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Bioconversion of pyridoxine to pyridoxamine through pyridoxal using a *Rhodococcus* expression system

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Pyridoxamine, which is a form of vitamin B_6, is a promising candidate for a prophylactic and/or remedy for diabetic complications. Pyridoxamine is chemically synthesized by an oxidative method in manufacturing. However, pyridoxamine production by bioconversion, which is generally preferable for environmental and energetic aspects, has been little investigated. Therefore, I aimed to produce pyridoxamine from pyridoxine, which is a readily and economically available starting material, by bioconversion using a *Rhodococcus* expression system. I found in the bioconversion of pyridoxine to pyridoxal, approximately 450 mM pyridoxal was produced from 500 mM pyridoxine using recombinant *Rhodococcus erythropolis* expressing the pyridoxine 4-oxidase gene derived from *Mesorhizobium loti*. Next, in the bioconversion of pyridoxal to pyridoxamine using recombinant *R. erythropolis* expressing the pyridoxamine-pyruvate aminotransferase gene derived from *M. loti*, the bioconversion rate was approximately 80% under the same conditions as pyridoxal production. Finally, in the bioconversion of pyridoxine to pyridoxamine through pyridoxal using recombinant *R. erythropolis* coexpressing the genes for pyridoxine 4-oxidase and pyridoxamine-pyruvate aminotransferase, the bioconversion rate was approximately 75%. Based on these findings, pyridoxamine production by bioconversion using a *Rhodococcus* expression system may be of interest for future industrial applications.

[**Key words:** Pyridoxamine; Pyridoxine; Coexpression; *Rhodococcus*; Pyridoxine 4-oxidase; Pyridoxamine-pyruvate aminotransferase; Bioconversion]

Pyridoxamine (PM), a type of vitamin B_6, interferes with the formation of advanced lipoxidation end-products (ALEs) and advanced glycation end-products (AGEs) (1,2). ALEs and AGEs are the major pathogenic factors in diabetic complications (3). For this reason, PM is a promising candidate for a prophylactic and/or remedy for diabetic complications.

PM is chemically synthesized using an oxidative method in manufacturing. The oxidative method changes pyridoxine (PN) to pyridoxal (PL), and then PL to PM through pyridoxaloxime, which is reduced with a Pd/C catalyst to PM (4). Bioconversion is generally preferable in the context of environmental and energetic aspects (5). As far as I know, no bioconversion of PM production from PN, which is a readily and economically available starting material, has been reported.

Mesorhizobium loti contains the degradation pathway of vitamin B_6 (6). In this study, two enzymes, pyridoxine 4-oxidase (PNO) and pyridoxamine-pyruvate aminotransferase (PPAT), were derived from *M. loti* and used for bioconversion. PNO, which is encoded by the *pno* gene (also known as *mll6785*), catalyzes the FAD (flavin adenine dinucleotide)-dependent oxidation of PN to PL (7). It is expressed in *Escherichia coli* and recombinant PNO has been purified and characterized (6). However, the expression of *pno* in *E. coli* (6) has the disadvantage that it needs coexpression of the chaperonins, GroEL and GroES. On the other hand, PPAT, which is the pyridoxal 5′-phosphate (PLP)-independent aminotransferase and is encoded by the *ppat* gene (also known as *mlr6806*), catalyzes the transfer of an amino group between PL and L-glutamate to PM and 2-oxoglutarate (8). In contrast to *pno*, *ppat* is expressed in *E. coli* without the chaperonins and the biochemical properties of the protein have been analyzed (8).

Many *Rhodococcus* strains contain a number of diverse enzymes beneficial for manufacturing industries (9–12). In particular, *Rhodococcus erythropolis* tolerates organic solvents (13) and has high intrinsic enzymatic activities including cofactor-regenerating enzymatic activity (14). I have recently described the construction of a *Rhodococcus* expression vector pRET11100 (14,15), which has the strong constitutive *TRR* promoter derived from *Lactobacillus plantarum* (16). The present study investigates the bioconversion of PN to PM through PL (Fig. 1), using a *Rhodococcus* expression system without chaperonins.

