**Thermococcus** sp. KS-1 PPIase as a fusion partner improving soluble production of aromatic amino acid decarboxylase

Takashi Koyanagi\(^*\), Ayumi Hara\(^1\), Kanako Kobayashi\(^1\), Yuji Habara\(^1\), Akira Nakagawa\(^2\), Hiromichi Minami\(^2\), Takane Katayama\(^3\) and Norihiko Misawa\(^2\)

**Abstract**

Peptidyl-prolyl cis-trans isomerase (PPIase, EC 5.2.1.8) catalyzes the racemization reaction of proline residues on a polypeptide chain. This enzyme is also known to function as a molecular chaperon to stabilize protein conformation during the folding process. In this study, we noted FK506 binding protein (FKBP)-type PPIase from a hyperthermophilic archaeon **Thermococcus** sp. strain KS-1 (PPIase KS\(^{-1}\)) to improve the solubility of *Pseudomonas putida* aromatic amino acid decarboxylase (AADC) that is an indispensable enzyme for fermentative production of plant isoquinoline alkaloids. AADC fused N-terminally with the PPIase KS\(^{-1}\) (PPIase KS\(^{-1}\)-AADC), which was synthesized utilizing *Escherichia coli* host, showed improved solubility and, consequently, the cell-free extract from the recombinant strain exhibited 2.6- to 3.4-fold elevated AADC activity than that from the control strain that expressed the AADC gene without PPIase KS\(^{-1}\). On the other hand, its thermostability was slightly decreased by fusing PPIase KS\(^{-1}\). The recombinant *E. coli* cells expressing the PPIase KS\(^{-1}\)-AADC gene produced dopamine and phenylethylamine from L-dopa and phenylalanine by two- and threefold faster, respectively, as compared with the control strain. We further demonstrated that the efficacy of PPIase KS\(^{-1}\)-AADC in solubility and activity enhancement was a little but obviously higher than that of AADC fused N-terminally with NusA protein, which has been assumed to be the most effective protein solubilizer. These results suggest that PPIase KS\(^{-1}\) can be used as one of the best choices for producing heterologous proteins as active forms in *E. coli*.

**Keywords:** Peptidyl-prolyl cis-trans isomerase (PPIase), Molecular chaperon, **Thermococcus** sp. strain KS-1, Aromatic amino acid decarboxylase, *Escherichia coli*

**Key points**

- Use of FKBP-type PPIase from **Thermococcus** sp. KS-1 as an effective molecular chaperon in *E. coli*.
- Solubility and activity enhancement in aromatic amino acid decarboxylase (AADC).
- Demonstration of the **Thermococcus** sp. KS-1 PPIase gene as a powerful fusion counterpart for the AADC gene.

**Introduction**

Peptidyl-prolyl cis-trans isomerase (PPIase, EC 5.2.1.8) is an enzyme that catalyzes the racemization reaction of amino acid proline (Fischer and Schmid 1999). This protein is also known to act as a molecular chaperon to stabilize the folding process and structure of protein through the cis-trans isomerization of proline residues in a polypeptide chain. PPIase widely distributes in organisms...
belonging to all of three domains, i.e., bacteria, archaea, and eukaryotes including mammals and plants, thus constituting the large family responsible for the universal role of protein stabilization in living organisms. PPIase comprises three subfamilies, cyclophilin, FK506 binding protein (FKBP), and parvulin, and all of these have been shown to exhibit similar enzymatic activities (Fischer and Schmid 1999; Furutani 2000; Galat 2003; Göthel and Marahiel 1999; Lu et al. 2007; Maruyama et al. 2004; Shaw 2002; Tong and Jiang 2015).

