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Effects of *tktA*, *aroF*^{FBR}, and *aroL* Expression in the Tryptophan-Producing *Escherichia coli*

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Abstract In order to analyze the effects of *tktA*, *aroF*^{FBR}, and *aroL* expression in a tryptophan-producing *Escherichia coli*, a series of plasmids carrying the genes were constructed. Introduction of *tktA*, *aroF*^{FBR}, and *aroL* into the *E. coli* strain resulted in approximately 10- 20 fold increase in the activities of transketolase, the feedback inhibition-resistant 3-deoxy-D-arabinoheptulsonate-7-phosphate synthase, and shikimate kinase. Expression of *aroF*^{FBR} in the *aroB* mutant strain of *E. coli* resulted in the accumulation of 10 mM of 3-deoxy-D-arabinoheptulsonate-7-phosphate (DAHP) in the medium. Simultaneous expression of *tktA* and *aroF*^{FBR} in the strain further increased the amount of excreted DAHP to 20 mM. In contrast, the mutant strain which has no gene introduced accumulated 0.5 mM of DAHP. However, the expression of *tktA* and *aroF*^{FBR} in a tryptophan-producing *E. coli* strain did not lead to the increased production of tryptophan, but instead, a significant amount of shikimate, which is an intermediate in the tryptophan biosynthetic pathway, was excreted to the growth medium. Despite the fact that additional expression of shikimate kinase in the strain could possibly remove 90% of excreted shikimate to 0.1 mM, the amount of tryptophan produced was still unchanged. Removing shikimate using a cloned *aroL* gene caused the excretion of glutamate, which suggests disturbed central carbon metabolism. However, when cultivated in a complex medium, the strain expressing *tktA*, *aroF*^{FBR}, and *aroL* produced more tryptophan than the parental strain. These data indicate that additional rate-limiting steps are present in the tryptophan biosynthetic pathway, and the carbon flow to the terminal pathway is strictly regulated. Expressing *tktA* in *E. coli* cells appeared to impose a great metabolic burden to the cells as evidenced by retarded cell growth in the defined medium. Recombinant *E. coli* strains harboring plasmids which carry the *tktA* gene showed a tendency to segregate their plasmids almost completely within 24 h.

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Key words: *tktA*, *aroF*, *aroL*, transketolase, shikimate kinase, tryptophan, *Escherichia coli*

In *Escherichia coli*, tryptophan, phenylalanine, and tyrosine are synthesized via the common biosynthetic route known as the shikimate pathway (Fig. 1) [22]. The aromatic amino acids are essential for providing animal nutrition and also very important as feed additives. Although many amino acids are produced using microorganisms [21], aromatic amino acids are difficult to be produced by direct fermentation, since complex regulatory mechanisms of the biosynthetic pathway make strain manipulation difficult, and high demand of energy for the biosynthesis of aromatic amino acids imposes a severe metabolic burden to the host organism [10, 11]. Improving strains by manipulating the terminal biosynthetic pathways can be successful to only a certain extent [12, 13, 14, 26, 27]. The production rate and yield of desired amino acids are greatly limited by the availability of carbon that is supplied from the central metabolism [for reviews, see 7]. Draths *et al.* reported that the carbon flux into the aromatic amino acid pathway could be increased by the overexpression of transketolase, which is the key enzyme of the pentose-phosphate pathway (Fig. 1) [5]. Patnaik *et al.* also showed, rather indirectly, that transketolase, feedback inhibition-resistant 3-deoxy-D-arabinoheptulsonate-7-phosphate synthase (DAHP synthase^{FBR}), and phosphoenol pyruvate synthase could increase flux to the biosynthetic pathway of aromatic amino acids [18, 19]. Snell *et al.* observed that the expression of DAHP synthase^{FBR} and transketolase in *E. coli* results in the accumulation of various shikimate pathway intermediates, indicating that multiple steps of the shikimate pathway might be rate-limiting when biosynthetic precursors are supplied in a sufficient amount [22].

