTACGGC-3' and 5'-TTCTTTCCTTAGGATCCTT-GATACATCTTG-3' primers against pPR13 [13] as a template. For *purF* promoter isolation, PCR was also performed with the 5'-GTTTGCTGCTCGGATCCTAAC-TGTTGCGAC-3' and 5'-GCTAAGGCCGGGATCCTTA-CTGCCCGCACG-3' primers against pMG26 [7] as a template. Each *purE* (189 bp) and *purF* (354 bp) PCR product was subcloned into the *Eco*RV site of pT7Blue®-vector (Novagen, North Ryde NSW 2113, Australia) to produce purFp-T and purEp-T plasmids. purFp-T and purEp-T were digested with *Bam*HI to produce *purF* and *purE* promoter fragments, which were ligated into the *Bam*HI site of pEKp1lacZ [11] to produce the purFp-lacZ and purEp-lacZ plasmids, respectively. The correct orientation of the *pur-lacZ* fusion of each plasmid was confirmed by nucleotide sequencing using a 5'-TCGAGCCATGGG-CCCCTAGG-3' primer. The *C. ammoniagenes* gDNA library (provided by Macrogen Co., Seoul, Korea) was generated by insertion of 2–3 kb sized *C. ammoniagenes* gDNA fragments into the *Hin*cII site of pUC118 (Mo Bi Tec, Gottingen, Germany).

### Media

Luria-Bertani (LB) medium (Miller, 1972) was used as a rich medium. Cells for the β-galactosidase assay were grown in a minimal medium (MM) containing salts, 800 nM $MgSO_4$, 960 nM anhydrous citric acid, 57 mM $K_2HPO_4 \cdot H_2O$, 168 mM $NaH(NH_4)PO_4 \cdot 4H_2O$), 2.5% casein hydrolysate (Sigma, St. Louis, MO, U.S.A.), 0.3% glucose, and 0.1 μg of thiamin. Where required, 100 μg/ml of adenine and/or guanine was added at each measurement that indicated *pur-lacZ* reporter activity. For plasmid selection, 100 μg/ml of kanamycin (Sigma) and ampicillin (Sigma) were added. To the medium, for the measurement of β-galactosidase activity, 2 μg/ml of 5-bromo-4-chloro-3-indoyl-D-galactosidase (X-gal; Sigma) was added.

### Functionality Test of the *C. ammoniagenes pur-lacZ* Reporters in *E. coli*

*E. coli* DH5α (*sup*E44 *lac*U169 [80 *lac*ZM15] *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1) was grown in LB medium at 37°C. The *purE-lacZ* and *purF-lacZ* plasmids were transformed into *E. coli* DH5α and selected on LB plates containing 50 μg/ml of ampicillin. The isolated transformants were streaked on an LB ampicillin plate containing 2 μg/ml of X-gal. The cell color was identified from photographs taken 16h after streaking onto the plate.

### *C. ammoniagenes* gDNA Library Screening in *E. coli*

DH5α *E. coli* cells harboring *pur-lacZ* reporter plasmids were transformed with a *C. ammoniagenes* gDNA library and selected on LB plates containing 100 μg/ml of kanamycin, 100 μg/ml of ampicillin, and 2 μg/ml of X-gal. After overnight incubation at 37°C, blue colonies were selected as positive clones containing a potential purine gene regulator. Plasmids containing *C. ammoniagenes* gDNA fragments were isolated from individual colonies and analyzed by digestion with both *Bam*HI and *Hin*dIII restriction enzymes, followed by analysis on 1% agarose gel. Both ends of the inserted *C. ammoniagenes* gDNA fragment were sequenced with the primers (sense: 5'-GTTTTCCCAGTCACGAC-3'; anti-sense: 5'-CAGGAA-ACAGCTATGAC-3'). The sequence data were translated

into six possible reading frames and compared against the protein database of the National Center for Biotechnology Information using the BLAST program (NCBI BLAST; http://www. ncbi.nlm.nih.gov/Blast.cgi).

