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Biotech Method

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Abbreviations:

3AB, 3-amino-benzoic acid; **DHS,** 3-dehydroshikimic acid; **E4P,** Erythrose 4-phosphate; **IPTG**, Isopropyl β-D-1-thiogalactopyranoside; **OD,** optical density; **PEP,** phosphoenolpyruvic acid.

Abstract

3-amino-benzoic acid (3AB) is an important building block molecule for production of a wide range of important compounds such as natural products with various biological activities.

In the present study, we established a microbial biosynthetic system for *de novo* 3AB production from the simple substrate glucose. First, the active 3AB biosynthetic pathway was reconstituted in the bacterium *Escherichia coli*, which resulted in the production of 1.5 mg/L 3AB. In an effort to improve the production, an *E. coli*-*E. coli* co-culture system was engineered to modularize the biosynthetic pathway between an upstream strain and an downstream strain. Specifically, the upstream biosynthetic module was contained in a fixed *E. coli* strain, whereas a series of *E. coli* strains were engineered to accommodate the downstream biosynthetic module and screened for optimal production performance. The best co-culture system was found to improve 3AB production by 15 fold, compared to the mono-culture approach. Further engineering of the co-culture system resulted in biosynthesis of 48 mg/L 3AB. Our results demonstrate co-culture engineering can be a powerful new approach in the broad field of metabolic engineering.

1 Introduction

3-amino-benzoic acid (3AB) is a benzoic acid with a *meta*-amino group. As one of the three regioisomers of aminobenzoic acid, 3AB possesses intriguing chemical properties that can be utilized for synthesis of complex molecules of important biological value. For example, 3AB serves as the precursor for making bioactive molecule tricaine mesylate which has been widely used as a fish anesthetic [1, 2]. In addition, known as a noncanonical amino acid, 3AB is an important building block molecule for biosynthesis of a large variety of natural products with diverse pharmaceutical properties, such as rifamycins, antimycins, grixazone platencin, etc [3]. However, the mechanism for 3AB biosynthesis is poorly understood, despite the fact that biosynthesis of 2-aminobenzoic acid and 4-aminobenoic acid has been well characterized.

Recently, a 3AB biosynthetic pathway was identified, which employs an aminotransferase/3AB synthase to convert 3-dehydroshikimic acid (DHS) to 3AB using L-glutamic acid as the amino donor [4, 5]. Notably, DHS is a key intermediate of the shikimate pathway that is ubiquitously used by bacteria, algae, fungi and plants for making aromatic amino acids [6, 7]. In addition, engineering of the shikimate pathway has been extensively reported for microbial production of several important biochemicals, including tyrosine, shikimic acid, muconic acid, 4-hydroxybenzoic acid [8-14]. As such, characterization of DHS-to-3AB conversion paved the way for *de novo* microbial biosynthesis of 3AB from simple carbon substrates via the shikimate pathway. In the present study, we successfully reconstituted the 3AB biosynthesis pathway in the bacterium *E. coli* by coupling the 3AB synthase, PctV, with the engineered shikimate pathway (Fig. 1A). To our knowledge, this is the first report of *in vivo* biosynthesis of 3AB from glucose in *E. coli*. The establishment of 3AB microbial biosynthesis platform is of significant research interest, as it could facilitate future heterologous biosynthesis of high-value natural products that use 3AB as a precursor.

On the other hand, in light of the low level of product synthesis achieved in an *E. coli* mono-culture expressing the 3AB pathway, and related issues with the functioning of the shikimate pathway and DHS synthesis identified recently in the synthesis of muconic acid [15], we took advantage of emerging co-culture engineering approaches to overcome these challenges and improve overall 3AB biosynthesis in *E. coli*. It has been previously shown that different modules of the complete biosynthetic pathway can be individually accommodated in separate *E. coli* strains in order to improve the biosynthetic performance of the overall system [15-17]. Here, we further demonstrate one important advantage of co-culture engineering, namely, effective screening and identification of suitable *E. coli* hosts for optimal accommodation of specific

biosynthetic modules. In fact, different *E. coli* strains, albeit of the same species, possess varying capabilities for supporting the functionality of different, especially heterologous, enzymes [18]. Therefore, selection of suitable *E. coli* hosts for biosynthesis of a specific product is considered case-dependent. As reconstitution of complete biosynthetic pathways in different *E. coli* strains is not a trivial task, extensive engineering efforts would be required in the optimization of various pathways in *E. coli*. In this study, we utilized the modular nature of co-culture engineering to rapidly identify a particular *E. coli* strain that markedly improved the efficiency of 3AB biosynthesis. The results of this work offer a new approach for host-pathway optimization and expand the applicability of co-culture engineering for product synthesis in the broad area of metabolic engineering.