MATERIALS AND METHODS

Strains and plasmids Actinomycete strains were obtained from the Institute of Applied Microbiology (Tokyo, Japan), the National Institute of Technology and Evaluation (Tokyo, Japan), and the Japan Collection of Microorganisms (Ibaraki, Japan). *E. coli* BL21(DE3) was obtained from Nippon Gene Co., Ltd. (Tokyo, Japan). These strains were cultured in Luria-Bertani (LB) medium (0.5% Bacto yeast extract, 1% Bacto tryptone, and 1% NaCl) in the presence or absence of the appropriate antibiotics. Ampicillin (100 μg/mL) and kanamycin (50 μg/mL) were used to select transformants in the culture media. The plasmids, pET-mll6785 (*pno* expression

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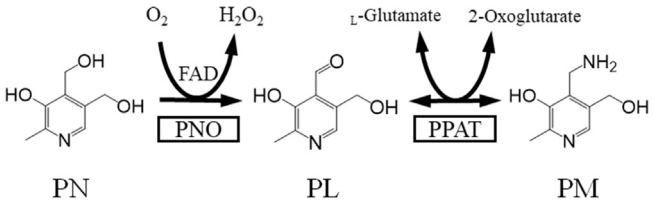

FIG. 1. Scheme for the bioconversion of PN to PM through PL. PL is produced from PN by PNO, while PM is produced from PL by PPAT. FAD is flavin adenine dinucleotide.

vector for *E. coli*), pET6806 (*ppat* expression vector for *E. coli*), and pRET11100 (*Rhodococcus* expression vector), were constructed as previously described (6,8,14).

Enzymes and chemicals All restriction enzymes and DNA modification enzymes were purchased from Toyobo Co., Ltd. (Osaka, Japan) and New England Biolabs, Inc. (Ipswich, MA, USA). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The synthetic oligonucleotides used in this study were purchased from Hokkaido System Science Co., Ltd. (Hokkaido, Japan).

Standard genetic manipulations and sequence analysis Cloning was performed by standard genetic manipulation techniques (17). Actinomycete strains were transformed using the method described by Yamamura (14). *E. coli* transformation was performed using the *E. coli* Transformation Buffer Set (Zymo Research Corp., Irvine, CA, USA). PCR fragments were prepared with KOD -Plus- (Toyobo Co., Ltd.), according to the manufacturer's instructions. Sequence analysis and assembly were performed using GENETYX Ver.12 (Genetyx Corp., Tokyo, Japan).

Molecular mass measurement Crude extracts from recombinant *R. erythropolis* were analyzed on SDS—PAGE (12% gel) by the method of Laemmli (18) and were visualized by staining with Coomassie Brilliant Blue R-250. Crude extracts were prepared by the method described by Yuan et al. (6). Size marker was used the SDS-PAGE Molecular Weight Standards (Broad Range) purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

Construction of *pno* and *ppat* expression vectors for *R. erythropolis* To construct *pno* and *ppat* expression vectors for *R. erythropolis*, the plasmid pRET11100 was used because it has a strong constitutive promoter and broad actinomycetes host range (14). The *pno* gene, which was derived from *M. loti*, was amplified with the primers: PNO3F50Pt: 5′-GATGGCTGCAGGATGACCAGGGCCAAGGTTGAGCACG CACCGAATTG-3' (*Pst* I site is underlined) and mllR(st)Kp: 5′-GGCCGGTAC CTTGTCGACTTAGTACTGTCGGGCGAAAGTCTCGG-3' (*Kpn* I site is underlined), from the plasmid pET-mll6785 using a Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany). The PCR fragment containing *pno* was digested with *Pst* I and *Kpn* I and then ligated into the *Pst* I and *Kpn* I sites of pRET11100 to prepare the plasmid pRET-PNO9, which is the *pno* expression vector for *R. erythropolis*. To prepare the *ppat* expression vector for *R. erythropolis*, the *M. loti*-derived *ppat* gene was amplified with the primers: mlr6806PtF1: 5′-GGAGACTGCAGGATGCGCTATCCCGAACATGCCGATCCGGTCATC-3' (*Pst* I site is underlined) and mlr6806KpR1200St: 5′-TCAGGGGTACCTTAATTAAGCTGAGGGAAA GTTCAGGCGTC-3' (*Kpn* I site is underlined), from the plasmid pET6806 and digested with *Pst* I and *Kpn* I. The DNA fragment containing *ppat* was ligated into the *Pst* I and *Kpn* I sites of pRET11100, and designated pRET-PPAT1.