**Liquid β-Galactosidase Assay**

*E. coli* cells containing *purE-lacZ* or *purF-lacZ* reporter plasmids together with the pUC118 vector or a plasmid with a potential regulatory gene were isolated as a single colony on an LB plate containing ampicillin and kanamycin. The individual transformants were inoculated into 5 ml of MM containing both kanamycin and ampicillin. A total of 0.3 ml of cells cultured overnight was inoculated into 5 ml of fresh MM with identical supplements. To measure the regulation levels of the *purF-lacZ* and *purE-lacZ* gene expression by purine, 100 μg/ml of adenine (Sigma) and/or 100 μg/ml of guanine (Sigma) were added to the MM. Cells were assayed for β-galactosidase activity as described previously [3, 5, 7]. All enzyme activities were determined at least three times.

## RESULTS

***C. ammoniagenes purE* and *purF* Promoters Expressed in *E. coli***

To generate reporter systems for the *purE* and *purF* promoters, putative *purE* and *purF* promoters [7, 13] were fused to the *lacZ* reporter. Promoters from *Corynebacteria* often function in *E. coli* [18, 23–25]. Therefore, firstly the functionality of the *C. ammoniagenes purE-* and *purF-lacZ* reporters was tested in the *E. coli* DH5α strain not expressing β-galactosidase activity. The *E. coli* strains transformed with either the *purE-lacZ* or *purF-lacZ* reporter plasmid grew as blue colored cells when streaked onto an LB kanamycin plate containing X-gal (Fig. 1). Cells transformed with the vector alone were white (Fig. 1).

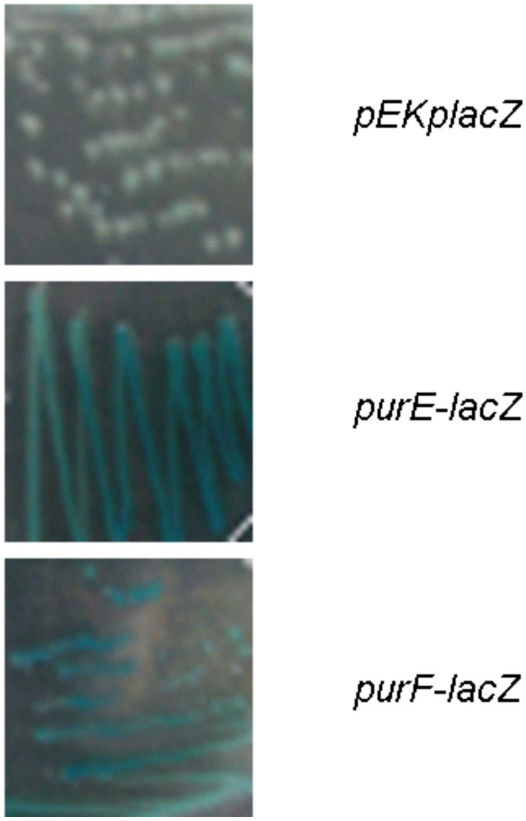


**Fig. 1.** Expression of *purF-lacZ and purE-lacZ* reporters in *E. coli* DH5α.

Individual colonies of *E. coli* DH5α cells containing each of the pEKpllacZ vector, *purE-lacZ*, and *purF-lacZ*, were streaked onto an LB agar plate containing 100 μg/ml of kanamycin, 100 μg/ml of ampicillin, and 2 μg/ml of X-gal. The plate was incubated for 16 h at 37°C and each section of the plate was photographed.

**Screening of *C. ammoniagenes* Genes Involved in Regulation of *purE-lacZ* and *purF-lacZ* Reporter Expression Using *E. coli* as a Host**

Because *purE-lacZ* and *purF-lacZ* reporters function well within *E. coli*, the *E. coli* strains were used that harbor either the *purE-lacZ* or *purF-lacZ* reporter for screening of a *C. ammoniagenes* gDNA library. It was expected that *C. ammoniagenes* gDNA clones repressing *purE-lacZ* or *purF-lacZ* would be obtained. The *E. coli* cells retaining the *purE-lacZ* or *purF-lacZ* plasmid were transformed with the *C. ammoniagenes* gDNA library. The *E. coli* cells containing library DNA together with the *purE-lacZ* or *purF-lacZ* reporter were selected on an LB plate containing kanamycin and ampicillin together with X-gal. Blue colonies were selected as positive cells potentially retaining a clone retaining either the *purE-lacZ* or *purF-lacZ* reporter with pUC118 that is involved in regulation of *pur-lacZ* reporter gene expression.

