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Enzymatic synthesis of nucleosides by nucleoside phosphorylase co-expressed in *Escherichia coli*

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Abstract: Nucleoside phosphorylase is an important enzyme involved in the biosynthesis of nucleosides. In this study, purine nucleoside phosphorylase and pyrimidine nucleoside phosphorylase were co-expressed in *Escherichia coli* and the intact cells were used as a catalyst for the biosynthesis of nucleosides. For protein induction, lactose was used in place of isopropyl β-D-1-thiogalactopyranoside (IPTG). When the concentration of lactose was above 0.5 mmol/L, the ability to induce protein expression was similar to that of IPTG. We determined that the reaction conditions of four bacterial strains co-expressing these genes (TUD, TAD, DUD, and DAD) were similar for the biosyntheses of 2,6-diaminopurine nucleoside and 2,6-diaminopurine deoxynucleoside. When the substrate concentration was 30 mmol/L and 0.5% of the recombinant bacterial cell volume was used as the catalyst (pH 7.5), a greater than 90% conversion yield was reached after a 2-h incubation at 50 °C. In addition, several other nucleosides and nucleoside derivatives were efficiently synthesized using bacterial strains co-expressing these recombinant enzymes.

Key words: Nucleoside phosphorylase, Lactose, Enzymatic synthesis
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1 Introduction

Nucleosides and nucleoside derivatives are important components of several antiviral and anticancer medications (de Clercq, 2001; Galmarini *et al*., 2002). Several nucleoside drugs, such as zidovudine, ribavirin, lamivudine, and capecitabine, are widely used in clinical practice. Historically, nucleosides and nucleoside derivatives have been commonly synthesized through chemical methods; however, this approach has resulted in serious pollution to the environment due to the large amount of toxic and hazardous reagents used in the production process.

Previous studies have reported that nucleosides and nucleoside derivatives, such as 5-methyluridine (Ishii *et al*., 1989), 5-fluorouridine (Hori *et al*., 1992), 2′-deoxyadenosine (Yokozeki and Tsuji, 2000), and adenine arabinoside (Wei *et al*., 2008), can be successfully synthesized enzymatically using wild-type nucleoside phosphorylase (NPase). However, these reactions required a large concentration of bacterial cells as the source of the enzyme, thus limiting the application of this approach.

Recently, NPase genes from a number of different microorganisms have been identified, cloned, and expressed (Takehara *et al*., 1995; Okuyama *et al*., 1996; Hamamoto *et al*., 1997a; 1997b; Lee *et al*., 2001; Esipov *et al*., 2002). These studies have focused on the cloning and expression of a single NPase gene in order to assess enzymatic activity, and few have addressed the role of these NPase genes in the biosynthesis of nucleosides.

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The majority of enzymatic reactions used for the synthesis of nucleosides have focused on the conversion between purine and pyrimidine nucleosides. In cases where a recombinant bacterium expressing a single plasmid was used as the catalyst, the culture of two strains was required in order to obtain the co-expression of purine nucleoside phosphorylase (PNPase)/uridine phosphorylase (UPase) or PNPase/thymidine phosphorylase (TPase) enzymes. Therefore, this approach had limitations in both the laboratory and industrial production of nucleosides.

In order to achieve a conversion between purine and pyrimidine nucleosides catalyzed by a single recombinant bacterium, we have developed strains of bacteria that express two genes on two individual plasmids (DUD and DAD) or strains that express a single plasmid where the two genes are expressed in tandem (TAD and TUD). Four types of recombinant bacteria were able to express two pairs of NPase recombinant proteins using this method. Using these strains as catalysts, we have successfully synthesized 2,6-diaminopurine nucleoside (DAPR), or 2,6-diaminopurine deoxynucleoside (DAPdR) from 2,6-diaminopurine (DAP) and uridine or thymidine (Fig. 1). In addition, we have successfully synthesized several additional nucleosides from these four cell lines using the optimal reaction conditions for DAPR or DAPdR.