Construction of the *pno* and *ppat* coexpression vector for *R. erythropolis* The *ppat* gene was amplified with the primers: mlr6806PtF1: 5′-GGAGACTGCAGGATGCGCTATCCCGAACATGCCGATCCGGTCATC-3' (*Pst* I site is underlined) and mlr6806KpR1200His: 5′-GAAAGTGGTACCGTCGGCGTCGAT-TACGGCCAGCGCCGCCTC-3' (*Kpn* I site is underlined), from the plasmid pET6806 and was digested with *Pst* I and *Kpn* I. The DNA fragment containing *ppat* was ligated into the *Pst* I and *Kpn* I sites of pRET11100, which was designated pRET-PPAT2. The *pno* gene was amplified with the primers: PNO9-FKp: 5′-CCGAGGAGGTATAC ATATGACCAGGGCCAAGGTTGAGCACGCACC-3' (*Kpn* I site is underlined) and R1135Bm: 5′-GGTTGGATCCGTCATCACCGAAACGCGCGAGGCAG-3′, from the plasmid pET-PNO9. The *pno*-containing PCR fragment was digested with *Kpn* I and *Xba* I and was ligated into the *Kpn* I and *Xba* I sites of pRET-PPAT2 to prepare the plasmid pRET-PPPN1 which is the *pno* and *ppat* coexpression vector for *R. erythropolis* (Fig. 2).

Screening of *R. erythropolis* strains as a host cell Recombinant *R. erythropolis* cells were inoculated into 5 mL of LB medium with kanamycin (50 μg/mL) and incubated at 25 °C with shaking for 3 days. The cells were harvested and suspended in 1 mL of 300 mM phosphate buffer (pH 7.0) containing 2% glucose and 100 mM PN and incubated at 30 °C with shaking for 10 h. Cell suspensions were then centrifuged at 12,000 × *g* for 5 min at 4 °C, and the resulting supernatants were analyzed by high-performance liquid chromatography (HPLC) as described below. Recombinant *E. coli* cells used in control experiments were incubated as previously described (6) and treated as described above.

Analysis of PN, PL, and PM by HPLC PN, PL, and PM were determined by HPLC on an Inertsil ODS-3 column (ϕ 4.6 × 75 mm; GL Sciences Inc., Tokyo, Japan).

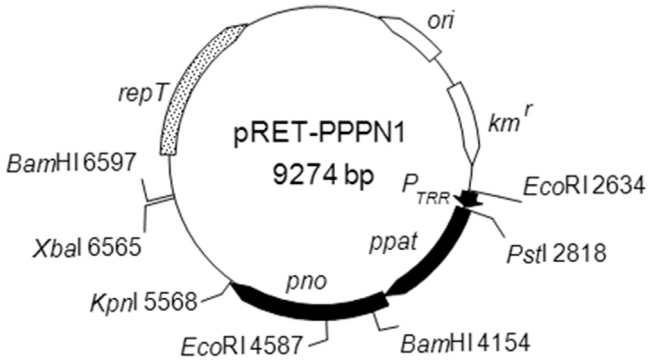

FIG. 2. Structure of pRET-PPPN1 co-expressing *pno* and *ppat*. *km*^{r} is the kanamycin resistance gene. P_{TRR} is the promoter *TRR*. *ppat* and *pno* are the gene encoding pyridoxamine-pyruvate aminotransferase (PPAT) and the gene encoding pyridoxine 4-oxidase (PNO), respectively. *repT* is the gene involved in plasmid replication in *R. erythropolis*. *ori* is ColE1 ori.