White colonies were re-streaked onto fresh plates to confirm the functionality of screened clones. Colonies with significant blue color were selected as positive cells retaining a gene for the putative purine gene regulator. Examples of the cell color for representative *E. coli* cells repressing *purE-lacZ* or *purF-lacZ* by the potential purine regulator are shown in Fig. 2. Plasmids containing a gene for the potential regulatory protein were isolated from the positive *E. coli* cells and analyzed by restriction enzyme digestion to determine the size of the insert DNAs and to eliminate independently obtained identical clones.

**Identification of *C. ammoniagenes* Genes Involved in Regulation and *purF-lacZ* Expression Within *E. coli***

The nucleotide sequences of the insert DNA fragments that showed strong *purE-lacZ* and *purF-lacZ* repression were determined by dideoxy nucleotide sequencing. The resulting nucleotide sequences were translated to amino acid sequences and compared to the NCBI database using

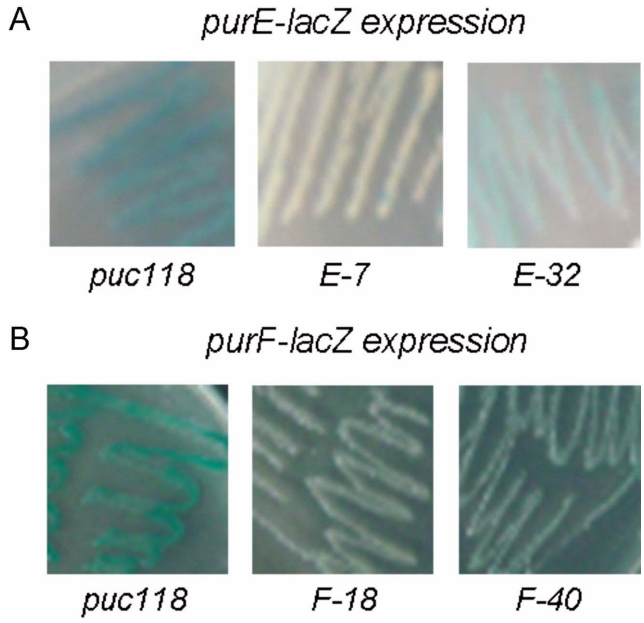


**Fig. 2.** Representative examples of *purF-lacZ* from *C. ammoniagenes* gDNA library clones in *E. coli*.
(A) *E. coli* DH5α transformants contain *purE-lacZ* together with pUC118, "E-7", or "E-32". (B) *E. coli* DH5α transformants contain *purF-lacZ* together with pUC118, "F-18", or "F-40". Plates streaked with individual transformants were incubated for 16 h at 37°C, then photographed.

the BLAST program to search homologous proteins (Table 1 and Table 2). The following clones were identified: clone "E-7" encoding a protein homologous to *E. coli* helicase; clone "E-22" encoding a protein homologous to the RNA polymerase alpha subunit of *C. glutamicum*; and clone "E-24" encoding a protein homologous to the Biotin operon repressor. Clones "E-26" and "E-33" possibly encoded proteins homologous to the genes involved in purine nucleotide biosynthesis. These genes include 5-phosphoribosyl-5-amino-4-imidazole carboxylase (E-26) and the phosphoribosylamino-imidazole carboxylase ATPase subunit (AIRC) in *C. ammoniagenes* (E-33) (Table 1).

Clone number "A-32" encoded a protein homologous to *B. subtilis* IMP cyclohydrolase for *purH* [8, 31]. The "F-18" and "F40" clones, which repress the *purF-lacZ* reporter, retained homologous amino acid sequences with either the *E. coli* MetE and MetF repressors [2] or a hypothetical transcriptional regulator of *Bacillus halodurans* (Table 2).