Synthetic biology has empowered bacterial production of complex compounds with high commercial value. There are high demands for efficiently producing recombinant proteins with high solubility as well as desired protein activity. Indeed, solubility of proteins is one of the most important factors in microbial applications such as production of pharmaceutical proteins and small molecule compounds with enzymes. However, heterologous overexpression is in many cases troublesome because of the unmatched pair use of heterologous protein genes and host cells, leading to the formation of inclusion body containing a large amount of denatured target proteins. Thus, in case of *Escherichia coli*, various types of protein stabilizer have been developed, e.g., DnaK/DnaJ/GroEL/ES (molecular chaperons co-expressed or fused with a target protein) (Bhandari and Houry 2009; Kyratsous et al. 2009), thioredoxin DsbA and DsbC (cysteine-bonds improvers fused to the N-terminus of a target protein) (Collins-Racie et al. 1995; Nozach et al. 2013), and N utilization substance A (NusA, high-performance protein solubilizer fused to the N-terminus of a target protein) (Davis et al. 1999). These systems can generally contribute to successful protein overproduction in the *E. coli* cells, but still have the probability that fails in the folding of soluble proteins case-dependently. It is thus desirable to retain the multiple options for functional expression systems as many as possible.

In this study, we employed the FKBP-type PPIase from a hyperhemophilic archaeon, *Thermococcus* sp. strain KS-1 (PPIase KS-1) (Furutani et al. 2000; Ideno et al. 2001, 2002, 2004; Iida et al. 1998; Misawa et al. 2011), as a molecular chaperon for attaining high-level production of *Pseudomonas putida* aromatic amino acid decarboxylase (AADC) in *E. coli* (Koyanagi et al. 2012). AADC catalyzes the reaction converting L-dopa into dopamine and requires pyridoxal phosphate for its enzymatic activity. Dopamine is an important neurotransmitter and is often used as a supplement for maintaining human body condition, thus, the effective expression of the AADC gene would benefit microbial production of this compound. Furthermore, the AADC reaction constitutes an important step for our recently developed plant isoquinoline alkaloid production system using engineered *E. coli*, since the isoquinoline backbone is built by the condensation of aromatic amine and aromatic aldehyde. Isoquinoline alkaloids have been known as pharmaceutically important compounds, therefore attaining high activity of a key enzyme AADC in isoquinoline alkaloid synthesis pathway inside the *E. coli* cells would directly be linked with future increased industrial production level of these useful compounds. We here demonstrated an archaeal FKBP-type PPIase KS-1 as a powerful fusion counterpart for the heterologous functional expression of the AADC gene in *E. coli* as the host cell.

**Methods**

**Bacterial strains and plasmids**

*E. coli* DH5α was generally used as a host for genetic manipulations, and *E. coli* BL21(DE3) was used as a host for protein overproduction. The plasmids pET-3a and pET-43.1a (Merck, Darmstadt, Germany) were used as cloning and expression vectors for the AADC and NusA-AADC structural genes respectively. The structural gene of AADC (aadc, GenBank accession no. BK006920.1) was amplified from the genomic DNA of *P. putida* KT2440 by the polymerase chain reaction (PCR) using PrimeStar GXL DNA polymerase (TakaraBio, Shiga, Japan) with a primer pair 5′-AAACCCCATATGACCCCCGAAACAA TTCCG-3′ and 5′-AAAGGATCCTAGCCCCTTGGAT CACGTCTCTG-3′ (The *NdeI* and *BamHI* restriction sites are underlined respectively). The amplified fragments (approximately 1.5 kbp) were treated with *NdeI* and *BamHI* and inserted into pET-3a digested by the same restriction enzymes. The 1.2 kbp fragment containing the *lacI* gene, amplified by PCR using pET-43.1a as a template and a primer pair 5′-GGCCGCAATTCCTTGG ATTCGGGACACCACCACTGAA-3′ and 5′-CCGGAA GGAATGTTGCTAGCATGCACCGCCCGCCA-3′, was inserted into *Spy/I* site of the resulting plasmid by using In-Fusion® HD Cloning Kit (15 bp homologous nucleotides in primers used for recombination are underlined). The constructed plasmid (pAADC) was used for overproduction of wild-type *P. putida* AADC. The *Thermococcus* sp. KS-1 FKBP-type PPIase gene (GenBank accession no. AB012209.1) was fused to N-terminus of AADC by inserting the *NdeI* and *BamHI* restriction sites are underlined respectively). After digestion with *SacI* and *XhoI*, the fragment was inserted into the resulting plasmid by using In-Fusion® HD Cloning Kit (15 bp homologous nucleotides in primers used for recombination are underlined).
into the similarly-cut pFusion-F87V to construct pPPlase
\( \text{KS}-1 \)-AADC. The NusA-AADC-expressing plasmid
(pNusA-AADC) was constructed by amplifying the \( aadc \)
gene from pPPlase \( \text{KS}-1 \)-AADC by using a primer pair
\( 5'-\text{GCTACTAGTCTGGTTCCGG-3'} \) and \( 5'-\text{CCCCTCGAGTCAAGCCTTGATACGT CCTG-3'} \) (The SpeI and
\( XhoI \) restriction sites are underlined respectively), and
the amplified fragments were inserted into pET-43.1a at
the \( SpeI \) and \( XhoI \) restriction sites. Summary of the expression con-
struct of pPPlase \( \text{KS}-1 \)-AADC was shown in Fig. 1. All
PCR-amplified regions were sequenced to confirm no
introduction of nucleotide errors.