In this report, we investigated the effects of *tktA*, *aroF*^{FBR}, and *aroL* expression in the tryptophan-producing *E. coli*

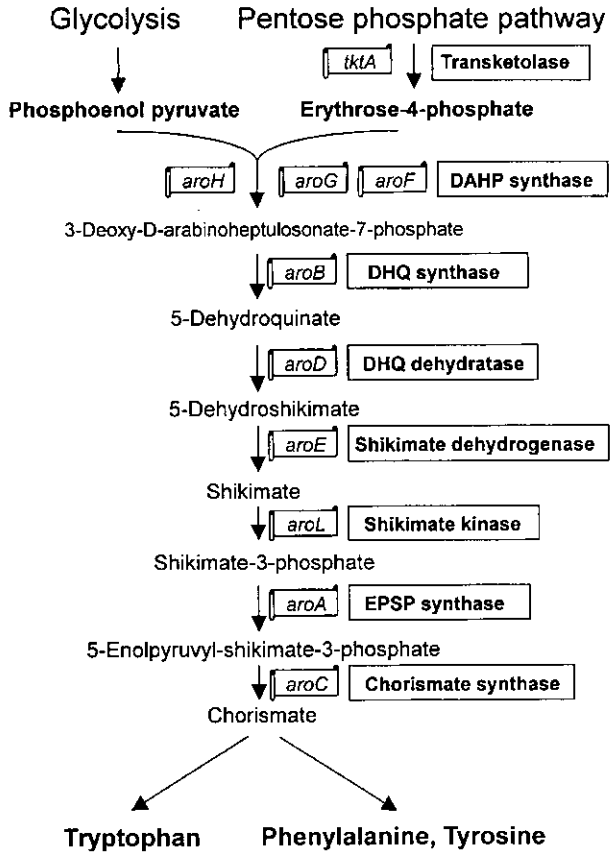

Fig. 1. Shikimate pathway and neighboring reactions for the biosynthesis of aromatic amino acids in *E. coli*.
Genes *tktA*, *aroF*, and *aroL* were specifically manipulated in this study. Abbreviations: DAHP, 3-Deoxy-D-arabinoheptulsonate-7-phosphate; DHQ, 5-Dehydroquinate; EPSP, 5-Enolpyruvyl-shikimate-3-phosphate.

CD42 strain. The genes encode transketolase, DAHP synthase^{FBR}, and shikimate kinase, respectively (Fig. 1). As described, genes *tktA* and *aroF*^{FBR} play critical roles in increasing carbon flux to the biosynthetic pathway of aromatic amino acids. We also look into the accumulation of shikimate pathway intermediates in the culture medium for the identification of potential rate-limiting steps. The physiological effect of transketolase expression in the *E. coli* CD42 is also reported.

MATERIALS AND METHODS

Bacterial Strains, Media, and Culture Conditions

Chromosomal DNA for the amplification of *tktA* and *aroL* genes was isolated from *E. coli* W3110 [1]. *E. coli* XL-1 Blue (Stratagene, U.S.A.) was used primarily for the construction and routine maintenance of plasmids. *E. coli* CD42 [8] is a derivative of *E. coli* CSH32 (*trpE*$^-$, *thi*$^-$; Cold Spring Harbor Laboratory). *E. coli* AB2847 [20] carries an *aroB351* mutation. *E. coli* strains were routinely grown in Luria-Bertani (LB) medium [15]. Tryptophan-production medium [8] contains 6% glucose, 2% $(NH_4)_2SO_4$, 0.4% K_2HPO_4, 0.4% K_2SO_4, 0.1% fumarate, 0.2% $MgCl_2$, 2.5% $CaCO_3$, and appropriate amount of trace elements. YE medium [5] contains 0.5% $(NH_4)_2SO_4$, 1.5% yeast extracts, 1.4% K_2HPO_4, 1.6% KH_2PO_4, 0.1% $MgSO_4$, and 1.5% glucose. Ampicillin was added to the final concentration of 100 μg/ml when it was necessary.

Construction of Plasmids

Plasmids used in this study are listed in Table 1. Plasmid carrying the *tktA* gene was constructed as follows: Gene *tktA* of *E. coli* W3110 was isolated by the polymerase chain reaction (PCR) using the primers of 5'GATCCAGA-GATTTCTGAAGCG3' and 5'AACACGCCTTATCTATTG3'. The primers were designed from the published *tktA* sequences [24]. The amplified 2.1-kb fragment containing the *tktA* ORF and the putative promoter region was cloned into the pCRII vector (Invitrogen, Carlsbad, U.S.A.). Gene *tktA* cloned into the vector was excised with *Bam*HI and *Xho*I, treated with Klenow fragments, and inserted into the *Eco*RI digested pAroF-PheA plasmid to yield pAroF-PheA-Tkt. Plasmid pAroF-PheA contained ColE1 replication origin, *aroF*^{FBR} preceded by the *lacI* promoter, *pheA*, and *lacI*. For the construction of plasmid pAroF-Tkt, plasmid pAroF-PheA-Tkt was digested with *Nco*I and *Not*I, treated with Klenow fragments, and self-ligated. Plasmid pAroF was generated by the deletion of *tktA* gene from pAroF-Tkt using *Eco*RI. Primers of 5'CCCGACCTTCACGTTGCA-TCA3' and 5'AGCTCGCGGATATGAATTGC3' were used for the PCR amplification of *aroL* and its promoter. The primers were designed from the published sequences [3,

Table 1. Plasmids used in this study.