The mobile phase was 0.1% trifluoroacetic acid-acetonitrile, the gradient of acetonitrile concentration was from 1 to 80% for 8 min, the flow rate was 1.0 mL/min, and the detection wavelength was at 294 nm.

Cultivation of recombinant *R. erythropolis* by a jar fermentor Recombinant *R. erythropolis* cells were propagated in LB medium as described by Kozono et al. (19). Recombinant *R. erythropolis* cells were inoculated into 5 mL of LB medium with kanamycin and incubated at 25 °C with shaking for 3 days. Five milliliters of seed medium were transferred into 3.6 L of LB medium in a 5 L jar fermentor (LS-5, Sakura Seiki Co., Ltd., Tokyo, Japan) and incubated at 30 °C with 500-rpm agitation, 1-vvm aeration, and a pH maintained at 7.0 ± 0.5 for 3 days.

Reactor bioconversion using the *Rhodococcus* expression system Bioconversion of PN and PL was performed in three ways. First, for the bioconversion of PN to PL, 3.5 L of the culture medium of recombinant *R. erythropolis* was harvested and suspended in 700 mL of tap water containing 500 mM (approximately 10%) PN and 0—2% glucose (standard reaction condition was 2% glucose). The cell suspension was transferred into a 1.8 L reactor (TBR-2-3, Sakura Seiki Co., Ltd.) and incubated at 27.5—40.0 °C with 500-rpm agitation, 0.1—1.5-vvm aeration, and a pH maintained at 5.5—7.5 for 2—6 days (standard reaction condition: 30 °C, 1 vvm, and pH 6.5 for 2 days). Second, for bioconversion of PL to PM, 12 L of the culture medium of recombinant *R. erythropolis* was harvested and suspended in 700 mL of tap water containing 100 mM PL and 400 mM L-glutamate. The cell suspension was transferred into a 1.8 L reactor and incubated at 30 °C with 500-rpm agitation, 1-vvm aeration, and a pH maintained at 6.5 for 2 days. Third, for the bioconversion of PN to PM through PL, 33 L of the culture medium of recombinant *R. erythropolis* was harvested and suspended in 700 mL of tap water containing 200 mM (approximately 4%) PN, 2% glucose, and 800 mM L-glutamate. The cell suspension was transferred into a 1.8 L reactor and incubated at 30 °C with 500-rpm agitation, 1-vvm aeration, and a pH maintained at 6.5 for 2 days.

Nucleotide sequence accession number The nucleotide sequences reported in this paper appear in the DDBJ/GenBank/EMBL nucleotide sequence databases under accession number NC_002678.

RESULTS

Construction of a *pno* expression vector for *R. erythropolis* and screening of *R. erythropolis* strains as a host cell PNO catalyzes the oxidation of PN to PL. The PNO enzyme from *M. loti* is monomeric and has a molecular mass of 56 kDa. It shows a high specificity for PN and no activity toward PM (6). For overexpression of *pno* in *R. erythropolis* cells, *pno* was inserted downstream of the *TRR* promoter of pRET11100. The plasmid pRET-PNO9 was transformed into many *R. erythropolis* strains for screening and identification of a host cell (Fig. 3A).

The strain with the lowest PL production was recombinant *R. erythropolis* JCM6827 (1.1 ± 0.1 mM), while the strain with the highest PL production was recombinant *R. erythropolis* JCM3191 (45.9 ± 4.6 mM). In control experiments using the recombinant *E. coli* BL21(DE3) harboring pET-mll6785, the production of PL was 0.05 ± 0.01 mM. The activity of recombinant *R. erythropolis* JCM3191 was 918 times higher than that of recombinant *E. coli*