**Media and chemicals**

Luria-Bertani (LB) (Difco) was routinely used for cul-
tivation of the \( E. coli \) strains. When the production of
dopamine and phenylethylamine was performed, M9-0.2
(w/v) % glucose minimal medium supplemented with 1
mM L-dopa or L-phenylalanine were used for cultivation.
Ampicillin was added to media at 100 µg/ml to maintain
the expression vectors in the \( E. coli \) cells. L-Dopa, dopa-
mine, L-phenylalanine, and phenylethylamine were pur-
chased from NakaraiTesque Co. (Kyoto, Japan).

**Overproduction of AADC, NusA-AADC, and PPlase
\( \text{KS}-1 \)-AADC**

The \( E. coli \) BL21(DE3) derivatives carrying pAADC,
pNusA-AADC, or pPPlase \( \text{KS}-1 \)-AADC were cultivated in
50 mL LB at 25 °C with shaking at 140 rpm, and when
the turbidity at 600 nm reached to 0.4–0.6, isopropyl-\( \beta \)-d-
thiogalactopyranoside (IPTG) was added at the final
concentration of 0.2 mM. Temperature was then changed
to 18 °C, and the cultivation was continued with shaking
for 18 h. The cells were harvested by centrifugation at
5000×g for 8 min, and were disrupted by ultrasonica-
tion in 50 mM potassium phosphate (pH 7.0) containing
4 mM 2-mercaptoethanol (2-ME) and 200 µM pyridoxal
5'-phosphate (PLP). Cell-free extract and insoluble cellu-
lar debris were separated by following centrifugation at
15,000×g for 10 min. The solubility of the proteins were
analyzed with SDS-PAGE by applying cell-free extracts
and cellular debris equivalent to 10 µg of the cells.

**AADC activity assay**

Cell-free extracts were evaluated for the AADC activity
by using the methods we described in the previous study
with some modifications (Koyanagi et al. 2012). The reac-
tion solution comprised 50 mM potassium phosphate
(pH 8.0), 2 mM 2-ME, 50 µM PLP and 1 mM L-dopa.
One milliliter of this reaction mixture was preincubated
for 5 min at 30 °C, followed by initiation of the reaction
by adding 20 µL of the cell-free extract, and stopping
the reaction by adding HCl at the final concentration of
0.1 M. The reaction products were analyzed by HPLC
equipped with a Discovery HS F5 column (Supelco, St.
Louis, MO). The elution was performed at a flow rate of
0.5 mL/min by increasing the concentration of acetonitrile
from 3 to 20% in 10 mM ammonium formate buffer
(pH 3.0), and the detection of L-dopa and dopamine was
performed by measuring the absorbance at 280 nm.