Plasmids	Characteristics	References
pCRII	Cloning vector for PCR products (Ap^r, Km^r)	Invitrogen
pDT1	pDT720 derivative containing ColE1 origin, *lacZ*, and *cer*, a plasmid partitioning locus	[8]
pAroF-PheA	pDT1 carrying P_{lac}*aroF*^{FBR}, *pheA*^{FBR}, and *lacI*	[16]
pAroF	pDT1 carrying P_{lac}*aroF*^{FBR} and *lacI*	This study
pCR-AroL	pCRII carrying *aroL*	This study
pAroF-Tkt	pDT1 carrying P_{lac}*aroF*^{FBR}, *tktA*, and *lacI*	This study
pAroF-AroL	pDT1 carrying P_{lac}*aroF*^{FBR}, *aroL*, and *lacI*	This study
pAroF-Tkt-AroL	pDT1 carrying P_{lac}*aroF*^{FBR}, *tktA*, *aroL*, and *lacI*	This study

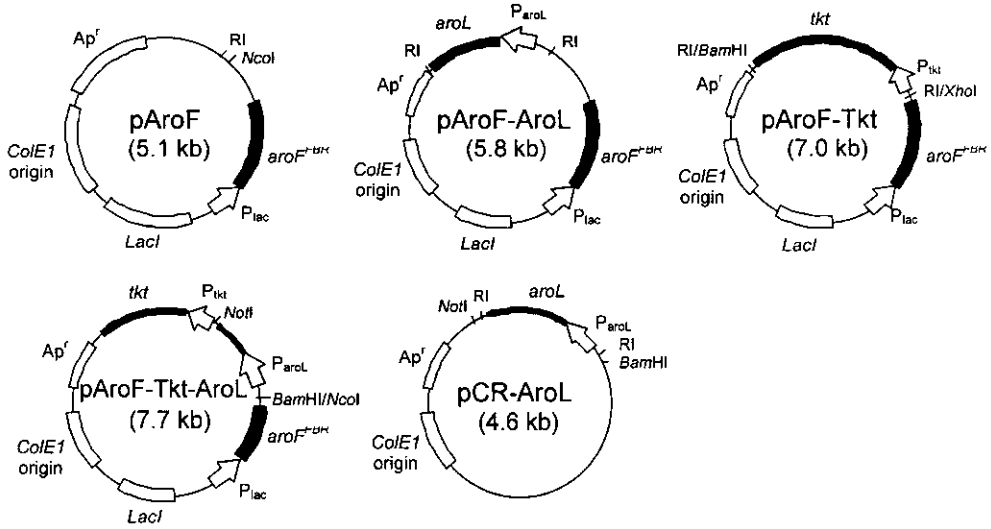

Fig. 2. Schematic illustration of recombinant plasmids used in this study.
Plasmids were constructed as described in the Materials and Methods. Abbreviation: RI, *Eco*RI; FBR, Feedback inhibition resistance; P_{lac}, *lac*UV5 promoter; P_{aroL}, promoter of *aroL* gene; P_{tkt}, promoter of *tktA* gene; Ap', ampicillin resistance.

4]. The resulting PCR product of 0.7-kb was cloned into the pCRII vector to yield pCR-AroL. Plasmid pAroF-Tkt-AroL was constructed as follows: Plasmid pCR-AroL was digested with *Not*I, treated with T4 DNA polymerase, and digested with *Bam*HI and the appropriate fragment was ligated into plasmid pAroF-Tkt. Plasmid pAroF-AroL was constructed by inserting *Eco*RI-digested *aroL* fragment of pCR-AroL into pAroF which was previously digested with the same restriction enzyme. The DNA fragments isolated by PCR were sequenced using an Amplitaq^{FS} sequencing kit (Perkin Elmer, Norwalk, U.S.A.) and ABI 310 genetic analyzer (Applied Biosystem Inc., Foster city, U.S.A.). Schematic illustration of plasmids constructed in this study is shown in Fig. 2.