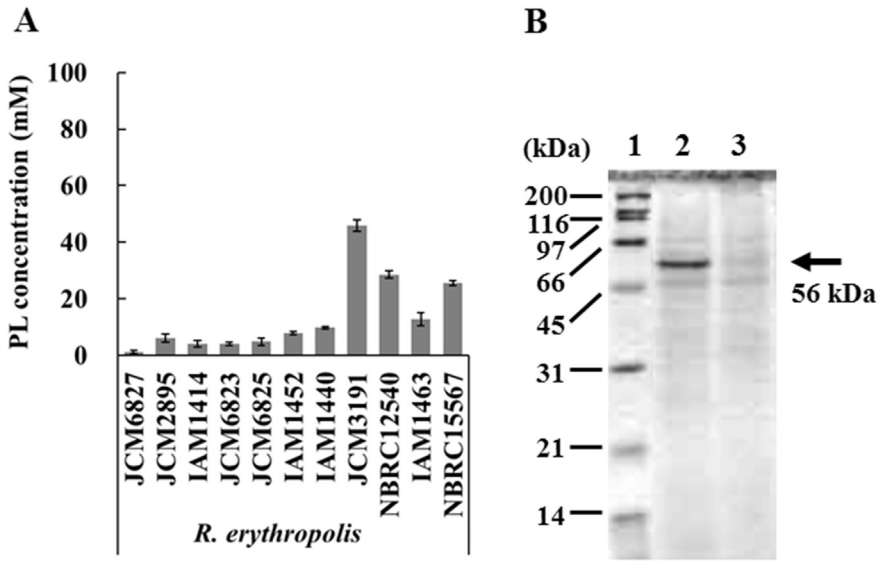

FIG. 3. Screening of *R. erythropolis* strains as a host cell. (A) PL concentrations of recombinant *R. erythropolis* strains harboring the pRET-PNO9 plasmid. Vertical bars indicate standard deviation (SD) from three independent experiments. (B) SDS-PAGE of crude extracts from recombinant *R. erythropolis* cells. Lane 1, standard proteins; lane 2, the crude extract from recombinant *R. erythropolis* JCM3191 harboring the pRET-PNO9 plasmid; lane 3, the crude extract from recombinant *R. erythropolis* JCM3191 harboring the pRET11100 plasmid.

BL21(DE3). The PL production of recombinant *R. erythropolis* JCM3191 with 5 μM FAD was 45.2 ± 3.9 mM and almost the same as that without FAD. All recombinant *R. erythropolis* strains harboring pRET11100 did not produce PL. Regarding PNO, it was overexpressed in *R. erythropolis* JCM3191. The protein had a molecular weight of 56 kDa, which is in agreement with the predicted molecular weight. The overexpressed PNO was detected as a soluble form on SDS-PAGE (Fig. 3B).

Optimization of reaction conditions for bioconversion of PN to PL The bioconversion of PN to PL using recombinant *R. erythropolis* JCM3191 harboring the plasmid pRET-PNO9 is an oxidation reaction requiring oxygen. PL production increased with increasing aeration; an optimal reaction aeration was determined to be >1 vvm (Fig. 4A). The effects of pH were also examined, and it was determined that a pH of 6.5 was optimal (Fig. 4B). Similarly, an optimal reaction temperature of 30.0–32.5 °C was determined based on evaluation of a range of temperatures from 27.5 to 40.0 °C (Fig. 4C). Further, the production of PL increased with increasing glucose as an energy source. The optimal reaction concentration of glucose was found to be >1.0% glucose. PL concentration increased over time by the addition of >1.0% glucose, and was approximately 450 mM in 84 h (Fig. 4D).

Construction of a *ppat* expression vector for *R. erythropolis* and conditional production of PM dependent on PL production PPAT is a pyridoxal 5′-phosphate-independent aminotransferase that catalyzes the transfer of an amino group between PL and L-glutamate to PM and 2-oxoglutarate. For overexpression of *ppat* in *R. erythropolis* cells, *ppat* was inserted downstream of the *TRR* promoter of pRET11100. The plasmid pRET-PPAT1 was transformed into *R. erythropolis* JCM3191. The recombinant *R. erythropolis* JCM3191 strain harboring the pRET-PPAT1 plasmid overexpressed *ppat*. The bioconversion rate of PL to PM using recombinant *R. erythropolis* JCM3191 was approximately 80% (Fig. 5A) using the same conditions as those of PL production (1 vvm, pH 6.5, and 30 °C). Overexpression of PPAT was detected as a soluble form on SDS-PAGE, with a molecular weight of 42 kDa, which is in agreement with the predicted molecular weight (Fig. 5B).