2 Materials and Methods

2.1 *E. coli* growth condition

LB medium was used for all DNA cloning manipulations. For 3AB biosynthesis, *E. coli* mono-cultures and co-cultures used in this study were cultivated in 14 mL culture tubes containing 2 mL glucose medium at 250 rpm under 37°C. 1 L glucose medium was composed of 20 g glucose, 2.0 g NH_4Cl, 5.0 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 7.3 g K_2HPO_4, 8.4 g MOPS, 0.5 g NaCl, 0.24 g $MgSO_4$, 0.5 g yeast extract, 40 mg tyrosine, 40 mg phenylalanine, 40 mg tryptophan, 10 mg 4-hydroxybenzoic acid and trace elements. The working concentrations of trace elements were 0.4 mg/L Na_2EDTA, 0.03 mg/L H_3BO_3, 1 mg/L thiamine, 0.94 mg/L $ZnCl_2$, 0.5 mg/L $CoCl_2$, 0.38 mg/L $CuCl_2$, 1.6 mg/L $MnCl_2$, 3.77 mg/L $CaCl_2$, and 3.6 mg/L $FeCl_2$. 50 mg/L kanamycin and 34 mg/L chloramphenicol were also supplemented to the glucose medium.

When needed, 0.1 mM IPTG was used to induce the gene expression under the control of the T7 promoter.

2.2 Plasmid and strain construction

The *pctV* gene from *Streptomyces pactum* was codon-optimized for *E. coli* expression, custom-synthesized and inserted into plasmid pET28a using *Nde/XhoI* sites, yielding pET28-V. To improve DHS assimilation, the *E. coli* transporter *shiA* gene was first PCR amplified from the chromosome of *E. coli* BL21(DE3) using primers CGTCTAGAAAGGAGGAACAGAC atgGACTCCACGCTCATCTCC and GCCTCGAGACTAGTtcaAGCGCGTTGACTGTCTTTC, digested with *XbaI/XhoI* and ligated with pET28-V treated with *SpeI/XhoI*. The resulting plasmid pET28-VS had the *pctV* and *shiA* genes both under the regulation of a T7 promoter. For construction of the constitutive expression plasmid, pET28-VS was digested with *XbaI/XhoI* and the resulting *pctV-shiA* cassette was transferred to a previously constructed plasmid pCYZ [15] treated with the same endonucleases. The resulting plasmid pdc-VS contained the *pctV* and *shiA* genes both under the control of the constitutive pyruvate decarboxylase promoter isolated from *Zymomonas mobilis*. All constructed plasmids were verified by DNA sequencing (Quintara Biosciences, CA).

In order to investigate the effect of strain selection on 3AB biosynthesis, 10 *E. coli* strains with different genotypes were chosen to compose 10 co-culture systems. Specifically, with the exception of XL2-Blue and XL10-Gold, these strains were transformed with plasmids pdc-VS and pGro7 (Clontech Laboratories, CA) to confer the kanamycin and chloramphenicol resistance. Both XL2-Blue and XL10-Gold have the chloramphenicol resistance genes integrated

in the chromosome and were only transformed with pdc-VS. All 10 transformed strains were co-cultivated with *E. coli* P5.2 that had been previously engineered to provide DHS precursor [9, 15]. Plasmid pGro7 also contained *E. coli* chaperone genes, but these genes' expression was completely un-induced in this study. Transformations were conducted according to the manufacturer's protocols. All plasmids and strains constructed for this study is summarized in Table 1.

2.3 3AB biosynthesis

For 3AB biosynthesis using mono-cultures, *E. coli* P5V or P5cV was first grown in LB medium at 37 °C. 40 μL of overnight LB culture was then inoculated into 2 mL glucose medium contained in 16 ml culture tube, followed by three-day cultivation at 250 rpm under 37 °C. For 3AB biosynthesis using *E. coli* P5V, 0.1 mM IPTG was supplemented at the time of inoculation.