**Regulation of *purE-lacZ* and *purF-lacZ* Reporters by *C. ammoniagenes* gDNA Clones in *E. coli***

The dependence of purines in the regulation of *purE-lacZ* and *purF-lacZ* was characterized by the candidate *C. ammoniagenes* gDNA clones involved in *pur* reporter repression. The 3 candidate clones ("E-7", "E-32", and "E-33") strongly repressed *purE-lacZ* expression and 2 candidate clones ("F-18" and "F-18") strongly repressed *purF-lacZ* expression. These clones were analyzed by transformation into *E. coli* DH5α followed by measurement of liquid β-galactosidase activity under both purine repressing and non-repressing conditions. In this assay, *E. coli* cells containing pUC118 together with either *purE-lacZ* or *purF-lacZ* were used as negative controls.

*E. coli* strains containing *purE-lacZ*/E-7, *purF-lacZ*/E-32, and *purE-lacZ*/E-33 reduced expression of β-galactosidase activity compared to cells containing *purE-lacZ* together with the pUC118 control vector (Table 3). The bacteria cells containing the *purE-lacZ* reporter together with non-repressible pUC118 were repressed 3.0- and 3.6-fold, respectively, by the addition of 100 μg/ml of adenine and 100 μg/ml of guanine. By the addition of both 100 μg/ml of adenine and 100 μg/ml of guanine, *purE-lacZ*/puC118 expression was repressed by 3.2-fold. Therefore, a synergistic increase in repression was not observed in the *purE-lacZ* promoter. Repression by 100 μg/ml of adenine and 100 μg/ml of guanine was less significant (2.0-fold for adenine and 2.6-fold for guanine) in cells containing the "E-32" clone together with *purF-lacZ* (Table 3). Regulation of the *purE-lacZ* reporter by adenine and guanine was abolished in cells harboring "E-7" and "E-33".

*E. coli* cells containing *purF-lacZ*/F-18 and *purF-lacZ*/F-40 repressed expression of β-galactosidase activity from 96.9 to 4.9 and 96.6 to 25.4, respectively, compared to cells containing *purF-lacZ*/pUC118 (Table 4). *E. coli* cells containing the *purF-lacZ*/pUC118 reporter reduced expression 3.1- and 5.7-fold by the addition of 100 μg/ml of adenine and 100 μg/ml of guanine, respectively. A

**Table 1.** Homology search for *C. ammoniagenes* gDNA clones repressing *purE-lacZ*.

| Clone number[a] | Species (strain) | Database search result | Identity (%) |
|---|---|---|---|
| E-7 | *E. coli* | Helicase, ATP-dependent | 13/33 (39) |
| E-22 | *C. glutamicum* (ATCC13032) | DNA-directed RNA polymerase alpha subunit | 211/250 (84) |
| E-24 | *E. coli* | Biotin operon repressor and biotin-[acetylCoA carboxylase] holoenzyme synthetase | 15/38 (39) |
| E-26 | *C. ammoniagenes* | 5'-phosphoribosyl-5-amino-4-imidazole carboxylase | 54/76 (71) |
| E-32 | *B. subtilis* | IMP cyclohydrolase | 19/61 (31) |
| E-33 | *C. ammoniagenes* | PhosphoribosylaminoimidazoleCarboxylaseATPase subunit | 15/22 (68) |

[a] *C. ammoniagenes* gDNA clones repressed expression of the *purE-lacZ* reporter genes.

**Table 2.** Homology search for *C. ammoniagenes* gDNA clones repressing *purF-lacZ.*

| Clone number[a] | Species | Database search result | Identity (%) |
|---|---|---|---|
| F-18 | *E. coli* | Repressor of *metE* and *metF*, B12-dependent homocysteine-N5-methyltetrahydrofolate transmethylase | 12/28 (42) |
| F-40 | *B. halodurans* | Transcriptional regulator (NP_241773) | 68/174 (39) |

[a]*C. ammoniagenes* gDNA clones repressed expression of the *purF-lacZ* reporter genes.