Bioconversion of PN to PM through PL To coexpress *ppat* and *pno* in *R. erythropolis* cells, *ppat* was inserted downstream of the *TRR* promoter of pRET11100, while *pno* was inserted downstream of *ppat*. The obtained plasmid was designated as pRET-PPPN1 (Fig. 2), which was then transformed into *R. erythropolis* JCM3191. The recombinant *R. erythropolis* JCM3191 strain harboring pRET-PPPN1 had bioconversion activity producing PM from PN. The PN concentration decreased over 5 h, while the PL concentration increased and PM production started. After 20 h from the start of the reaction, PN and PL concentrations decreased and PM concentration increased. After 48 h from the start of the reaction, the PM concentration was 145 mM (bioconversion rate of 75%), while PN and PL concentrations were <1 mM. The production of PM from 400 mM PN was approximately 150 mM, which is almost the same as that from 200 mM PN. PM was efficiently produced by bioconversion of PN (Fig. 6).

DISCUSSION

R. erythropolis strains have broad metabolic diversity and an array of unique enzymatic capabilities that are beneficial for several manufacturing industries (10,20). Several scientists developed many genetic tools to analyze *Rhodococcus* (21) including gene disruption systems (11,22,23); however, little study has been done to actually screen *R. erythropolis* strains as a host cell. Screening *R. erythropolis* strains, I demonstrated that *R. erythropolis* JCM3191 was a good host for PL production (Fig. 3A), and that the activities of PL production were significantly different between *R. erythropolis* strains. In fact, the activity of recombinant *R. erythropolis* JCM3191 was 41 times higher than that of recombinant *R. erythropolis* JCM6827. Moreover, the activity of recombinant *R. erythropolis* JCM3191 was 918 times higher than that of recombinant *E. coli* BL21(DE3). The PL production of recombinant *R. erythropolis* JCM3191 was almost the same as that without FAD. *R. erythropolis* JCM3191 may have sufficient amounts of FAD for PNO. Although these differences of activities may be attributed to the broad metabolic diversities of *R. erythropolis* strains, I was not able to obtain data on differences of intrinsic enzyme activities (e.g., catalase activity). Further, *R. erythropolis* may lack vitamin B_6