Enzyme Assay

Shikimate kinase was assayed as described by Moldave and Coggins [2]. Cell extracts were prepared as follows. *E. coli* strains harboring various recombinant plasmids were cultivated in YE or tryptophan-production media for 12 h at 37°C. When necessary, IPTG was added to a final concentration of 0.1 mM at the OD_{600} of 0.5. Cells were harvested by centrifugation, washed twice with 25 mM of diethanolamine-HCl buffer (pH 9.0), and disrupted by ultrasonic treatment. Cell debris were removed by centrifugation at 20,000 ×g for 10 min. Shikimate kinase was assayed in a 0.5 ml mixture containing 0.1 M of shikimate, 0.5 M of $MgCl_2$, 1 M of NaF, 0.5 M of ATP, and 0.1 M of DTT. The reaction was stopped by adding 10% TCA and protein precipitates were removed by centrifugation at 20,000 ×g for 10 min. The supernatant was filtered, injected into an HPX-87H anion exchange column (Bio-Rad, U.S.A.), and separated in the Alliance HPLC System (Waters, Milford, U.S.A.) at the flow rate of 0.5 ml/min using mobile phase of 4 mM H_2SO_4. The peak area corresponding to shikimate was used for the calculation of the activity. One unit of enzyme oxidized 1 μmol of shikimate to shikimate-5-phosphate per 1 min. The activity of DAHP synthase^{FBR} was measured as previously described [6]. Transketolase was assayed by the method described by Iida *et al.* [9].

Fermentation and Determination of Metabolites

For the shake flask fermentation, cells were grown aerobically in 500 ml baffled flasks containing 50 ml of YE or tryptophan-production medium. When necessary, IPTG was added to a final concentration of 0.1 mM at the OD_{600} of 0.5. Samples of each 1 ml were taken and analyzed for cell growth. For the analysis of metabolic intermediates, 0.5 ml of the culture supernatant was taken, filtered through a 0.22 μm acetate filter, and analyzed by HPLC as described above. Tryptophan and DAHP were measured as described [6, 23]. Organic acids were measured as described [25]. Reagents including shikimate, erythrose-4-phosphate, ribose-5-phosphate, and xylose-1-phosphate were purchased from Sigma Co. (St. Louis, U.S.A.).

Determination of Plasmid Stability

E. coli CD42 strains harboring various recombinant plasmids were grown in the antibiotic-free tryptophan-production media and samples were taken at an appropriate time interval. LB plates with no added antibiotics were spread with the sample and incubated at 37°C for 24 h. Colonies of 100 were randomly chosen and patched onto LB plates containing ampicillin to identify colonies which are sensitive to the antibiotics.

Table 2. Specific activities of transketolase, DAHP synthase^{FBR}, and shikimate kinase in *E. coli* CD42 strains harboring recombinant plasmids[1].

Strains	Specific activity (Units/mg)		
	DAHP synthase^{FBR}	Transketolase	Shikimate kinase
E. coli CD42	0.43	0.20	0.31
E. coli CD42/pAroF	5.03	0.56	0.27
E. coli CD42/pAroF-Tkt	4.32	4.59	0.20
E. coli CD42/pAroF-Tkt-AroL	4.53	4.18	3.86
E. coli CD42/pAroF-AroL	4.60	0.41	3.49
E. coli CD42/pCR-AroL	0.38	0.38	3.73

[1]Each strain was cultivated in the 50 ml of tryptophan-production medium for 18 h. Cells were harvested, disrupted, and assayed for the activity as described in the Materials and Methods.

RESULTS AND DISCUSSION

Expression of *tktA* and *aroF*^{FBR} Genes Results in the Accumulation of Shikimate in Tryptophan-Producing *E. coli*