**Production of dopamine and phenylethylamine
by recombinant \( E. coli \) strains**

The AADC- and PPlase \( \text{KS}-1 \)-AADC-expressing \( E. coli \)
cells were evaluated for their dopamine and phenyleth-
ylamine production ability by adding 1 mM L-dopa and
L-phenylalanine to medium as substrates, respectively.
The cells were cultivated in 50 mL M9 minimal medium
at 25 °C with shaking at 140 rpm, and IPTG was added at
the concentration of 0.1 mM when the turbidity at 600
nm reached to 0.4–0.6. The samples of 1 mL were with-
drawn at the times indicated and centrifuged for 1 min
at 15,000×g, and the concentrations of dopamine and
phenylethylamine were measured by HPLC. Detection
of L-dopa and dopamine was performed as described
above, whereas L-phenylalanine and phenylethylamine
were fluorescent-labeled by AccQ-tag (contained in
AccQ-Fluor™ Reagent Kit, Waters, Milford, MA) prior
to the HPLC analysis. AccQ-tag amino acid analysis col-
umn (Waters) was used to separate the compounds, and
fluorescence was detected with excitation wavelength at
250 nm and emission at 395 nm. Elution program was set
according to the manufacturer’s instruction.
Results

Overproduction of AADC and comparison of solubility among the expression systems

E. coli BL21(DE3) carrying pAADC, pNusA-AADC, or pPPIase KS−1-AADC were cultivated in LB and the expression of target genes from the T7 promoter were induced by IPTG. In the PPIase KS−1-AADC fusion construct, a 14 amino acids linker (TSLVRRGSHMEFEL) was introduced between two structural genes of the proteins, since this length had been found to be effective for stabilizing the Bacillus megaterium P450 BM3 (variant F87V) when expressed in E. coli BL21(DE3) (Misawa et al. 2011). The cell-free extracts and the cell debris were analyzed for their protein contents by SDS-PAGE (Fig. 2A). The soluble form of wild-type AADC was detectable, but the significant amount was seen in the insoluble fraction (pAADC). On the other hand, NusA-AADC was highly soluble as compared to the wild-type protein and only a little amount appeared as insoluble form (pNusA-AADC). PPIase KS−1-AADC was also produced almost in the soluble fraction, and the amount of the protein detected in the insoluble fraction was similar to the case of NusA-AADC (pPPIase KS−1-AADC). These results indicated that PPIase KS−1 greatly improved the solubility of AADC, and the effectiveness of PPIase KS−1 was comparable to the NusA system, which is regarded as one of the most efficient protein solubilizer generally used among the E. coli overexpression constructs.

The AADC activity was measured using these cell-free extracts, and the results were shown in Fig. 2B. The activities for NusA-AADC and PPIase KS−1-AADC were significantly higher than AADC without fusion partner, well reflecting the high solubility of the NusA and PPIase KS−1 systems. Although the thickness of protein bands were almost similar between NusA- and PPIase KS−1-fusions, the AADC activity toward L-dopa was significantly higher in PPIase KS−1-AADC as compared to NusA-AADC (1750 ± 240 vs. 1360 ± 70 nmole/min/mg protein respectively), thus indicating that the efficiency of the PPIase KS−1 system is comparable or even superior to NusA.