For 3AB biosynthesis using co-cultures, *E. coli* P5.2 and the selected downstream cells were first separately grown in the LB medium at 37 °C overnight. The cell density of these cultures were measured individually. For cultivation in culture tube, 40 μL of overnight *E. coli* P5.2 LB culture and appropriate amounts of overnight culture of one of the ten downstream strains were inoculated to 2 ml glucose medium in amounts chosen such as to constitute a desired inoculation ratio. For shake flask cultivation, 30 ml glucose medium was inoculated with 600 μL of P5.2 culture and 900 μL XL10-VS culture. After bioproduction was completed, 3AB concentrations of these co-culture systems were analyzed for comparison. The real-time ratios of the two constituent cells in the P5.2:XL10-VS co-culture were determined by a phenotype assay. Specifically, the co-culture samples were diluted using sterile water and grown on a LB agar plate containing 50 mg/L kanamycin. 30 colonies were randomly picked from the plate and re-

streaked on a new LB agar plate containing 10 mg/L tetracycline. XL10-VS could resist both kanamycin and tetracycline, whereas P5.2 could only grow on kanamycin plates. Therefore, the colonies that grew on both plates were XL10-VS, and its ratio to P5.2 could be calculated accordingly.

2.4 Analytical methods

Cell growth was monitored by measuring cultures' optical density at 600 nm (OD_{600}). DHS and 3AB quantification was performed based on a previously described method [15]. Specifically, cell cultures were centrifuged at 13,000 g for 2 min, and filtered through 0.2-μm-pore-size polytetrafluoroethylene membrane syringe filters (VWR International). 1 mL of the resultant cell-free broth was then supplemented with 10 μL 1 g/L *p*-coumaric acid internal standard, followed by LC/MS/MS analysis by an Applied Biosystems API2000 system equipped with a Waters symmetry 5 μm C8 column.

3 Results

3.1 Low 3AB Biosynthesis by *E. coli* mono-cultures

The complete 3AB biosynthetic pathway is composed of, (a) a pathway for the formation of the precursor DHS via the *E. coli* endogenous shikimate pathway, and, (b) the single step for DHS-to-3AB conversion via the heterologous 3AB synthase PctV isolated from *Streptomyces pactum* (Fig. 1A). We first constructed *E. coli* strain P5.1 that carried the engineered shikimate pathway genes in the chromosome as well as a plasmid for enhancing the shikimate pathway

flux. The exact mechanism by which this mutant plasmid, isolated by applying global transcription machinery engineering [9], enhances the shikimate pathway flux is not understood. However, it has been shown that this strain is capable of over-producing the shikimate pathway intermediate DHS. Subsequently, we over-expressed the codon-optimized PctV enzyme in P5.1 to achieve *de novo* biosynthesis of 3AB from glucose. In order to address the issue of DHS extracellular secretion that has been identified before, we also over-expressed an *E. coli* membrane-bound DHS importer, the ShiA permease [15, 19, 20]. The inducible T7 promoter was utilized to express PctV as well as ShiA in strain P5.1.

The resulting strain P5v produced merely 1.5 mg/L of 3AB at 37 °C, whereas DHS accumulated to 51 mg/L. Since heterologous enzyme folding and activity in *E. coli* can be affected by expression temperature, we also investigated 3AB production at lower temperatures. At 30 °C, 3AB production was reduced to 0.7 mg/L, and accumulation of 55 mg/L DHS was observed. At 20 °C, 3AB and DHS concentrations reached 0.3 and 68 mg/L, respectively. As lowering cultivation temperature did not help PctV activity, 37°C was chosen as the cultivation temperature for all subsequent experiments. In order to remove the need for inducible promoter, we also constructed a new strain P5cV that used a constitutive *Zymomonas mobilis* pyruvate decarboxylase promoter for expression of PctV and ShiA. Such constitutive expression strategy generate a comparable 3AB production of 1.4 mg/L.

3.2 Improvement and optimization of 3AB Biosynthesis by *E. coli*-*E. coli* co-cultures

In light of the low 3AB production by P5v mono-culture, we introduced the previously reported *E. coli*-*E. coli* co-culture strategy to improve biosynthetic performance. Specifically, we

divided the complete biosynthetic pathway into two modules. The upstream module was accommodated in *E. coli* strain P5.2 (*E. coli* P5.1 carrying a kanamycin-resistant plasmid) which was previously engineered for DHS formation; the downstream module containing the 3AB synthase PctV and the DHS transporter ShiA was accommodated in a separate *E. coli* strain.

It has been previously shown that co-culture engineering can provide important advantages in the production of important biochemicals [15]. Here, we further show that co-culture engineering can also facilitate effective selection of appropriate *E. coli* hosts that best accommodate a desired biosynthetic module. To this end, we screened 10 different *E. coli* strains as hosts that contain the downstream module of 3AB biosynthesis. Because of different genotypes, these *E. coli* strains have varying metabolic capabilities for supporting biosynthesis of different metabolites. For example, BL21(DE3) is generally considered as an expression host for heterologous enzymes. BL21 Star (DE3) has a mutated RNaseE enzyme that helps improve mRNA stability and increase target protein yield. SHuffle T7 is a strain engineered to promote the folding of disulfide-bonded proteins. Thus, the variation of these strains' genotype can generate different 3AB production performance in the co-culture systems. Notably, employment of the co-culture approach helped us eliminate the need to engineer the pathway for the synthesis of the precursor in each strain. Specifically, we did not have to reconstruct the shikimate pathway in all 10 *E. coli* strains to over-produce the precursor DHS for making 3AB, which is clearly an important benefit of co-culture engineering.