4.8-fold decrease in expression of *purF-lacZ* was observed after the addition of both 100 μg/ml of adenine and 100 μg/ml of guanine (Table 4). In cells harboring the "F-18" clone (*purF-lacZ*/F-18), repression of *purF-lacZ* gene expression by adenine and guanine was reduced from 3.1- to 2.6-fold and from 5.7- to 2.0-fold, respectively, compared to the *purF-lacZ*/pUC118 cells. The repression of *purF-lacZ* gene expression was nearly abolished by the addition of adenine (from 3.1- to 1.2-fold) and reduced by the addition of guanine (from 5.7- to 3.6-fold) in cells harboring the "F-40" clone (*purF-lacZ*/F-40) (Table 4).

## DISCUSSION

Ten enzymatic steps are required for the *de novo* synthesis of IMP from PRPP in bacteria [reviewed in 37–39]. IMP is further converted to AMP and GMP after two additional enzymatic steps [37–39]. In *E. coli*, the common regulatory protein PurR is involved in the global regulation of genes for the *de novo* synthesis of purine [4–6, 37] and other nucleotides [1, 3, 33, 36, 37]. PurR binds to a 16-bp palindromic sequence that overlaps the -35 promoter region of the *pur* genes and inhibits transcription [4–6, 14, 37]. Hypoxanthine and guanine serve as two co-repressors for *pur* gene regulation by PurR [4, 29]. In *B. subtilis,* the expression of the *pur* operon is also subject to initiation of transcription by *Bacillus* PurR [9, 34, 35] and by a transcription termination mechanism [20]. *Bacillus* PurR also binds to its operator site followed by inhibition of transcription [9, 30, 32]. A second protein encoded by *yabJ*, which is located in an operon of *purR*, has been suggested for acting together with PurR [30].

To isolate genes for regulatory factors associated with *de novo* purine nucleotide biosynthesis in *C. ammoniagenes*, a genetic screening method using *E. coli* as a host was developed. *C. ammoniagenes purE-lacZ* and *purF-lacZ* reporter genes were expressed in *E. coli*, indicating that the two *C. ammoniagenes* genes for purine nucleotide biosynthesis were transcribed using *E. coli* transcription machinery. The promoters of *Corynebacteria* retained the putative -35 and -10 sites often expressed in *E. coli* [22–25]. *C. ammoniagenes* gDNA library clones repressing either the *purF-lacZ* or *purE-lacZ* reporter genes were screened based on a phenotype reduced to a blue color on plates containing X-gal. Putative amino acid sequence analysis of the clones involved in negative regulation of the purine gene reporters allowed identification of homologous proteins.

Genes repressing *purE-lacZ* include genes homologous to the potential regulatory protein of the *E. coli* biotin repressor [2], and the genes for purine nucleotide biosynthesis, including *B. subtilis* IMP cyclohydrolase [9, 28, 38], and the *C. ammoniagenes* 5'-phosphoribosyl-5-aminoimidazole (AIR) carboxylase ATPase subunit [18]. It is unknown how the metabolic enzymes required for the *de novo* synthesis of purine nucleotides are involved in the regulation of *purE-lacZ* reporter gene expression. Because *purH* encodes AIR carboxylase, it is possible that AIR carboxylase is involved in the regulation of gene expression, although the physiological role of such autoregulation is unknown. It is also unknown how and why these genes are regulated by their own products. However, such a process might be required for efficient and tight regulation of the production of important purine nucleotides at the level of gene expression and enzymatic level regulation [12, 21].