Thermostability of PPIase KS−1-fused AADC

In the previous study, thermostolerance of the B. megaterium P450 BM3 (variant F87V) protein was improved by the existence of PPIase KS−1 at N-terminus (Misawa et al. 2011). Based on this result, PPIase KS−1-fused AADC was tested for its thermostability by incubating the cell-free extracts prepared from the E. coli strain expressing the wild-type or PPIase KS−1-fused AADC gene (Fig. 3). PPIase KS−1-fused P450 BM3 heterologously produced in the E. coli cells restored the enzymatic activity at 43.9% upon 20-min heat treatment of cell-free extract at 60°C, while the recombinant protein without PPIase KS−1-fusion recorded not more than 27.7%. PPIase KS−1-AADC, however, was found to be weaker than non-fused AADC against heat in this study, as shown in Fig. 3. We heat-treated the cell-free extract from 45 to 60 °C for 30 min, but the residual activity of PPIase KS−1-AADC was clearly inferior to that of AADC. This result indicates that thermostability of PPIase KS−1-fusion is protein-species dependent.
Dopamine and phenylethylamine production by the PPIase KS₁−AADC overproducing strain

Next, we evaluated the production ability of aromatic amines (dopamine and phenylethylamine) of the strains expressing the wild-type AADC and PPIase KS₁−AADC genes. AADC is capable of converting L-phenylalanine into phenylethylamine, though the reaction is weaker than converting L-dopa to dopamine (Koyanagi et al. 2012). M9 minimal media containing 1 mM L-dopa or L-phenylalanine were used in this experiment, since L-dopa and dopamine are unstable in the complete medium LB and easily converted into black melanin-like pigment during cultivation. Figure 4A, B show the conversion of L-dopa and L-phenylalanine into dopamine and phenylethylamine, respectively. Wild-type AADC-producing E. coli strain exhibited the dopamine production achieving the final concentration of 0.6 mM at 42 h after the start of cultivation, but PPIase KS₁−AADC-expressing strain produced higher amount of 0.7 mM at earlier time point around 18 h (Fig. 4A). At this time point, the accumulation level of dopamine was approximately 2.6-fold higher for PPIase KS₁−AADC-producing strain than wild-type AADC-producing strain. Phenylethylamine accumulation was also higher in PPIase KS₁−AADC-producing strain (1.1 mM at 46 h cultivation) than that of wild-type AADC-producing strain (0.3 mM at the same time point, 3.4-fold lower than PPIase KS₁−AADC). The latter strain was capable of producing phenylethylamine not more than 0.6 mM even after 138 h from the start of cultivation. PPIase KS₁−AADC fusion thus elevated the production level of active AADC molecules, leading to the increment of dopamine and phenylethylamine accumulation.

Discussion

In this study, we obtained results that the solubility of P. putida-derived AADC linked to FKBP-type PPIase KS₁ at the N-terminus was significantly increased in recombinant E. coli cells, as compared with the non-fused protein. A similar result has been shown in P450 BM3 (variant F87V) from Bacillus megaterium (Misawa et al. 2011), indicating that function of FKBP-type PPIase KS₁ as a chaperon is useful for the production of soluble proteins with active forms. In addition, PPIase KS₁ showed comparable solubilizing efficiency with NusA, which is involved in the heterologous expression system of E. coli with the highest solubilizing capacity so far reported. Another FKBP-type PPIase of E. coli origin, SlyD, has been evaluated as a N-terminal fusion counterpart of various aggregation-prone heterologous proteins including Candida antarctica lipase B (CalB), and its high solubilizing activity was confirmed in E. coli (Han et al. 2007; Seo et al. 2009). Geitner et al. (2013) expressed the gene encoding an active parvulin domain (Par2) of the E. coli periplasmic prolyl isomerase SurA as a chimeric protein with chaperone domain of SlyD, and found that the folding activity was dramatically increased to 1500-fold higher than the wild-type SurA. SlyD and PPIase KS₁ are both classified into the FKBP-C superfamily protein, but both are derived from distinct domains, i.e., bacteria and archaea, respectively, and shared only 30% identity in their amino acid sequences. Despite these significant differences, both proteins seem to act as similarly effective folding enhancer when used as the N-terminal fusion counterpart in E. coli.