2 Materials and methods

2.1 Chemicals

The nucleosides and bases were kindly supplied by Shanghai Biocaxis Chemicals Co., Ltd., including DAP, hypoxanthine, thymine, uracil, 5-fluorouracil, azauracil, 5-fluorocytosine, guanine, adenine, guanosine, adenosine, thymidine, uridine, 5-methyluridine, inosine, 1H-1,2,4-triazole-3-formamide (TCA), ribavirin, 2′-deoxyribavirin, uracil arabinoside, etc.

Xanthine oxidase and a standard substrate were purchased from Sigma (USA). Tryptone and yeast extract were purchased from Oxoid (UK). Other reagents and solvents were of commercial quality and were not further purified before use.

2.2 Plasmids and strains

The expression plasmids and the strains used in this study are listed in Table 1. *Taq* DNA polymerase, T4 ligase, and all restriction enzymes used for DNA

Table 1 Plasmids and *Escherichia coli* strains

Plasmid/ strain	Description
Plasmid	
pMD-18T	Cloning vector, pUC18 derivative, Amp^r
pET-11a	pBR322-origin vector, T7 promotor, Amp^r
pET-30a	pBR322-origin vector, T7 promotor, Kan^r
p11D	pET-11a carrying *deoD*, Amp^r
p30U	pET-30a carrying *udp*, Kan^r
p30A	pET-30a carrying *deoA*, Kan^r
p30D	pET-30a carrying *deoD*, Kan^r
pAD	pET-30a carrying *deoA* and *deoD*, Kan^r
pUD	pET-30a carrying *udp* and *deoD*, Kan^r
Strain	
JM109	*F′ traD36 proA*$^+$*B*$^+$ *lacI*^{q} *Δ(lacZ)M15/ Δ(lac-proAB) glnV44 e14*$^-$ *gyrA96 recA1 relA1 endA1 thi hsdR17*
BL21(DE3)	F-*ompT hsdS*B ($r_B^- m_B^-$) *gal dcm* (DE3)
DAD	BL21(DE3) (p30A, p11D)
DUD	BL21(DE3) (p30U, p11D)
TAD	BL21(DE3) (pAD)
TUD	BL21(DE3) (pUD)

Amp^r: apramycin resistance; Kan^r: kanamycin resistance. *deoA* encodes TPase; *deoD* encodes PNPase; *udp* encodes UPase

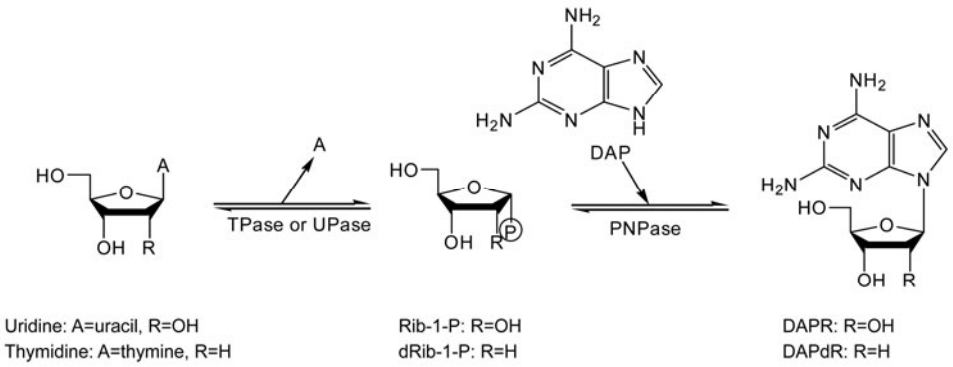

Fig. 1 Proposed mechanism for the production of DAPR or DAPdR

cloning were purchased from TaKaRa (Japan), while the GeneClean kit for DNA purification was purchased from Generay Biotech (Shanghai, China).