Next, the constitutive expression plasmid pdc-VS carrying *pctV* and *shiA* genes, was introduced into *E. coli* strains JM109, K12 (DE3), Genehogs, XL1-Blue, XL2-Blue, XL10-Gold, BW25113, BL21(DE3), BL21 Star (DE3) and SHuffle T7, respectively. The resulting strains carrying the downstream module of the pathway, including JM109-VS, K12-VS, Genehogs-VS,

XL1-VS, XL2-VS, XL10-VS, BW-VS, BL-VS, STAR-VS, SH-VS, were grown together with the upstream cell P5.2 at an inoculation ratio of 1:2 (upstream:downstream) for 3AB production. Both upstream and downstream strains were resistant to kanamycin and chloramphenicol, allowing for coordinated growth under the same selective pressure. In order to properly compare the 3AB biosynthesis by these *E. coli-E.coli* co-cultures, the 3AB concentration was normalized by the co-culture cell density. As shown in Fig. 1B, normalized production of 3AB varied greatly among the 10 downstream hosts. The best performer for expressing the downstream module was XL10-VS (XL10-Gold carrying the 3AB synthase and the ShiA transporter) whose specific 3AB production reached 3.4 mg/L/OD in a 2 ml test tube culture. Surprisingly, no DHS accumulated in the P5.2:XL10-VS co-culture at the end of cultivation. In contrast, use of the other downstream *E. coli* strains resulted in significantly higher DHS accumulation and lower 3AB concentrations, although the production profiles of these strains were also quite different from each other. It should be noted that, although there was clear 3AB production improvement between P5v mono-culture and P5.2:XL10-VS co-culture, we did not have a control mono-culture of XL10-Gold containing the complete 3AB biosynthesis pathway. While such control would be a proper control to better indicate the efficacy of the co-culture, the re-construction of XL10-Gold to build similar biosynthetic capability to *E. coli* P5 was highly challenging and was not performed due to the complexities in strain engineering.

We proceeded to optimize the P5.2:XL10-VS co-culture system to further improve 3AB production. To this end, different inoculation ratios between P5.2 and XL10-VS were investigated. As shown in Fig. 2A, 3AB concentration as well as specific production level indeed fluctuated under four different inoculation conditions which decided the relative strength of the upstream and downstream biosynthetic modules. The highest 3AB production of 22 mg/L was

achieved at the inoculation ratio of 1:1.5. Compared with the mono-culture strategy, the 3AB concentration was increased by15-fold. Notably, there was no residual DHS under all inoculation ratios.

The 3AB biosynthesis was then carried out in shake flask to further improve the overall production performance. Specifically, *E. coli* P5.2 and XL10-VS were inoculated at the determined optimal ratio of 1:1.5 into the glucose medium, followed by 6 days' cultivation. As shown in Fig. 2B, the cell density and 3AB titer in the co-culture system both increased gradually with time. The highest 3AB production reached 48 mg/L at day 6, which is 2 times higher than the test tube production. The XL10-VS percentage of the whole population fluctuated only between 60% and 70% throughout the production process, indicating the stable nature of the P5.2:XL10-VS co-culture system.

4 Discussion

Using the previously identified 3AB synthase, we achieved heterologous 3AB biosynthesis in engineered *E. coli*. The complete biosynthetic pathway was first reconstituted in one single *E. coli* cell. However, the low activity of 3AB synthase PctV resulted in poor DHS-to-3AB conversion, although there was high level accumulation of precursor DHS. In order to improve 3AB production, we utilized an *E. coli*-*E. coli* co-culture system comprising one strain for DHS provision and a second one for completion of the DHS-to-3AB conversion. This strategy allowed us to rapidly screen a variety of different *E. coli* strains, and identify the best performer XL10-Gold which helped increase the 3AB final concentration.

