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A Study on the Production of Aromatic Amino Acids by *Escherichia coli.*

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*Escherichia coli*에 의한 방향족 아미노산 생산에 관한 연구

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A series of *Escherichia coli* mutants were exmined for ability to convert glucose and ammonium salts into phenylalanine. This enabled the biochemical changes having major effects on phenylalanine yield, and interactions between mutations, to be identified. Changes to the common pathway of aromatic biosynthesis having a major effects include desensitization of the first enzyme (3-deoxy-D-arabinoheptulosonate synthase) to end-product inhibition, and removal of repression of enzyme synthesis. It is suggested that the 3-deoxy-D-arabino-heptulosonate synthase Phe isoenzyme has a more importnat effect on yield. Similarly, removal of repression and end-product inhibition on the phenylalanine terminal pathway increased yield, and changes to both common and branch pathways were synergistic. Blockage of the typrosine and tryptophan pathways had minor effects on phenylalanine yield, and a mutation affecting aramatic amino acid transport (aroP) decreased yield. With multiple-mutation strains high specific rates of product formation (ie 0.1-0.17g phenylalanine/g cells/h) were obtained.

This paper is concerned with the genetic manipulations which may be used to remove restrictions on phenylalanine biosynthesis in *Echerichia coli* with the objective of developing industrial processes for conversion of cheap substrates such as glucose and ammonium salts into the aromatic amino acid. It arises out of a similar work which was being pursued relating to tryptohan hyperproduction.[15]

A summary of the most relevant controls acting on phenylalanine biosynthesis in *E. Coli* is given in Fig. 1. From considering the scheme shown in Fig. 1, it is apparent there are three main factors to consider in manipulating flow down these pathways.[16] Firstly, several enzymes of the common pathway of aromatic biosynthesis leading to chorismate, and enzymes of the phynylalanine terminal pathway which branches from chorismate are sensitive to repression of enzyme synthesis. Secondly, both the 3-deoxy-D-heptulosonate7-phosphate (DAHP) synthase (E.C.4.1.2.15) isoenzymes at the start of the common pathway and chorismate mutase P(E.C.5.4.99.5)-prephenate dehydratase (E.C.4.2.1.51.)-abbreviate CMP-PDH-at the start of the phenylalanine pathway are sensitive to feedback inhibition. Thirdly, chorismate may be diverted down pathways other than the phenylalanine pathway.

In this paper, the relative importance of these factors is demonstrated, and some information was also obtained concerning to role of a transport system involved in phenylalanine uptake, specified by the gene aroP[2]

Materials and Methods

Organisms

All bacterial strains used in this work are derivative of *E. coli* K-12. A list of the strains mentioned in this report, and some details of their properties are given in Table 1.

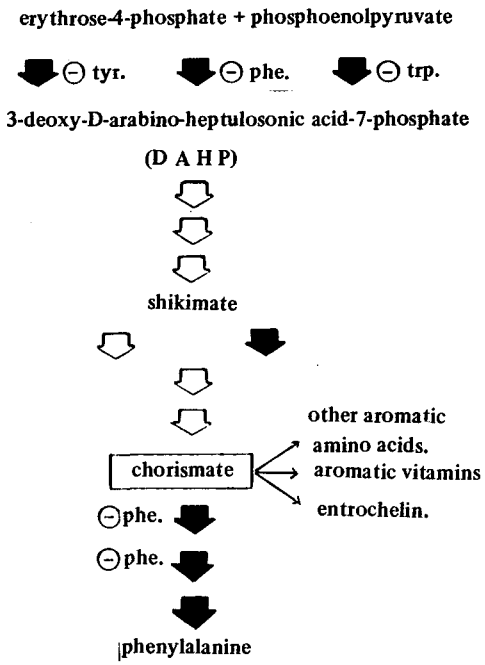

Fig. 1. Outline of the controls acting on the biosynthetic pathways leading to phenylalanine formation.
Filled in arrows represent repressible enzymes and unfilled arrows, constitutively formed enzymes.
symbols represent effector of inhibition.