2.3 Creation of bacterial strains co-expressing two plasmids

Three NPase genes were amplified from an *E. coli* K-12 strain by the polymerase chain reaction (PCR). All PCR primers used in the reactions are listed in Table 2. The PCR procedure was as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 92 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 90 s. A final elongation cycle was performed for 10 min at 72 °C. The PCR products were resolved on a 0.9% (w/v) agarose gel. The target strand was recovered, purified, and inserted into the pMD18-T vector. DNA sequencing was performed by Invitrogen (Shanghai, China). Assembly and analysis of DNA sequences were performed on Lasergene (Version 7.1.0, DNAStar Inc., Madison, WI). The basis local alignment search tool (BLAST) from the National Center for Biotechnology Information BLAST website was used for database searches. Once the gene sequences were confirmed, the *udp* and *deoA* genes were cloned separately into the kanamycin resistant expression vector pET-30a to create recombinant plasmids pET-30a-*udp* (p30U) and pET-30a-*deoA* (p30A), respectively. Gene *deoD* was cloned into the multi-cloning site of the ampicillin resistant expression vector pET-11a to construct the recombinant plasmid pET-11a-*deoD* (p11D). Both the p11D and p30U plasmids were transformed into *E. coli* BL21(DE3) to create a strain expressing both PNPase and UPase simultaneously (DUD). Similarly, the p11D and p30A plasmids were transformed into *E. coli* BL21(DE3) to create a strain expressing both PNPase and TPase simultaneously (DAD).

2.4 Creation of bacterial strains containing a single plasmid with two genes expressed in tandem

In order to construct plasmids that expressed *deoD* in tandem with *deoA* or *udp*, the *deoD* gene was first amplified with *Sac*I and *Hin*dIII restriction enzyme sites flanking the 5′ and 3′ ends, respectively. This gene fragment was subsequently inserted downstream of the *deoA* and *udp* genes located in plasmids p30A and p30U, respectively. In addition, a ribosome binding site sequence (rbs; parentheses in Table 2) was added to the upstream sequence of the 5′ primer so that each NPase gene had an individual rbs, in order to ensure transcription. The PCR procedure used was equivalent to that used for the construction of the two plasmid systems. The PCR-amplified *deoD* gene was resolved on a 0.9% agarose gel, purified, and digested with restriction enzymes *Sal*I and *Hin*dIII. The digested product was then inserted downstream of the *udp* and *deoA* genes in the p30U and p30D vectors, respectively, to obtain two plasmids expressing these genes in tandem: pET-30a-*udp*-*deoD* (pUD) and pET-30a-*deoA*-*deoD* (pAD) (Fig. 2). Each plasmid was then transformed into *E. coli* BL21(DE3) to create the recombinant strains TUD and TAD, respectively.

2.5 Preparation of intact cells

The Luria-Bertani (LB) culture medium contained 10 g of peptone, 5 g of yeast extract, and 5 g of NaCl in a final volume of 1 L of distilled water. The medium was adjusted to pH 7.0 with 2 mol/L NaOH.

A total of 30 ml of the LB medium was placed into 250 ml flasks and inoculated with individual colonies from strains DUD, TUD, DAD, and TAD that had been subcultured on LB agar plates. The DUD and DAD agar plates contained 50 μg/ml of kanamycin sulfate and 50 μg/ml of sodium carbenicillin, while the

Table 2 Primers used for the construction of co-expressing strains

Primer	Sequences 5′→3′, restriction enzyme site*	GenBank accession No.
udp(+)	GGGAATTCCATATGTCCAAGTCTGATG, *Nde*I	CP 000948
udp(−)	GCGGATCCTTACAGCAGACGACGCGCC, *Bam*HI	
deoA(+)	GGGAATTCCATATGTTTCTCGCACAAG, *Nde*I	NC 000913
deoA(−)	GCGGATCCTTATTCGCTGATACGGCGATAG, *Bam*HI	
deoD(+)	GGTACCCATATGGCTACCCCACACATTAATGC, *Nde*I	NC 000913
deoD(−)	GCGGATCCTTACTCTTTATCGCCCAGCAGAAC, *Bam*HI	
deoD(+)	GGAGCTC(GAAGGAG)ATGGCTACCCCAC, *Sac*I	NC 000913
deoD(−)	TTAAGCTTTTACTCTTTATCGCCCAGC, *Hin*dIII	

* Underlined. rbs: (GAAGGAG)