XL10-Gold differs from other *E. coli* strains in that it possesses high competency for receiving large foreign DNA molecules (the Hte phenotype), a property that could be hardly associated with high DHS conversion capability. Other genotypic features of XL10-Gold, such as *supE44* (reducing the undesired nonsense translation termination) and *recA1* (increasing plasmid stability), are not unique compared with the other nine screened strains. The exact reason why XL10-Gold is a superior strain for supporting the 3AB biosynthesis remains unclear. On the other hand, the other 9 strains also showed varied 3AB production performance and DHS accumulation. These findings demonstrate that the genotypic difference between *E. coli* strains can lead to largely altered capability for supporting heterologous enzyme's activity and hence altered biosynthetic performance. Therefore, it is further demonstrated that the success of a biosynthetic process is dependent upon selection of appropriate *E. coli* hosts. To this end, co-culture engineering shows outstanding advantage, as its modular nature allows for rapid and effective identification of the best strains for one particular enzyme or a pathway module, as opposed to laborious reconstruction of the entire biosynthetic pathway in all strains to be screened. It should be noted that imbalanced growth rate and growth competition between the constituent cells in the co-culture could lead to domination of the population by one cell. The employment of two *E. coli* strains, rather than two different species, helped address the issue of one co-culture member outgrowing the other. In this study, this strategy resulted in maintenance of the cell-to-cell ratio within an acceptable range. In conclusion, the biosynthesis improvement achieved in the present study highlights this approach's potential for heterologous production of value-added biochemicals.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Table 1 Plasmids and *E. coli* strains used in this study

Plasmid	Description	Source
pET28a	T7 promoter, Kan^R	Novagen
pHACM-rpoA14	carrying a mutated alpha subunit of RNA polymerase for enhancing the shikimate pathway	[9]
pET28-V	pET28a carrying the codon-optimized *pctV* gene from *Streptomyces pactum*	This study
pET28-VS	pET28-V carrying the *E. coli shiA* gene	This study
pCYZ	carrying a constitutive *Zymomonas mobilis* pyruvate decarboxylase promoter, Kan^R	[15]
pdc-VS	pCYZ carrying the *pctV* and *shiA* gene under the control the constitutive promoter	This study
pII	low copy number plasmid expressing weak green fluorecence protein, Kan^R	[21]
pGro7	carrying *E. coli* chaperone groES-groEL genes, CmR (*)	Clontech
Strain	Description	Source
P5	*E. coli* K12 Δ*pheA* Δ*tyrRlacZ*::$P_{LtetO-1}$-$tyrA^{fbr}aroG^{fbr}$ *tyrR*::$P_{LtetO-1}$-$tyrA^{fbr}aroG^{fbr}$ *hisH*(L82R) Δ*aroE* Δ*ydiB*	[15]
P5.1	P5 carrying plasmid pHACM-rpoA14	This study
P5.2	P5.1 harboring plasmid pII	[15]
P5V	P5.1 containing pET28-VS	This study
P5cV	P5.1 containing pdc-VS	This study

JM109	F$^-$ *traD36 proA$^+$B$^+$ lacI^q Δ(lacZ)M15/ Δ(lac-proAB) glnV44 e14$^-$ gyrA96 recA1 relA1 endA1 thi hsdR17*	NEB
K12(DE3)	F$^-$ *λ$^-$ ilvG- rfb-50 rph-1*	[9]
Genehogs	F$^-$ *endA1 mcrA* Δ*(mrr-hsdRMS-mcrBC) φ80lacZ* Δ*M15* Δ*lacX74 recA1 araD139* Δ*(ara-leu)7697 galU galK rpsL (Str^R) nupG fhuA::*IS2	Life Technologies
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac F′[proAB lacIqZΔM15 Tn10 (Tet^R)]*	Stratagene
XL2-Blue	*endA1 supE44 thi-1 hsdR17 recA1 gyrA96 relA1 lac [F´ proAB lacIqZΔM15 Tn10 (Tet^R) Amy Cm^R]*	Stratagene
XL10-Gold	*endA1 glnV44 recA1 thi-1 gyrA96 relA1 lac Hte Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 Tet^R F'[proAB lacI^{q}ZΔM15 Tn10(Tet^R) Amy Cm^R]*	Stratagene
BW25113	*Δ(araD-araB)567 Δ(rhaD-rhaB)568 ΔlacZ4787 (::rrnB-3) hsdR514 rph-1*	CGSC at Yale University
BL21(DE3)	F$^-$ *ompT gal dcm lon hsdS$_B$(r$_B^-$ m$_B^-$) (DE3)*	NEB
BL21 Star (DE3)	F$^-$ *ompT hsdSB (r$_B$-, m$_B$-) gal dcm rne131 (DE3)*	Life Technologies
SHuffle T7	F$^-$ *lac, pro, lacIQ / Δ(ara-leu)7697 araD139 fhuA2 lacZ::T7 gene1 Δ(phoA)PvuII phoR ahpC* galE (or U) galK λatt::*pNEB3-r1-*cDsbC (*Spec^R, *lacI^q) ΔtrxB rpsL150(*Str^R*) Δgor Δ(malF)3*	NEB
JM109-VS	JM109 containing plasmid pdc-VS and pGro7	This study
K12-VS	K12(DE3) containing plasmid pdc-VS and pGro7	This study
Genehogs-VS	Genehos containing plasmid pdc-VS and pGro7	This study
XL1-VS	XL1-Blue containing plasmid pdc-VS and pGro7	This study
XL2-VS	XL2-Blue containing plasmid pdc-VS	This study
XL10-VS	XL10-Gold containing plasmid pdc-VS	This study
BW-VS	BW25113 containing plasmid pdc-VS and pGro7	This study
BL-VS	BL21(DE3) containing plasmid pdc-VS and pGro7	This study
STAR-VS	BL21 Star (DE3)containing plasmid pdc-VS and pGro7	This study
SH-VS	SHuffle T7 containing plasmid pdc-VS and pGro7	This study