Chemicals

Chorismic acid was prepared according to the method of Gibson.[8] Barium prephenate was prepared from chorismate by a previously described method.[6]

Buffers

Buffer 56 of Monod et al[12] and Tris-hydrochloride buffer prepared by the methods of Dawson and Elliott[5] were used.

Media

A solution of trace elements was added to media. The solution consisted of 3M $(NH_4)_6$ $(M_0O_7)_{24}$,400M H_3BO_3, 10M $CuSO_4$, 80M $MnCl_2$ and 10M $ZnSO_4$. Medium MMB, minimal medium, has been described previously.[4] Medium MMT5 consisted of, per liter, NH_4Cl, 1.93g; KSO_4, 0.122g; $MgCl_2$ 0.27g; K_2HPO_4, 0.153g; Sodium citrate, 0.294g; $FeCl_3$, 0.032g and trace element solution, lml. The PH was ajusted to 6.5 and the medium was sterilized by membrane filteration. The formulation of MMT5 was based on yield coefficients reported by Neidhardt et. al.[13] Media were supplemented with glucose and auxotrophic requirements as described previously.[4,15]

Growth of Cells

Cells were grown at 37℃ as detailed in the earlier report.[3,4] The results for shaken-flask experiments are the mean of values for duplicated flasks. These are expressed as yield of phenylanine per mass of cells, which showed good reproducibility.

Measurement of Cell Mass

Biomass level was determined turbidometrically,[15] calibrated in terms of dry weight per liter.

Analysis of Culture fluids

The concentration of Phenylalanine in culture broth was determined by a microbiological method using a phenylalanine auxotrophic and streptomycin resistant strain of *E. Coli*. Culture medium for the bioassay contained, in a total volum of 10.5ml, 0.5ml of an appropriately diluted sample, 0.12g glucose, 6.0mg streptomycin, and 0.3mg of washed cells of the phenylalanine auxotroph in 8ml buffer 56. After 15h culture at 37°C with reciprocal shaking, turbidity was measured at 670nm. The assay did not respond to phenyl pyruvic acid. Glucose assays were by the dinitrosalycylic acid method.[11]

Enzyme assay

The conditions for the prephenate dehydratase assay were those of Im and Pittard.[9]

Calcuations

Yp/x is defined as Δ(phenyl alanine) ÷ Δ(cell mass). Specific growth rate K is defined as Δ LN (cell mass) ÷ Δ(time). Q(phenly-alanine) was calculated as K x Yp/x for growing cells or alternatively as Δ (phenylalanine) ÷ (Δ(time) × Δ (cell mass)) for slowly growing or non-growing cells.

Results

Strain construction

The first stage in construction of phenyl alanine hyperproducing strains was the assembly of a collection of strains carrying mutations affecting different aspects of aromatic amino acid metabolism. Many of the mutations were at loci that had been well characterized by other workers, including the *tyrR* locus which was concerned with repression of the synthesis of several cell components such as DAHP synthases Tyr and Phe, shikimate kinase (E.C.2.7.1.71) and aromatic amino transferase[7,10,14], and the pheO locus, an operator for the CMP-PDH structural gene.[9]

Mutations, designated *aroF*(FBI^R) and *aroG*(FBI^R), causing DAHP synthase isoenzymes Tyr and Phe respectively to be desensitized to end-product inhibition were characterised