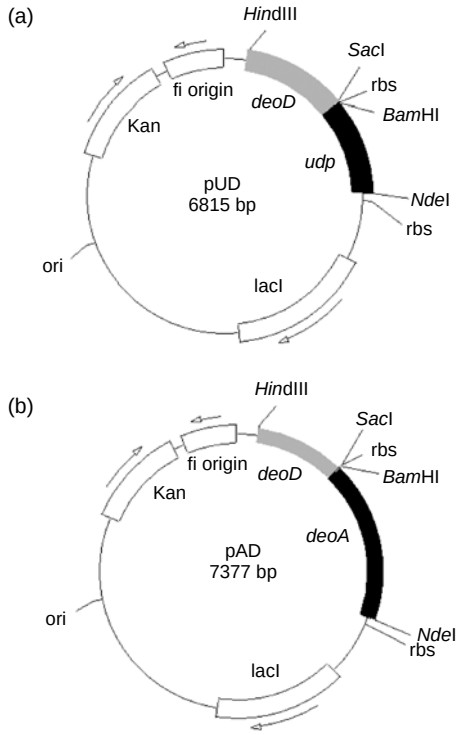

Fig. 2 Gene maps of recombinant plasmids of pUD (a) and pAD (b)

TUD and TAD plates contained 50 μg/ml of kanamycin only. After aerobic cultivation at 37 °C for 15 h, 1.0 ml of the cultured broth was transferred to 30 ml of the same medium without antibiotic and placed into a 500-ml flask. These cultures were incubated under aerobic conditions at 37 °C for 3–4 h. When the optical density at 660 nm (OD_{660}) reached 0.6 (in approximately 3–4 h), 0.5 mmol/L lactose or isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the broth. The strains were then cultured at 37 °C for an additional 6 h. After the incubation, the cells were harvested by centrifugation (10000×*g* for 15 min) and washed twice with 100 mmol/L Tris-HCl buffer (pH 7.0). The cells were then stored at −20 °C for use as intact cells in the reactions.

2.6 Enzyme assays

UPase activity was assayed according to the method described by Saunders *et al*. (1969). Briefly, an assay solution containing 0.5% (w/v) of prepared, intact recombinant bacterial cells, 10 mmol/L uridine, and 100 mmol/L potassium phosphate buffer (pH 7.0) was incubated in a total volume of 2 ml at 50 °C for 20 min. The reaction was terminated by adding 1 mol/L of ice-cold NaOH (a total of 2 ml). The reaction mixture was centrifuged and the supernatant was diluted 50-fold with NaOH (pH 12). The TPase assay was performed in a similar manner as the UPase assay, with the exception that 10 mmol/L uridine was replaced by 10 mmol/L thymidine and the incubation time was decreased to 10 min.

The PNPase assay was performed using the method described by Kalckar (1947) and was the same as that for UPase assay, with the exception that 10 mmol/L uridine was replaced by 10 mmol/L inosine and the total incubation time was 10 min. The reaction mixture was centrifuged and the supernatant was diluted 50-fold with 100 mmol/L potassium phosphate buffer (pH 7.5). Xanthine oxidase was added to the diluted supernatant, as previously described by Kalckar and Klenow (1948), and incubated for 1 h at room temperature.

The activities of all enzymes were determined spectrophotometrically by measuring the absorption at 290 nm relative to a blank control. The blank solution was the same as the assay solution except that cells were added after the addition of NaOH. The specific activities of each enzyme were expressed as the change of 0.01 in the absorption of the reaction mixture at 290 nm per concentration of intact recombinant bacterial cells.

2.7 Standard reaction

The standard reaction mixture was composed of 30 mmol/L nucleoside and base as well as 25 mg of intact recombinant bacterial cells (0.5%, w/v) in a total volume of 5 ml of 50 mmol/L potassium phosphate buffer (pH 7.0). The reaction was carried out at 50 °C with shaking and was terminated by boiling the samples.

2.8 Reaction product assay

Nucleosides and bases were analyzed at room temperature (25 °C) by high-performance liquid chromatography (HPLC) (Agilent 1200, USA) with ultraviolet (UV) detection at 254 nm using a Hypersil ODS-2 5 μm column (4.6 mm×250 mm) with a solvent containing 5% (v/v) acetonitrile and 95% (v/v) water and a flow rate of 1.0 ml/min.