We tested the effects of expressing transketolase and DAHP synthase^{FBR} in tryptophan-producing *E. coli* CD42 strain. For this purpose, we constructed plasmids pAroF expressing DAHP synthase^{FBR} and pAroF-Tkt expressing both DAHP synthase^{FBR} and transketolase (Table 1). As shown in Table 2, introduction of plasmids pAroF and pAroF-Tkt into *E. coli* CD42 resulted in an approximately 10-fold and 20-fold increase in the specific activities of DAHP synthase^{FBR} and transketolase, respectively, which suggests the efficient expression of the introduced genes. To analyze the effect of *tktA* and *aroF*^{FBR} expression on tryptophan production, we analyzed the recombinant strains by using the shake-flask fermentation. The results indicated that the growth of the recombinant strains in the tryptophan-production medium was severely affected (see below) and the amount of tryptophan produced in the medium was lower than that of the *E. coli* CD42 strain (Fig. 6). To ensure an appropriate carbon flow to the shikimate pathway, the plasmids were introduced into *E. coli* AB2847 strain which carried an *aroB* mutation and then it was analyzed for the accumulation of DAHP. In theory, increased carbon flow to the shikimate pathway in the *aroB* mutant strain should be reflected by an increase in the excreted DAHP [5, 17, 18, 19]. As shown in Fig. 3, *E. coli* AB2847 carrying pAroF, thus expressing DAHP synthase^{FBR}, accumulated about 10 mM of DAHP. Simultaneous expression of *tktA* and *aroF*^{FBR} using pAroF-Tkt in the strain further increased the amount of the excreted DAHP as much as 20 mM. The mutant strain by itself accumulated 0.5 mM of DAHP. The final cell yields were almost comparable for all three strains. These results are in good agreement with the findings of Draths *et al.* [5], in which they suggest that the carbon flux to the shikimate pathway could be increased by the expression of transketolase and DAHP synthase^{FBR}.

Despite the efficient expression of transketolase and DAHP synthase^{FBR}, we postulated that a certain metabolic step leading to tryptophan might be a rate-limiting due to the fact that any increase in the excreted tryptophan was not observed. Previous report indicated that, when DAHP synthase^{FBR} was overexpressed in wild type *E. coli*, reaction catalyzed by 3-dehydroquinate synthase became the rate-limiting step for the biosynthesis of aromatic amino acid [17]. For this reason, we analyzed the culture supernatants for the presence of metabolic intermediates excreted during the fermentation process. As shown in Fig. 4, *E. coli* CD42 expressing DAHP synthase^{FBR} accumulated 0.5 mM of shikimate (Panel B in Fig. 4). Additional expression of transketolase using plasmid pAroF-Tkt further increased the amount of accumulated shikimate to 1.4 mM (Panel C in Fig. 4). Interestingly almost no detectable amount of shikimate was accumulated in the supernatant of *E. coli* CD42 strain lacking the plasmids (Panel A in Fig. 4). These results indicate that, when transketolase and DAHP

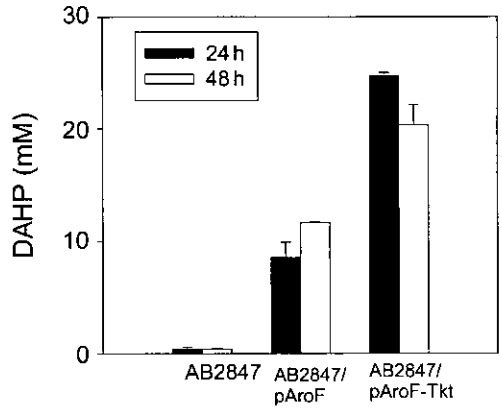

Fig. 3. Accumulation of 3-deoxy-D-arabinoheptulsonate-7-phosphate (DAHP) by *E. coli* AB2847.

The strain carries a mutation in the *aroB* gene. AB2847/pAroF and AB2847/pAroF-Tkt designate *E. coli* AB2847 strains carrying pAroF and pAroF-Tkt, respectively. Each strain was cultivated in a 50 ml of YE medium [5] supplemented with 6% glucose for 24 or 48 h. DAHP present in the culture medium was measured as described in the Materials and Methods.