**Table 3.** Regulation of *purE-lacZ* by purine in *E. coli* containing *C. ammoniagenes* gDNA clones.

| Strain \ Medium | β-galactosidase activity[a] | | | | Regulation fold[b] | | |
|---|---|---|---|---|---|---|---|
| | Minimal medium (MM) | MM+Ade | MM+Gua | MM+Ade+Gua | Ade | Gua | Ade+Gua |
| *purE-lacZ*/pUC118 | 192.8±2.2 | 64.7±0.8 | 53.5±0.9 | 61.2±0.7 | 3.0 | 3.6 | 3.2 |
| *purE-lacZ*/E-7 | 42.1±0.2 | 33.7±0.4 | 37.7±0.2 | 26.2±1.0 | 1.2 | 1.1 | 1.6 |
| *purE-lacZ*/*E-32* | 97.6±0.1 | 48.3±0.8 | 38.0±5.4 | 36.1±0.5 | 2.0 | 2.6 | 2.7 |
| purE-lacZ/E-33 | 38.2±0.3 | 30.1±0.4 | 30.8±0.4 | 43.6±0.4 | 1.3 | 1.2 | −1.14 |

[a]Bacteria were grown in minimal medium supplemented with 100 μg/ml of adenine (Ade) and/or guanine (Gua). The activity in Miller units is the average±SD of 3 independent experiments.
[b]Each value was estimated by dividing the β-galactosidase activity of cells grown in minimal medium by the activity of cells grown in a purine rich medium.

**Table 4.** Regulation of *purF-lacZ* by purine in *E. coli* containing *C. ammoniagenes* gDNA clones.

| Strain \ Medium | β-galactosidase activity[a] | | | | Regulation fold[b] | | |
|---|---|---|---|---|---|---|---|
| | Minimal medium (MM) | MM+Ade | MM+Gua | MM+Ade+Gua | Ade | Gua | Ade+Gua |
| *purF-lacZ*/ pUC118 | 96.9±2.3 | 31.3±2.0 | 17.1±1.2 | 20.0±3.5 | 3.1 | 5.7 | 4.8 |
| *purF-lacZ*/A-18 | 4.9±2.3 | 1.9±5.0 | 2.5±0.7 | 1.6±2.7 | 2.6 | 2.0 | 3.1 |
| *purF-lacZ*/A-40 | 25.4±2.4 | 22.7±0.5 | 7.1±0.9 | 10.7±2.7 | 1.2 | 3.6 | 2.4 |

[a]DH5α *E. coli* cells containing *purF-lacZ* together with a vector or a *C. ammoniagenes* gDNA library clone were grown in minimal medium (MM) supplemented with 100 μg/ml of adenine and/or guanine. The β-galactosidase activity was measured as described in Materials and Methods. The activity in Miller units is the average±SD of 3 independent experiments.
[b]Each value was estimated by dividing the β-galactosidase activity of cells grown in minimal medium by the activity of cells grown in a purine rich medium.

Autoregulation of *purR* transcription by PurR has been identified in *E. coli* [15, 29]. One of the clones that repressed *purE-lacZ* shows a sequence homology with the DNA-directed RNA polymerase alpha subunit of *C. glutamicum*. By searching *C. ammoniagenes* gDNA clones repressing *purF-lacZ,* two clones were obtained that were homologous to the *E. coli metE* and *metF* repressors [2] and a hypothetical *Bacillus halodurans* transcriptional regulator. These genes are potential transcriptional regulators of the *purF* promoter. The expressions of *purE-lacZ* and *purF-lacZ* were lowered by adenine and guanine, indicating that the *C. ammoniagenes purE* and *purF* genes are subject to purine regulation, even in *E. coli*. The reduction of purine-dependent regulation of *purE-lacZ* or *purF-lacZ* in cells harboring the regulatory gene indicates that overexpression of the potential regulators and pre-reduced gene expression can be regulated by adenine and guanine. The regulation of *Corynebacteria* purine genes in *E. coli* by purine further indicates that the gene regulation mechanism of purine nucleotide biosynthesis is similar in these bacteria.

In this study, we have developed a *purE-lacZ* and *purF-lacZ* reporter system that can be used for screening genes involved in the regulation of purine nucleotides. We have also isolated *C. ammoniagenes* genes involved in the regulation of purine nucleotide biosynthesis. Further characterization of the regulatory genes for purine *de novo* biosynthesis is underway. Isolation of the potential genes regulating the *de novo* synthesis of purine nucleotides is potentially useful for production of industrial strains of *C. ammoniagenes* that produce high levels of IMP and GMP.

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