3 Results

3.1 Co-expression of NPase genes in recombinant bacterial strains

The NPase genes that were co-expressed on individual plasmids were markedly over-expressed in the recombinant *E. coli* BL21(DE3) strain after induction by IPTG (Fig. 3). Monomeric PNPase (approximately 26 kDa) and TPase (approximately 43 kDa) were detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Lane 1 in Fig. 3a and Lane 3 in Fig. 3b, respectively). In the strain co-expressing PNPase and UPase, the monomers of these proteins could not be separated visually by SDS-PAGE (Lane 2 of Fig. 3a and Lane 1 of Fig. 3b, respectively), since they both had a similar molecular weight (PNPase approximately 26 kDa; UPase approximately 27 kDa). All four strains expressing the two plasmids together or expressing the individual plasmid, where the genes were expressed in tandem, produced some inclusion bodies in the preparations (Lanes 3 and 4 of Fig. 3a and Lanes 2 and 4 of Fig. 3b, respectively).

3.2 NPase gene expression induced by lactose and IPTG

IPTG is an analogue of lactose and is commonly used as an inducer of the lactose operon. However, IPTG is very expensive and is not cost-effective for a pilot scale production. Therefore, we used lactose as the inducing agent and achieved comparable yields in protein expression (Table 3).

As described in Table 3, IPTG sufficiently induced recombinant gene expression when used at a concentration between 0.1 and 1.0 mmol/L. Slight differences in expression were obtained when lactose was used. When the lactose concentration was less than 0.5 mmol/L, poor expression was observed in all of the recombinant strains. However, when the lactose concentration was greater than 0.5 mmol/L, the recombinant protein yields were similar to those obtained with IPTG. The lactose was quickly exploited as a carbon source in the bacteria when used at low concentrations. Moreover, an increase in the concentration of lactose did not give better induction results or increase the yield of the target protein, but did increase

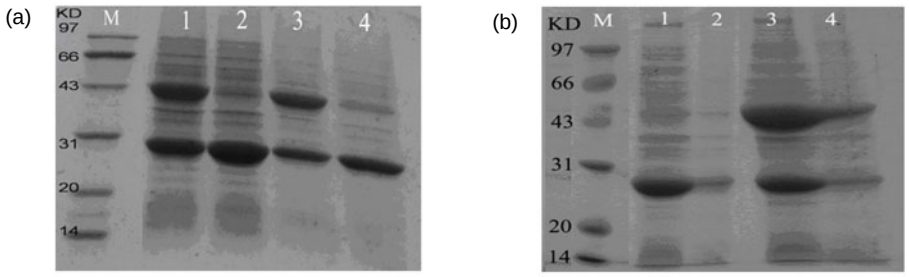

Fig. 3 SDS-PAGE of recombinant strains containing double plasmids (DAD and DUD) (a) and tandem co-expression plasmid (TAD and TUD) (b)

SDS-PAGE was performed in a 12% (w/v) polyacrylamide gel (PAG). (a) M: protein marker; Lanes 1 and 2: total proteins of DAD or DUD induced by IPTG; Lane 3: precipitate after sonication of DAD; Lane 4: precipitate after sonication of DUD; (b) M: protein marker; Lane 1: total proteins of TUD induced by IPTG; Lane 2: precipitate after sonication of TUD; Lane 3: total proteins of TAD induced by IPTG, Lane 4: precipitate after sonication of TAD

Table 3 Effect of lactose and IPTG on enzyme activity

Inducer	*C* (mmol/L)	Enzyme activity (U/mg of recombinant bacterial cells)							
		DUD		DAD		TAD		TUD	
		PNPase	UPase	PNPase	TPase	PNPase	TPase	PNPase	UPase
IPTG	0.1	214	3641	520	5300	282	6637	227	3465
	0.3	209	3756	550	5498	289	6598	214	3562
	0.5	224	3710	536	5521	296	6600	205	3514
	1.0	210	3512	521	5315	279	6614	220	3419
Lactose	0.1	27	291	30	288	25	331	26	318
	0.3	157	2456	488	3785	176	4987	143	2895
	0.5	221	3689	513	5477	279	6632	216	3531
	1.0	207	3635	520	5410	281	6651	229	3499
None-inducer		26	287	29	295	27	299	23	311

the total biomass concentration (data not shown). Since the induction effect of lactose was similar to IPTG, 0.5 mmol/L lactose was used as the inducer for protein expression in subsequent experiments.