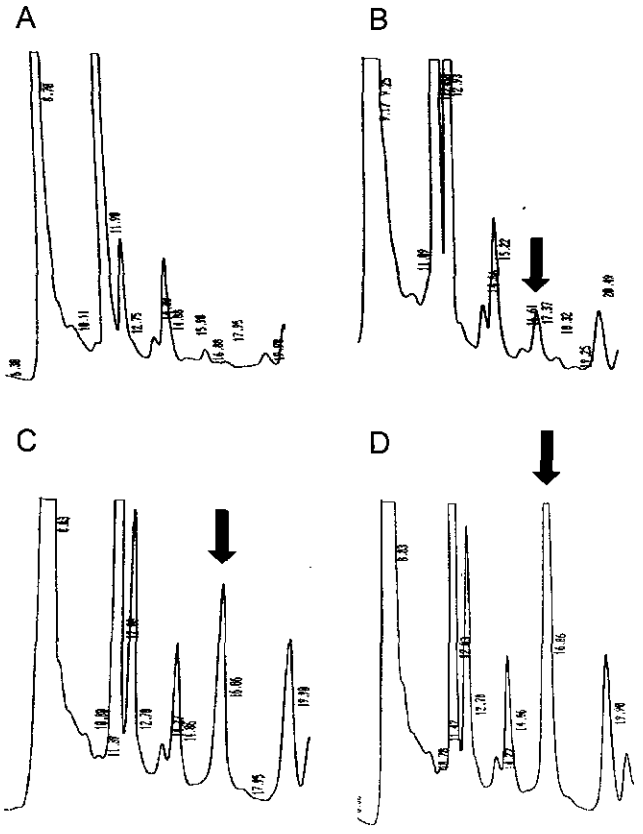

Fig. 4. Accumulation of shikimate by *E. coli* CD42.
E. coli CD42 harboring various recombinant plasmids were cultivated in the 50 ml of YE medium supplemented with 1.5% glucose for 24 h. Culture supernatant was analyzed with HPLC for organic acids. Panels: A, *E. coli* CD42; B, *E. coli* CD42/pAroF; C, *E. coli* CD42/pAroF-Tkt; D, *E. coli* CD42/pAroF-Tkt spiked with 1 mM shikimate. Peaks corresponding to shikimate are marked by arrows.

synthase^{FBR} are expressed, the reaction catalyzed by shikimate kinase becomes a rate-limiting in the strain. In fact, this may explain indirectly the reason for the lack of an increase in the amount of the excreted tryptophan observed in the *E. coli* CD42 strain carrying pAroF or pAroF-Tkt. The result may also explain partly the findings of Snell *et al.* who speculated the presence of multiple rate-limiting steps in the shikimate pathway [22]. With an exception of shikimate, no other metabolic intermediates of the shikimate pathway, such as chorismate, DAHP, and anthralinate, were detected in our experiments (data not shown).

Expression of *aroL* Decreases the Accumulated Shikimate

Since the expression of transketolase and DAHP synthase^{FBR} led to the accumulation of shikimate without affecting the tryptophan production, we speculated that the reaction step catalyzed by shikimate kinase might be a rate-limiting. Assuming that the expression of shikimate kinase in the strain may lead to the increased production of tryptophan, we cloned *aroL* gene which encodes shikimate kinase,

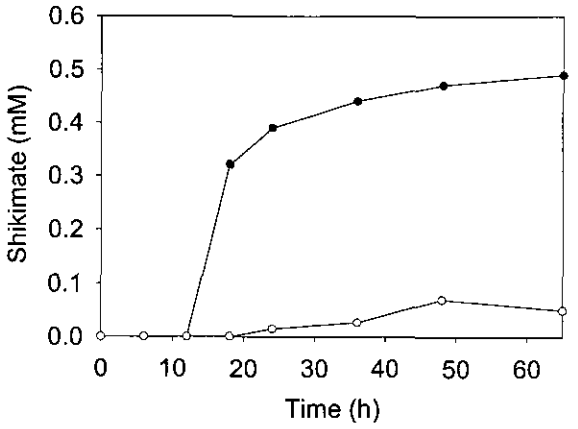

Fig. 5. Removal of shikimate by the expression of shikimate kinase.
E. coli CD42/pAroF-Tkt (●) and *E. coli* CD42/pAroF-Tkt-AroL (○) were cultivated in the 50 ml of tryptophan-production medium for 65 h and the amount of accumulated shikimate was analyzed with HPLC as described in the Materials and Methods.

and expressed its enzyme in *E. coli* CD42. As shown in Table 2, introduction of plasmids pAroF-AroL and pAroF-Tkt-AroL increased the activity of shikimate kinase approximately 10 fold as compared with that of the parental strain. As the next step, we analyzed the effect of shikimate kinase on tryptophan production in tryptophan-production medium. As shown in Fig. 5, the increased expression of shikimate kinase drastically lowered the amount of accumulated shikimate. *E. coli* CD42 carrying pAroF-Tkt-AroL accumulated less than 0.1 mM of shikimate after 48 h of cultivation. The result indicates that the accumulation of shikimate was caused by the insufficient amount of shikimate kinase and it could be removed by a high expression of the *aroL* gene.