It was found that the thermal stability of AADC was slightly weakened by fusing with PPIase KS₁−AADC although elevated thermal stability was observed in case of using P450 BM3 (variant F87V) instead of AADC (Misawa et al. 2011). The reason for the unfavorable result is unknown at the present, but, since the structure of...
the protein becomes bulkier than the wild-type AADC, by fusing PPIase \(_{KS-1}\), it is possible that denaturation or decomposition of the PPIase \(_{KS-1}\) and AADC fusion protein were promoted due to intense structural change provoked by heat treatment. This result is also conflicting with improvement of protein solubility and activity, and future follow-up survey is required.

However, \textit{Thermococcus} sp. KS-1 PPIase is sufficiently small, only 17.5 kDa, when compared to NusA that is a large protein of 54.9 kDa, so that the use of such a small PPIase \(_{KS-1}\) protein as a fusion counterpart is obviously advantageous and the size of a plasmid constructed can be reduced. In recent synthetic biology, it is necessary to simultaneously express a large number of heterologous genes in the same cell, therefore the compactness of PPIase \(_{KS-1}\) should surely be useful. \textit{P. putida} AADC is a protein used in the synthetic-biological production of plant isoquinoline alkaloids using \textit{E. coli} cells (Kim et al. 2013; Matsumura et al. 2017; Minami et al. 2008; Nakagawa et al. 2011, 2012, 2014, 2016). Since L-dopa is unstable in production media (Nakagawa et al. 2011), rapid conversion from L-dopa to dopamine seems to be an important reaction step for effectively forming the isoquinoline skeleton. Thus, improved solubility of this enzyme may further contribute not only to microbrial production of bioactive compound dopamine, but also to future industrial production of pharmaceutically important isoquinoline alkaloids. Thus, \textit{Thermococcus} sp. KS-1 PPIase can be a good choice for improving the production property of heterologous proteins, which contribute to an important issue in synthetic biology to efficiently provide starting materials or intermediates.

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**Authors’ contributions**
TK and NM conceived and designed this study. TK, AH, KK and YH conducted experiments. TK and NM analyzed the data. AN, HM, TK and NM supervised this study. TK wrote the original draft of the manuscript, and NM edited it. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interest.

**Author details**
1 Department of Food Science, Ishikawa Prefectural University, Nonoi, Ishikawa 921-8836, Japan. 2 Research Institute for Biosources and Biotechnology, Ishikawa Prefectural University, Nonoi, Ishikawa 921-8836, Japan. 3 Graduate School of Biostudies, Kyoto University, Sakyo-ku, 606-8501 Kyoto, Japan.