3.3 Optimal reaction conditions for DAPR or DAPdR production

The optimal reaction conditions for DAPR or DAPdR production from DAP and uridine or thymidine were investigated using the intact recombinant cells from strains TUD, TAD, DUD, and DAD. The optimal substrate concentration was found to be between 10 and 40 mmol/L (Fig. 4a). TUD was able to catalyze the transformation of 90% of uridine and DAP into DAPR. In addition, TAD catalyzed the conversion of 77% of thymidine and DAP into DAPdR, which were slightly higher than those catalyzed by DUD and DAD (83% and 75%, respectively).

Since the four strains of recombinant bacteria produced a large amount of the enzymes, the reactions only required 0.5% (w/v) of intact, recombinant cells as the NPase source (Fig. 4b). In previous studies, approximately 50 g/L of wild-type intact cells were needed to carry out the reactions, which was an amount much larger than that used in this study. The optimal pH for the biosynthesis reactions was found to be between 6 and 8 (Fig. 4c). When an acidic or alkaline buffer was used, a low yield of DAPR or DAPdR was obtained. Unlike other wild-type bacteria, the optimal temperature for the enzymatic synthesis of nucleosides by these strains was approximately 50 °C (Fig. 4d), which was approximately 10 °C lower than wild-type stains previously used. When the recombinant strains were incubated at 70 °C, a very low yield of DAPR or DAPdR was obtained. In addition, adjusting the phosphate concentration between 10 and 300 mmol/L had little effect on the biosynthesis of DAPR or DAPdR.

3.4 Process of biosynthesis of DAPR or DAPdR

A time course of DAPR or DAPdR production, when 5 mg/ml of intact recombinant cells were used, is shown in Fig. 5. DAPR and DAPdR were efficiently produced and had a yield of 90% and 80%, respectively, when 30 mmol/L DAP and 30 mmol/L