Effects of *tktA*, *aroF*^{FBR}, and *aroL* Expression on Tryptophan Production and Cell Growth

We analyzed growth and tryptophan production by *E. coli* CD42 carrying plasmids pAroF, pAroF-Tkt, pCR-AroL, and pAroF-Tkt-AroL. Although the expression of *tktA*, *aroF*^{FBR}, and *aroL* in *E. coli* CD42 could diminish the amount of accumulated shikimate (Fig. 5), the situation still did not led to the increase in the amount of tryptophan (panel C in Fig. 6). Furthermore, the amount of excreted tryptophan was even lower than that of the parental *E. coli* CD42. The amount of excreted phenylalanine and tyrosine was unchanged and negligible. A decrease in the amount of excreted tryptophan appears to be caused by the slow growth of the recombinant strains in the tryptophan-production medium as shown in the panel A of Fig. 6. Strains expressing transketolase, such as *E. coli* CD42 carrying pAroF-Tkt or pAroF-Tkt-AroL, showed even longer lag periods. The final yield of the cell was far below that of the parental strain. Interestingly enough, *E. coli* CD42

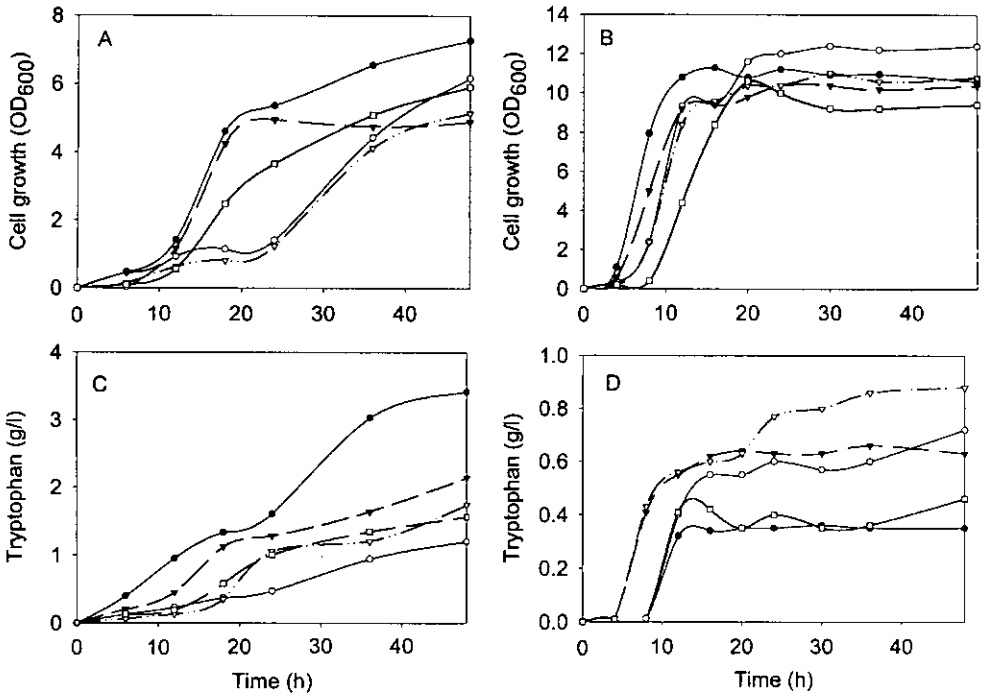

Fig. 6. Cell growth and tryptophan production by *E. coli* CD42 harboring various recombinant plasmids.
Panels: A, Cell growth in tryptophan-production medium; B, Cell growth in YE medium supplemented with 6% glucose; C, Tryptophan production in tryptophan-production medium; D, Tryptophan production in YE medium supplemented with 6% glucose. Symbols: ●, *E. coli* CD42; ○, *E. coli* CD42/pAroF-Tkt; ▽, *E. coli* CD42/pAroF-Tkt-AroL; ▼, *E. coli* CD42/pAroF; □, *E. coli* CD42/pCR-AroL.

strains carrying pAroF-Tkt or pAroF-Tkt-AroL accumulated increased amounts (5–10 mM) of glutamate in the growth medium as identified by the HPLC analysis (data not shown). Since glutamate, pyruvate, and anthranilate are produced from glutamine and chorismate by anthranilate synthase (a tryptophan biosynthetic enzyme), this may suggest that the biosynthetic step catalyzed by anthranilate phosphoribosyl transferase could be rate-limiting. Considering the fact that transketolase plays a pivotal role in the pentose-phosphate pathway, there is an another possibility that metabolic flux in the central carbon metabolism may be disturbed by the overexpression of transketolase, and as a result, the carbon flow could be redirected into the TCA cycle yielding the accumulation of glutamate. By the same token, the situation may lead in part to the retarded cell growth due to the disturbed central metabolism and shortage of proper metabolic precursors. Decreased and controlled expression of the introduced genes may be necessary to reduce the metabolic load.