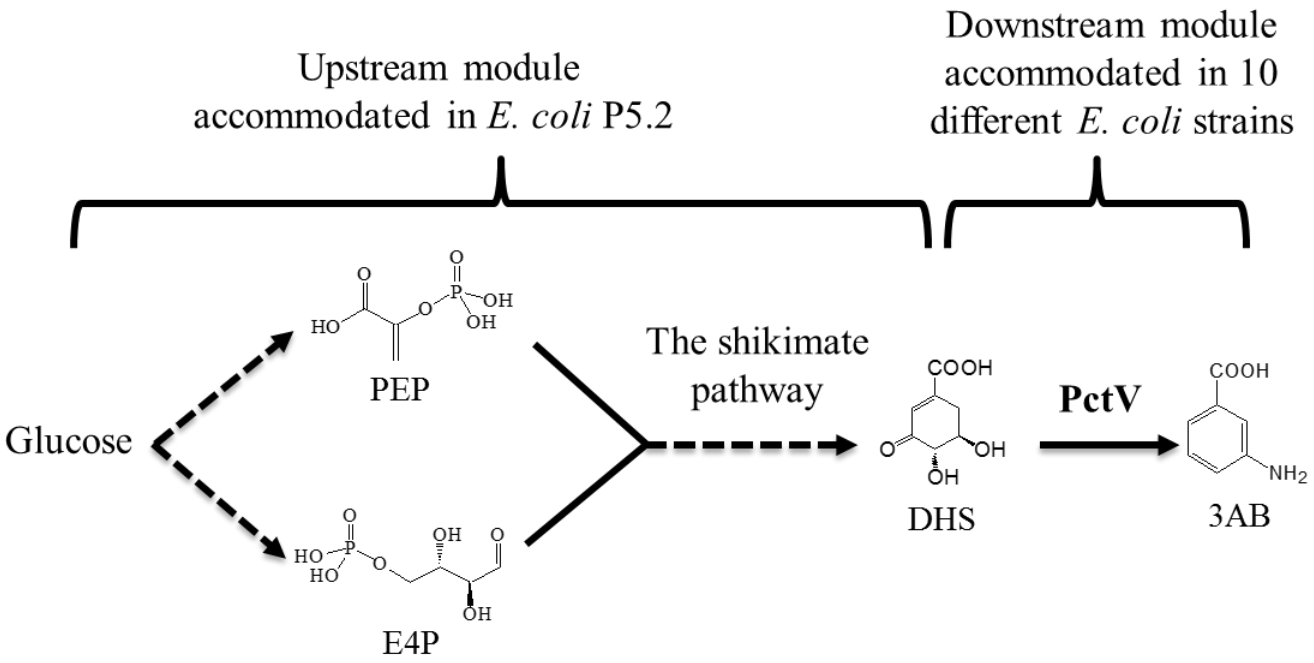
* pGro7 was used primarily for conferring the chloramphenicol resistance to *E. coli*; no chaperone gene expression was induced.