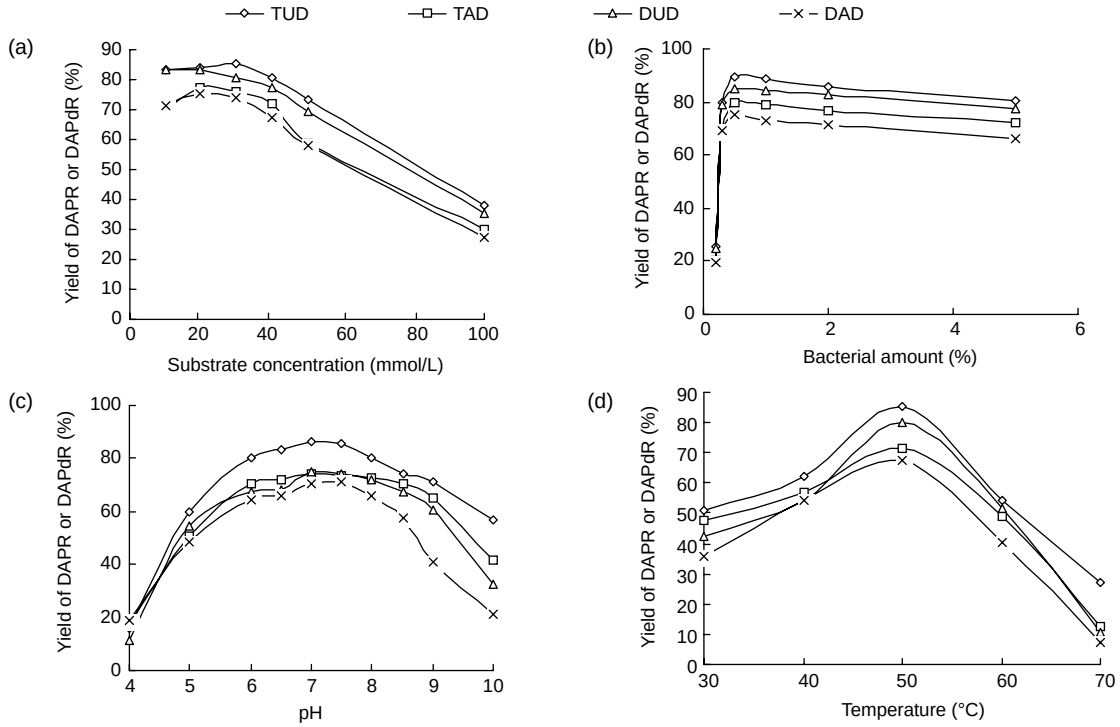

Fig. 4 Effects of substrate concentration (a), amount of wet cells (b), pH (c), and temperature (d) on the production of DAPR or DAPdR by recombinant bacteria

uridine or thymidine were used in a 2-h incubation. Very little difference in DAPR production was observed when TUD and DUD were used as reaction catalysts. Similar results were observed for DAPdR production by TAD and DAD.

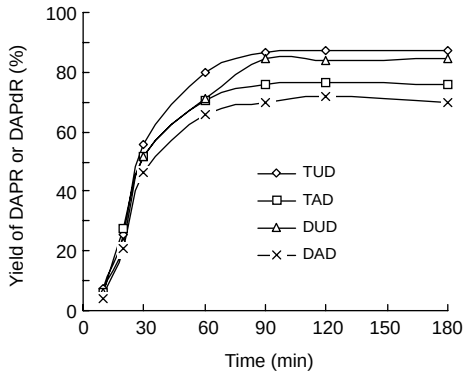

Fig. 5 Time course of DAPR or DAPdR production by intact cells of four kinds of recombinant bacteria
A total of 30 mmol/L DAP and 30 mmol/L uridine or thymidine were used as the substrates and 0.5% wet cells were used as the enzyme source

3.5 Production of other ribonucleosides catalyzed by TUD or DUD

Under the above optimal reaction conditions, other ribonucleosides were biosynthesized by TUD or DUD. The data were shown in Table 4.

Data in Table 4 show that the biosynthesis of nucleosides by both TUD and DUD produced good yields. However, the synthesis of purine nucleosides from pyrimidine nucleosides produced a higher yield than the synthesis of pyrimidine nucleosides from purine nucleosides. Guanosine was a better ribose donor than inosine since guanine had very poor solubility in water. In addition, arabinose nucleosides and cytidine could not be transformed into any other nucleosides by TUD or DUD.

3.6 Production of other deoxynucleosides catalyzed by TAD or DAD

Under the above optimal reaction conditions, other deoxynucleosides were biosynthesized by TAD or DAD. The data were shown in Table 5.

Table 4 Conversion yield of nucleosides catalyzed by recombinant strain TUD or DUD

Ribose donor	Ribose acceptor	Product	Conversion yield (%)	
			TUD	DUD
Inosine	Thymine	5-methyluridine	51.4	50.2
	Uracil	Uridine	53.5	51.3
	5-fluorouracil	5-fluorouridine	48.4	48.7
	Azauracil	Azauridine	45.1	47.8
Guanosine	Thymine	5-methyluridine	65.2	68.4
	Uracil	Uridine	63.2	61.4
	5-fluorouracil	5-fluorouridine	67.4	61.5
	Azauracil	Azauridine	61.8	59.4
	TCA	Ribavirin	72.7	70.5
	5-fluorocytosine	5-fluorocytidine	0	0
	Cytosine	Cytidine	0	0
Uridine	Adenine	Adenosine	80.4	81.7
	Guanine	Guanosine	5.7	4.8
	DAP	DAPR	89.4	84.2
	TCA	Ribavirin	83.5	81.9
Uracil arabinoside	Adenine	Adenine arabinoside	0	0

Table 5 Conversion yield of deoxynucleoside catalyzed by recombinant strain TAD or DAD

Deoxyribose donor	Deoxyribose acceptor	Products	Conversion yield (%)	
			TAD	DAD
Thymidine	Adenine	2′-deoxyadenosine	87.4	85.7
	DAP	DAPdR	84.1	83.2
	Hypoxanthine	2′-deoxyinosine	52.7	51.3
	TCA	2′-deoxyribavirin	79.5	83.4
	Guanine	2′-deoxyguanosine	5.4	6.2