However, when complex YE medium was employed, certain recombinant strains, such as *E. coli* CD42 carrying pAroF-Tkt-AroL, showed a 2-fold increase in tryptophan production as compared to that of the parental *E. coli* CD42 strain (panel D in Fig. 6). Even though the amount of tryptophan produced in YE medium was 0.2–1 g/l and far below that of the tryptophan-production medium, growth retardation caused by the expression of transketolase as seen in the experiments using tryptophan-production medium was not observed (panel B in Fig. 6). Judging from the growth rate and tryptophan production in YE medium, the carbon flow to the aromatic amino acid biosynthetic pathway appeared to be increased by the expression of DAHP synthase^{FBR}, transketolase, and shikimate kinase. The increased production of tryptophan in the medium may be due to the direct supply of metabolic intermediates from the YE medium. The situation may also have imposed a less metabolic burden to the cell yielding almost normal cell growth. These data may suggest that, if cell growth could be recovered in the tryptophan-production medium, the combined expression of transketolase, DAHP synthase^{FBR}, and shikimate kinase would enhance tryptophan production in *E. coli* CD42 strains. However, the data also suggest that one or more steps after the one that was mediated by shikimate kinase could still be rate-limiting.

Plasmid Instability Caused by the Expression of Transketolase

Since the overexpression of transketolase appeared to impose severe metabolic burden to the host cell as evidenced by the retarded cell growth in the tryptophan-production medium, we analyzed the stability of various plasmids in *E. coli* CD42. As shown in Fig. 7, plasmids containing the *tktA* gene, such as pAroF-Tkt and pAroF-Tkt-AroL, were almost completely lost in the host strain within 12 h of

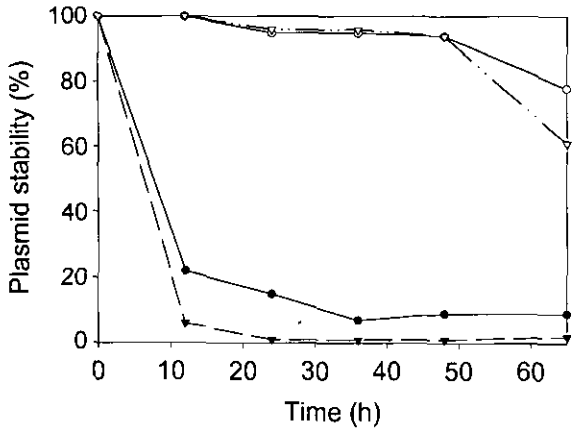

Fig. 7. Stability of recombinant plasmids containing *tktA*, $aroF^{FBR}$, and *aroL*.
Symbols: ▼, *E. coli* CD42/pAroF-Tkt-AroL; ●, *E. coli* CD42/pAroF-Tkt; ▽, *E. coli* CD42/pAroF-AroL; ○, *E. coli* CD42/pAroF. Plasmid stability represents the ratio of ampicillin resistant colonies versus viable colonies on LB plates lacking ampicillin after 12 h of incubation.

cultivation. However, plasmids lacking *tktA*, such as pAroF and pAroF-AroL, were maintained stably in the host even after 48 h of cultivation. When the promoter of *tktA* was replaced with *lac* promoter and the expression of transketolase was minimized by the *lacI* repressor gene expression, recombinant plasmids containing the modified *tktA* gene were stably maintained in the host (unpublished data). These results, in conjunction with the cell growth data, indicate that the overexpression of transketolase may be toxic to the host cell, and as the result, the segregation of the recombinant plasmid has been accelerated. Decreasing the level of *tktA* expression in the recombinant strain may help to resolve the problem. Similarly, growth retardation caused by the expression of transketolase was observed in *Corynebacterium glutamicum* [10]. Although there are many differences in the cellular physiology between *C. glutamicum* and *E. coli*, it appears that the overexpression of transketolase causes difficulties in both microorganisms.

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