Figure Legends

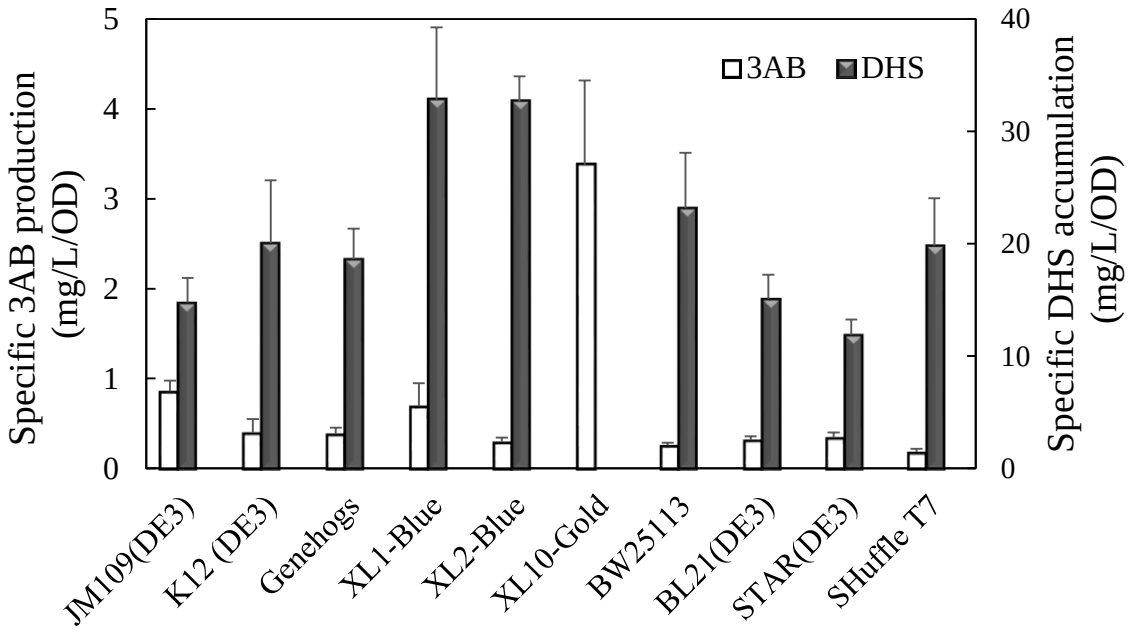
Figure 1. Design and application of co-culture engineering strategy for 3AB biosynthesis. (**A**) Diagram of 3AB biosynthesis pathway. The whole pathway is divided into the upstream module and the downstream module. The upstream module is responsible for making DHS from glucose, whereas the downstream module is responsible for converting DHS to the final product of 3AB. (**B**) Identification of appropriate downstream *E. coli* strains for 3AB biosynthesis. 10 *E. coli* strains harboring the downstream module of the 3AB biosynthetic pathway were co-cultured with the upstream strain P5.2 at an inoculation ratio of 1:2. The production was normalized by the cell-density of the co-cultures for comparison. Error bars represent s.e.m. from three repeat experiments.

Figure 2. Engineering of P5.2:XL10-VS co-culture for improving 3AB production. (**A**) Optimization of the P5.2:XL10-VS inoculation ratio for 3AB production. *E. coli* P5.2 and XL10-VS were inoculated into the co-culture system with the inoculation ratio of 1:0.5, 1:1, 1:1.5 and 1:2. No DHS intermediate was observed at any of the inoculation ratio. (**B**) 3AB production by P5.2: XL10-VS co-culture in shake flask. Time profiles of 3AB concentration, cell density and XL10-VS percentage are represented by circle, square and triangle, respectively. P5.2 and XL10-VS was inoculated at a ratio of 1:1.5. Error bars represent s.e.m. from three repeat experiments.