A poor yield was obtained when TAD or DAD catalyzed the synthesis of 2′-deoxyguanosine from guanine and thymidine. This was most likely due to the poor solubility of guanine in water. In contrast, TAD or DAD catalyzed the synthesis of 2′-deoxy-purine nucleosides from thymidine and corresponding bases with a high yield. This particular finding has strong practical implications, since thymidine can be commercially synthesized at a low price. Otherwise, other deoxy-purine nucleosides would not have been able to be easily produced through common chemical methods.

4 Discussion

It has been previously shown that several nucleosides can be synthesized from other nucleosides by nucleoside phosphorylase (Utagawa, 1999). However, in practice, a purine nucleoside such as 2′-deoxyadenosine (Yokozeki and Tsuji, 2000) has been synthesized from a pyrimidine nucleoside, and a pyrimidine nucleoside such as 5-methyluridine (Hori *et al*., 1992) has been synthesized from a purine nucleoside. Therefore, two sets of nucleoside phosphorylases (PNPase and UPase, or PNPase and TPase) should be required for the reaction to proceed. In previous studies (Takehara *et al*., 1995; Okuyama *et al*., 1996; Hamamoto *et al*., 1997a; 1997b; Lee *et al*., 2001; Esipov *et al*., 2002), only a single NPase was used, and therefore the two sets of NPases would be needed for the interconversion between purine and pyrimidine nucleosides.

In this study, four versions of *E. coli* strains were created which contained NPase genes, two containing the co-expressed plasmids pET-11a-*deoD* and pET-30a-*udp* (DUD) or pET-11a-*deoD* and pET-30a-*deoA* (DAD), and two expressing a single plasmid pET-30a-*udp-deoD* (TUD) or pET-30a-*deoA-deoD* (TAD) where the two genes were expressed in tandem. The DUD, TUD, DAD, and TAD recombinant stains were capable of producing large amounts of PNPase/UPase and PNPase/TPase, respectively. Although some inclusion bodies formed during the production of these proteins, the activity of the NPase was not compromised.

We found that lactose, which has no toxicity in humans or animals, was more practical as an inducer of protein expression than IPTG and had a lower cost. In addition, when lactose was used at a concentration greater than 0.5 mmol/L, it showed a similar induction profile as IPTG.

Because PNPase/UPase and PNPase/TPase were highly expressed in the recombinant bacteria, only single strain was used in the biosynthesis of nucleosides, i.e., DAPR and DAPdR (Okuyama *et al*., 2003). The synthesis of DAPR from DAP and uridine was catalyzed by TUD or DUD with a yield of 90% or 83%. The synthesis of DAPdR from DAP and thymidine was catalyzed by TAD or DAD with a yield of 77% or 75%. Uridine and thymidine were used as (deoxy)ribosyl donors since they are pyrimidine nucleosides and are easily separated from purine analogues in the reaction mixture. In addition, these components are commercially produced and readily available.

For the biosyntheses of DAPR and DAPdR, we found that the optimal reaction conditions were to use a buffer at pH 7.0, a final substrate concentration of 30 mmol/L, 0.5% of the intact, recombinant bacterial cells, and an incubation temperature of 50 °C for 2 h. Only a small amount of the intact recombinant bacterial cells were required in the reaction, though the substrate concentration was slightly lower.

We found that several other nucleosides could be synthesized with TAD, TUD, DUD, or DAD, such as 5-methyluridine, 5-fluorouridine, uridine, adenosine, azauridine, ribavirin, 2′-deoxyadenosine, 2′-deoxyinosine, and 2′-deoxyribavirin. The very low yield of the guanine transformation into guanosine or 2′-deoxyguanosine was due to the low solubility of guanine in water. However, guanosine was the best ribose donor (also the reason of poor solubility of guanine in water). In addition, cytosine could not be converted into cytidine by TUD or DUD, and in contrast to previous studies, the arabinose nucleoside could not be synthesized by TUD or DUD.

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