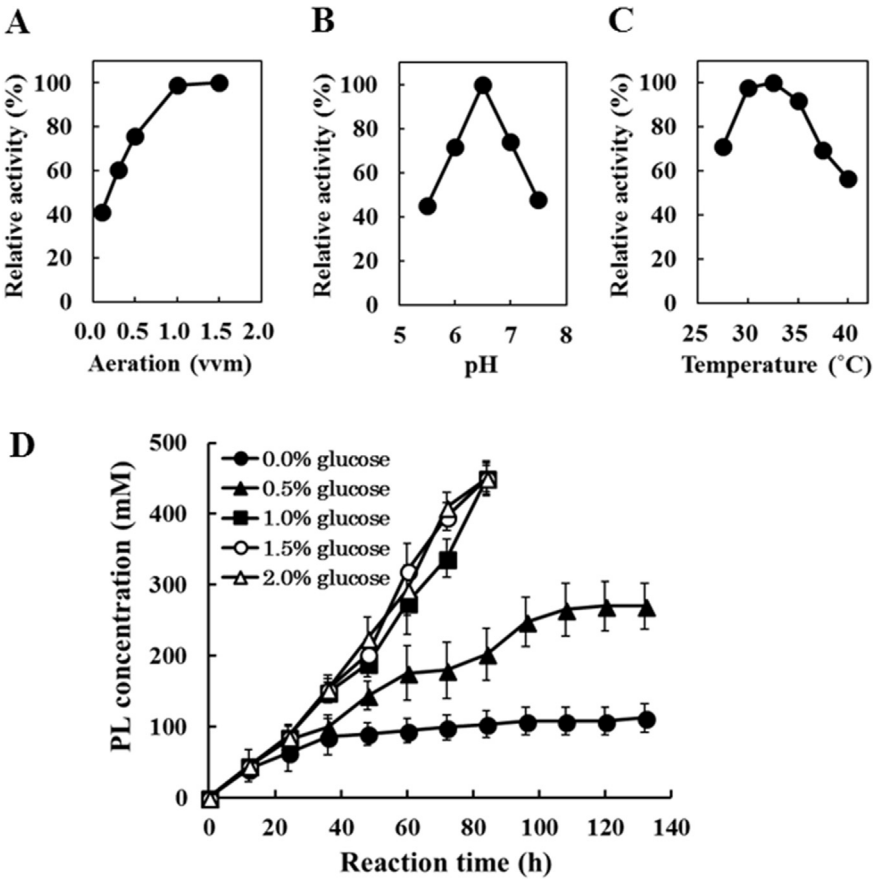

FIG. 4. Effects of aeration, pH, temperature, and glucose concentrations on the bioconversion of PN to PL by recombinant *R. erythropolis* JCM3191 harboring the pRET-PNO9 plasmid. (A) Effect of aeration. Activity was assayed under standard reaction conditions, except for the aeration. (B) Effect of pH. Activity was assayed under standard reaction conditions, except for pH. pH was adjusted with 28% sodium hydroxide (NaOH). (C) Effect of temperature. Activity was assayed under standard reaction conditions, except for the temperature. (D) Effect of glucose addition. Activity was assayed under standard reaction conditions, except for reaction time and glucose concentration. Vertical bars indicate standard deviation (SD) from three independent experiments. Closed circles, closed triangles, closed squares, open circles, and open triangles indicate no glucose, 0.5% glucose, 1.0% glucose, 1.5% glucose, and 2.0% glucose, respectively.

metabolic enzymes, such as PNO and PPAT, as all recombinant *R. erythropolis* strains harboring pRET11100 did not produce PL.

The characterization of purified PNO has been described by Yuan et al. (6), in which the optimal reaction pH and temperature of purified PNO were found to be between pH 8.0 and 8.5 and 40 °C, respectively. However, little study has been done to examine the optimal reaction pH and temperature of the whole-cell reaction. I determined that the optimal reaction pH and temperature of the

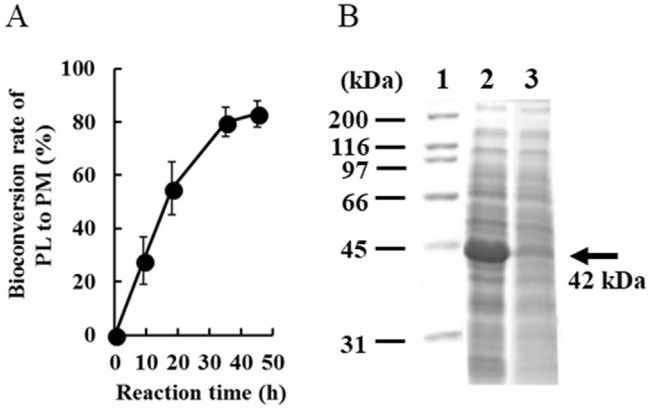

FIG. 5. Bioconversion of PL to PM using recombinant *R. erythropolis* JCM3191 harboring the pRET-PPAT1 plasmid. (A) Bioconversion rate of PL to PM of recombinant *R. erythropolis* JCM3191 harboring the plasmid pRET-PPAT1. Vertical bars indicate standard deviation (SD) from three independent experiments. (B) SDS-PAGE of crude extracts from recombinant *R. erythropolis* cells. Lane 1, standard proteins; lane 2, the crude extract from recombinant *R. erythropolis* JCM3191 harboring the pRET-PPAT1 plasmid; lane 3, the crude extract from recombinant *R. erythropolis* JCM3191 harboring the pRET11100 plasmid.