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**References**

- Bhandari V, Houry WA (2015) Substrate interaction networks of the Escherichia coli chaperones: trigger factor, DnaK and GroEL. Adv Exp Med Biol 883:271–294. https://doi.org/10.1007/978-3-319-23603-2_15
- Collins-Racie LA, McColgan JM, Grant KL, DiBlasio-Smith EA, McCoy JM, LaValie ER (1995) Production of recombinant bovine enterokinase catalytic subunit in Escherichia coli using the novel secretory fusion partner DsbA. Biotechnology (NY) 13:982–987. https://doi.org/10.1038/ntbio0995-982
- Davis GD, Elsee C, Newham DM, Harrison RG (1999) New fusion protein systems designed to give soluble expression in Escherichia coli. Biotechnol Bioeng 65:382–388. https://doi.org/10.1002/(SICI)1097-0290(19991120)65:4<382::AID-BIT2>3.0.CO;2-I
- Fischer G, Schmid FX (1999) Peptidyl-prolyl cis-trans isomerases in molecular chaperones and folding catalysts. Harwood Academic, Amsterdam, pp 461–489
- Furutani M, Ideno A, Iida T, Maruyama T (2000) FK506 binding protein from a thermophilic archaeon, Methanothermus thermolithothrophicus, has chaperone-like activity in vitro. Biochemistry 39:453–462. https://doi.org/10.1021/bk9911076
- Galat A (2003) Peptidylprolyl cis/trans isomerases (immunophilins): biological diversity-targets-functions. Curr Top Med Chem 3:1315–1347. https://doi.org/10.2174/1568026034541862
- Göthel SF, Marahiel MA (1999) Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. Cell Mol Life Sci 55:423–436. https://doi.org/10.1007/s000180050299
- Geitner AJ, Varga E, Wehmer M, Schmid FX (2013) Generation of a highly active folding enzyme by combining a parvulin-type prolyl isomerase from SurA with an unrelated chaperone domain. J Mol Biol 425:4089–4098. https://doi.org/10.1016/j.jmb.2013.06.038
- Han KY, Song JA, Ahn KY, Park JS, Seo HS, Lee J (2007) Solubilization of aggregation-prone heterologous proteins by covalent fusion of stress-responsive Escherichia coli protein, SlyD. Protein Eng Des Sel 20:543–549. https://doi.org/10.1093/protein/gzm055
- Ideno A, Furutani M, Iida T, Kurosawa Y, Maruyama T (2002) FK506 binding protein from the hyperthermophilic archaeon Pyrococcus horikoshii suppresses the aggregation of proteins in Escherichia coli. Appl Environ Microbiol 68:464–469. https://doi.org/10.1128/AEM.68.2.464-469.2002
- Ideno A, Furutani M, IWabuchi T, Iida T, Iba Y, Kurosawa Y, Sakuraba H, Ohshima T, Kawara-bayashi Y, Maruyama T (2004) Expression of foreign proteins in Escherichia coli by fusing with an archaeal FK506 binding protein. Appl Microbiol Biotechnol 64:99–105. https://doi.org/10.1007/s00253-003-1459-4
- Ideno A, Yoshida T, Iida T, Furutani M, Maruyama T (2001) FK506-binding protein of the hyperthermophilic archaeum, Thermococcus sp. KS-1, a cold-shock-inducible peptidyl-prolyl cis-trans isomerase with activities to trap and refold denatured proteins. Biochem J 357:465–471. https://doi.org/10.1042/0264-6021:3570465
- Iida T, Furutani M, Nishida F, Maruyama T (1998) FKBP-type peptidyl-prolyl cis-trans isomerase from a sulfur-dependent hyperthermophilic archaeon, Thermococcus sp. KS-1. Gene 222:249–255. https://doi.org/10.1016/S0378-1119(98)00484-3
- Koyanagi T, Nakagawa A, Sakurama H, Yamamoto K, Sakurai N, Takagi Y, Minami H, Katayama T, Kumagai H (2012) Eukaryotic-type aromatic amino acid decarboxylase from the root colonizer \textit{Pseudomonas putida} is highly specific for 3,4-dihydroxyphenyl-L-alanine, an allelochemical in the rhizosphere. Microbiology 158:2965–2974. https://doi.org/10.1099/mic.0.062463-0
Kim JS, Nakagawa A, Yamazaki Y, Matsumura E, Koyanagi T, Minami H, Katayama T, Sato F, Kumagai H (2013) Improvement of reticuline productivity from dopamine by using engineered *Escherichia coli*. Biosci Biotechnol Biochem 77:2166–2168. https://doi.org/10.1271/bbb.130552

Kyroudis CA, Silverstein SJ, Delong CR, Panagiotidis CA (2009) Chaperone-fusion expression plasmid vectors for improved solubility of recombinant proteins in *Escherichia coli*. Gene 440:9–15. https://doi.org/10.1016/j.gene.2009.03.011

Lu KP, Finn G, Lee TH, Nicholson LK (2007) Prolyl cis-trans isomerization as a molecular timer. Nat Chem Biol 3:619–629. https://doi.org/10.1038/nchembio.2007.35