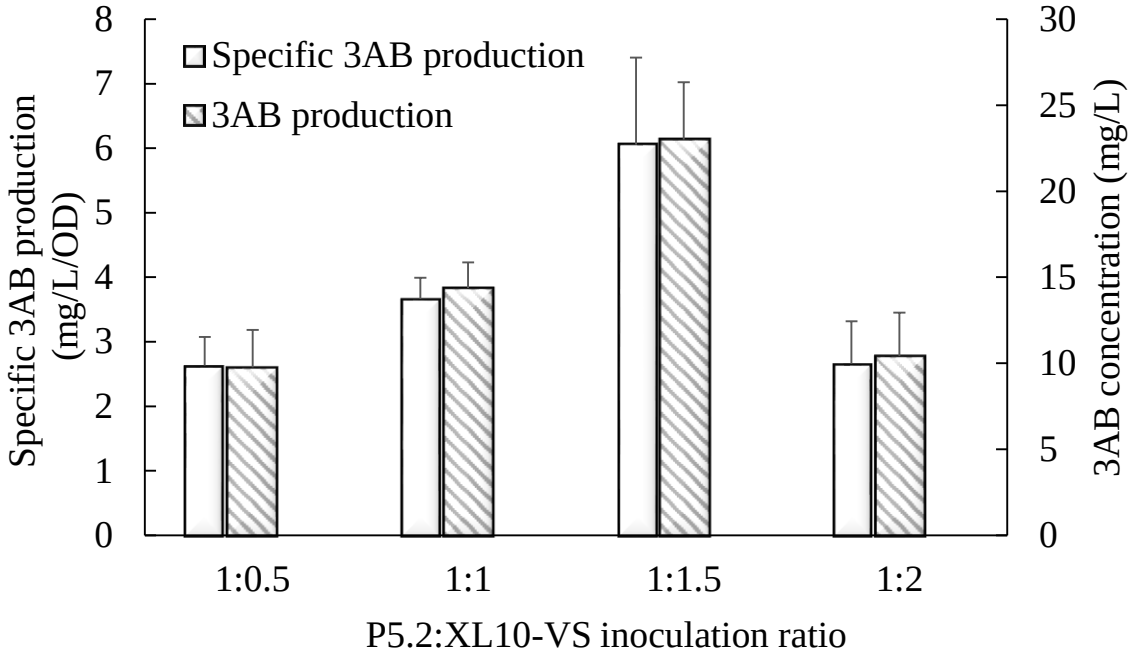
A


Upstream module
accommodated in E. coli P5.2
Downstream module
accommodated in 10
different E. coli strains
Glucose
PEP
E4P
The shikimate
pathway
DHS
PctV
3AB

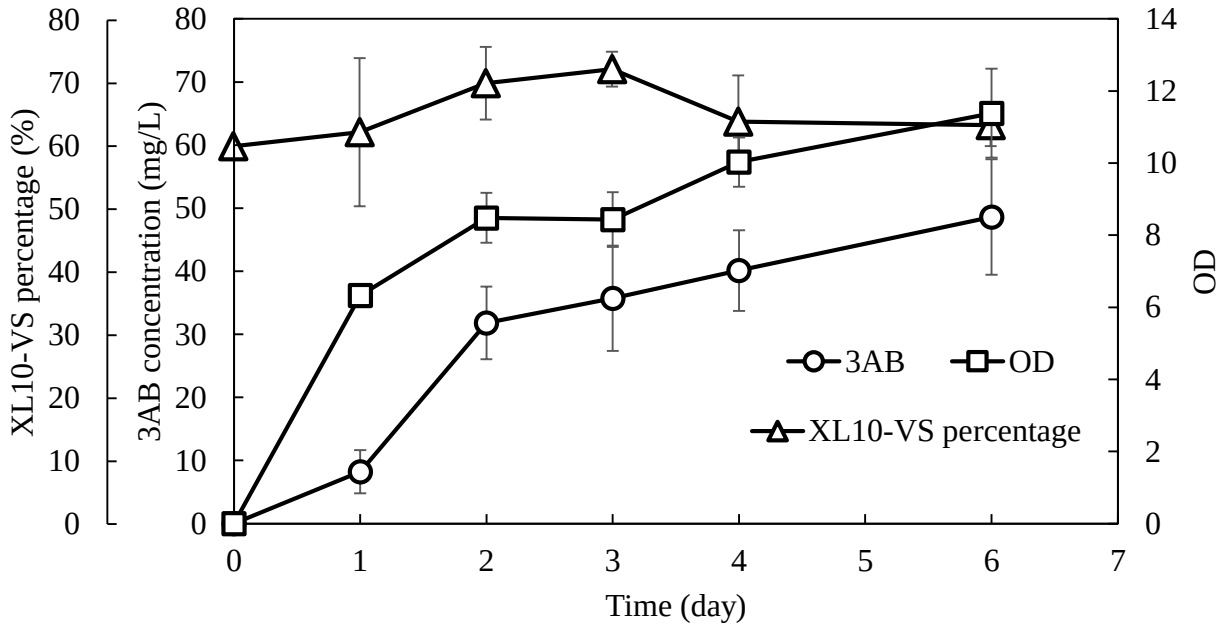
B


Specific 3AB production
(mg/L/OD)
Specific DHS accumulation
(mg/L/OD)
3AB
DHS
0
1
2
3
4
5
0
10
20
30
40
JM109(DE3)
K12 (DE3)
Genehogs
XL1-Blue
XL2-Blue
XL10-Gold
BW25113
BL21(DE3)
STAR(DE3)
SHuffle T7

A


Specific 3AB production
3AB production
Specific 3AB production (mg/L/OD)
3AB concentration (mg/L)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
1:0.5
1:1
1:1.5
1:2
P5.2:XL10-VS inoculation ratio

B


XL10-VS percentage (%)
3AB concentration (mg/L)
OD
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
7
Time (day)
3AB
OD
XL10-VS percentage