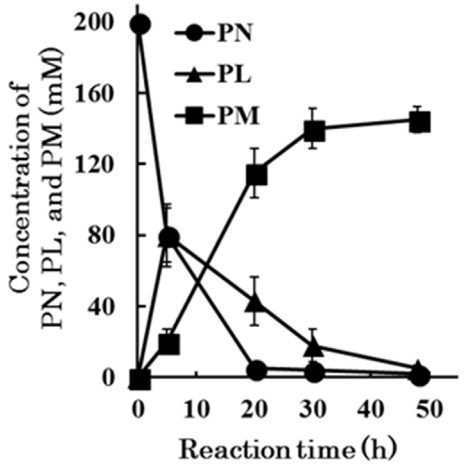

FIG. 6. Bioconversion of PN to PM through PL using the recombinant *R. erythropolis* JCM3191 strain harboring the pRET-PPPN1 plasmid. Vertical bars indicate standard deviation (SD) from three independent experiments. Closed circles, closed triangles, and closed squares indicate PN, PL, and PM concentrations, respectively.

whole-cell reaction were pH 6.5 and 30.0–32.5 °C, respectively, which differ from those of purified PNO. These differences in optimal reaction pH and temperature may be due to differences in membrane permeability of the substrate and enzyme stability. Further, the production of PL increased with increasing aeration requiring >1 vvm, suggesting bioconversion of PN to PL is an oxidation reaction needing active aeration.

Previously, I described the possibility that *R. erythropolis* has high intrinsic enzymatic activities (e.g., cofactor-regenerating enzymatic activity) that increased by the addition of glucose (14). For this reason, glucose was added in the bioconversion of PN to PL in the *Rhodococcus* expression system. The production of PL increased by the addition of glucose and the bioconversion rate was approximately 90% from 500 mM PN (approximately 10% PN). Although I have not yet collected sufficient data to explain this phenomenon, intrinsic enzymatic activities such as catalase activity in *R. erythropolis* may account for the production increase by glucose addition.

The expression level of *ppat* in recombinant *R. erythropolis* JCM3191was almost the same as that in *E. coli* described by Yoshikane et al. (24). With the aim of PN bioconversion to PM through PL using *R. erythropolis* JCM3191 coexpressing *ppat* and *pno*, PM was produced from PL using this recombinant strain expressing *ppat* using the same conditions for PL production (1 vvm, pH 6.5, and 30 °C). The bioconversion rate of PL to PM was approximately 80%.

Using recombinant *R. erythropolis* JCM3191 harboring pRET-PPPN1, thereby possessing the bioconversion activity to produce PM from PN, the production of PM in this strain was 145 mM from 200 mM PN. PM production from 400 mM PN was comparable to that found from 200 mM PN, suggesting there is little substrate inhibition below 400 mM PN. After reaction completion, the total concentration of vitamin B_6 (PN, PL, and PM) from 200 mM PN was approximately 145 mM (PN, <1 mM; PL, <1 mM; PM, 145 mM). This observed decrease in the total amount may be due to PL forming a Schiff base with primary amines such as proteins, L-glutamate, and PM (4,24).

If PL does not accumulate during the bioconversion reaction of PN to PM through PL, there is a possibility that the amount of PM production will increase. Further study to investigate increasing PM production will be conducted by making *ppat* activity higher than *pno* activity so that PL is not accumulated during the reaction. Recently, I reported the weak constitutive promoters, *hsp* and *1200rep* (14), the compatible and stable plasmid pRET1202, and the high copy plasmid pRET1129 derived from pRET1102 (15). Furthermore, we previously reported many mutated PPATs with high activity toward L-glutamate (8). Thus, the coexpression vector pRET-PPPN1 expressing *ppat* and *pno* can be reconstructed using these promoters, plasmids, and mutated PPATs.

In conclusion, using a *Rhodococcus* expression system, approximately 145 mM PM was produced from 200 mM PN through PL demonstrating a bioconversion rate of 75%. Moreover, recombinant *R. erythropolis* overexpressing *pno* produced approximately 450 mM PL from 500 mM PN (approximately 10% PN). Based on these findings, pyridoxamine production by bioconversion using a *Rhodococcus* expression system may be of interest for future industrial applications.

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