Maruyama T, Futurani M (2000) Archaeal peptidyl prolyl cis-trans isomerases (PPlases). Front Biosci 5:D821–836. https://doi.org/10.2741/maruyama

Maruyama T, Suzuki R, Futurani M (2004) Archaeal peptidyl prolyl cis-trans isomerases (PPlases) update 2004. Front Biosci 9:1680–1720. https://doi.org/10.2741/1361

Matsumura E, Nakagawa A, Tomabechi Y, Koyanagi T, Kumagai H, Yamamoto K, Katayama T, Sato F, Minami H (2017) Laboratory-scale production of (S)-reticuline, an important intermediate of benzylisoquinoline alkaloids, using a bacterial-based method. Biosci Biotechnol Biochem 81:396–402. https://doi.org/10.1080/09168451.2016.1243985

Minami H, Kim JS, Ikezawa N, Takemura T, Katayama T, Kumagai H, Sato F (2008) Microbial production of plant benzylisoquinoline alkaloids. Proc Natl Acad Sci USA 105:7395–7398. https://doi.org/10.1073/pnas.0802981

Misawa N, Nomura M, Ootomatsu T, Shimizu K, Kaido C, Kikuta M, Ideno A, Ikenaga H, Ogawa J, Shimizu S, Shindo K (2011) Bioconversion of substituted naphthalenes and β-eudesmol with the cytochrome P450 BM3 variant F87V. Appl Microbiol Biotechnol 90:147–157. https://doi.org/10.1007/s00253-010-3064-7

Nakagawa A, Matsumura E, Koyanagi T, Katayama T, Kawano N, Yoshimatsu K, Yamamoto K, Kumagai H, Sato F, Minami H (2016) Total biosynthesis of opiates by stepwise fermentation using engineered *Escherichia coli*. Nat Commun 7:10390. https://doi.org/10.1038/ncomms10390

Nakagawa A, Matsuzaki C, Matsumura E, Koyanagi T, Katayama T, Yamamoto K, Sato F, Kumagai H (2014) (S)-tetrahydropapaveroline production by stepwise fermentation using engineered *Escherichia coli*. Sci Rep 4:6605. https://doi.org/10.1038/srep06605

Nakagawa A, Minami H, Kim JS, Koyanagi T, Katayama T, Sato F, Kumagai H (2011) A bacterial platform for fermentative production of plant alkaloids. Nat Commun 2:326. https://doi.org/10.1038/ncomms1327

Nakagawa A, Minami H, Kim JS, Koyanagi T, Katayama T, Sato F, Kumagai H (2012) Bench-top fermentative production of plant benzylisoquinoline alkaloids using a bacterial platform. Bioeng Bugs 3:49–53. https://doi.org/10.4161/bbug.3.1.18446

Nozach H, Fruchart-Gaillard C, Fenaille F, Beau F, Ramos OH, Douzi B, Saez NJ, Moutiez M, Servent D, Gondry M, Thai R, Cunisasse P, Vincentelli R, Dile V (2013) High throughput screening identifies disulfide isomerase DisBC as a very efficient partner for recombinant expression of small disulfide-rich proteins in E. coli. Microb Cell Fact 12:2–16. https://doi.org/10.1186/1475-2859-12-37

Seo HS, Kim SE, Han KY, Park JS, Kim YH, Sim SJ, Lee J (2009) Functional fusion mutant of *Candida antarctica* lipase B (CaB) expressed in *Escherichia coli*. Biochim Biophys Acta 1794:519–525. https://doi.org/10.1016/j.bbabio.2008.12.007

Shaw PE (2002) Peptidyl-prolyl isomerases: a new twist to transcription. EMBO Rep 3:521–526. https://doi.org/10.1093/embo-reports/kvf118

Tong M, Jiang Y (2015) FKS06-binding proteins and their diverse functions. Curr Mol Pharmacol 9:48–65. https://doi.org/10.2174/187446720866615059113541