Table 1. A list of bacterial strains used

Strain	Relevant genotype [a]	Origin or reference
AT 2273	tyrA352	A.J. Pittard
JP 2250	aroF363 TyrA382 phe0352	G. Baldwin
NST5	AT2273 aroF363 pheA (FBI^R)	This work By transduction, selecting for Tyr^+ strain involved selection for 3-fluorotyrosine resistance
NST9	JP2250 $pheO^+$ pheA (FBI^R)	This work By trasduction involving NST5 as a donor.
NST16	JP2250 pheA (FBI^R)	This work By transduction NST9 as donor
JP2229	$aroF^+$ (nadA– aroG– gal) 50 aro+1367 tyrR366 tyrA4 pheAl n tna-2 thr-352 lacV5 his-4	ref (15)
JP2235	JP2229 aroF394 (FBI^R)	ref (15)
JP2241	JP2235 trpE401 malT382	ref (14)
NST21	JP2241 phe0352 pheA (FBI^R)	The work, by transduction with NST16 as donor.
NST22	NST21 $trpE^+$	This work, by transduction
NST26	NST21 $nadA^+$ aroG397 (FBI^R) gal^+	This work, by transduction
NST29	NST21 $tyrA^+$	This work, by transduction
NST30	NST29 $trpe^+$	This work, by transduction
NST33	NST26 leu^+	This work, by transduction
NST37	NST33 $argF^+$ $proA^+thr^+$ his^+	This work, by transduction, in several steps
NST43	JP2235 $pheA^+$	This work, by transduction
NST44	NST43 $nadA^+$ $aroC^+$ gal^+	This work, by transduction
NST45	NST $nadA^+$ aroG397 (FBI^R) gal^+	This work, by transduction
NST47	JP2229 $pheA^+$	This work, by transduction
NST50	NST26 leu^+ aroP: Tn10	This work by transduction
NST54	NST $nadA^+$ arog397 (FBI^R)gal^+	This work by transduction
NST71	NST zci-1005:Tn10	This work, by transduction
NST72	NST37 zci-1005:: Tn10 $tyrR^+$	This work, by transduction

a. Symbols are as described by Bachmann et al[1] The symbol FBI^R indicates mutations that cause loss of sensitivity to feedback inhibition.

b. All transductions involved bacteriophage Plkc.

c. Zci-1005::Tn10: symbol for insertion of transposon Tn10 at a site of unknown function

during investigation of tryptophan hyper production[15].

It should also be mentioned that a mutation [*pheA*(FBI^R)] causing the phenylalanine pathway enzyme CMP-PDH to be desensitized to feedback inhibition was isolated specifically for this work (See Table 1). Transduction analysis established its chromosomal location; enzyme assays showed that it caused both enzyme activities of the bifunctional CMP-PDH complex to lose sensitivity to phenylalanine inhibition (details not presented).

The next stage in strain construction involved the use of gene-trasfer methods to construct multiple-mutation strains as outlined in Table 1. Construction of strains of defined genotype was greatly facilitated by the use of baceriophage *Plkc* for transduction and background data on gene location.[1] Chromosomal locations of genes relevant to this study, and markers with which they can be co-transduced are shown in Fig. 2.

Batch Kinetics

Typical kinetics for phenylalanine formation by one of the higher yielding multiple-mutation strains under controlled conditions in a fermentor are illustrated in Fig. 3. The strain used was NST 37, which has the genotype *aroG*(FBI^R) *aroF*(FBI^R) *tyrR pheA*(FBI^R) *pheO tyrA trpE.* It can be seen

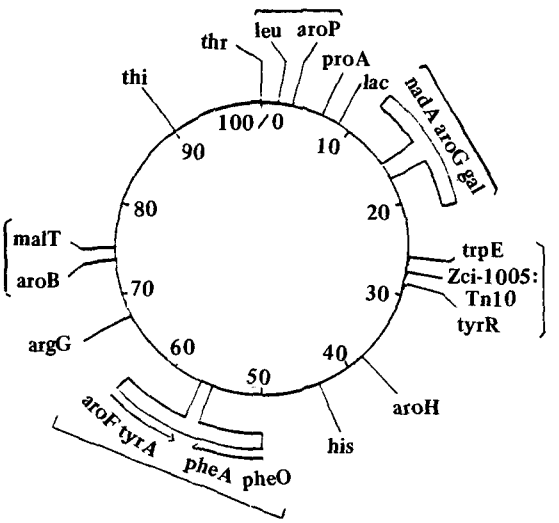

Fig. 2. Genetic linkage map for E. coli showing the loci relevant to this work.

from the figure that a very significant proportion of cell metabolism has been diverted towards phenylalanine formation.

This can be seen more clearly from the kenetic parameters given in Table 2 that have been calculated from the date shown in Fig. 3. The Yp/x values in this table show that with these strains the yield of phenylalanine is well in excess of the yield of cells. it can also be inferred that although values of Q(phenylalanine) are higher during exponential phase of growth than during stationary phase, kinetics of product formation are not strictly growth associated.

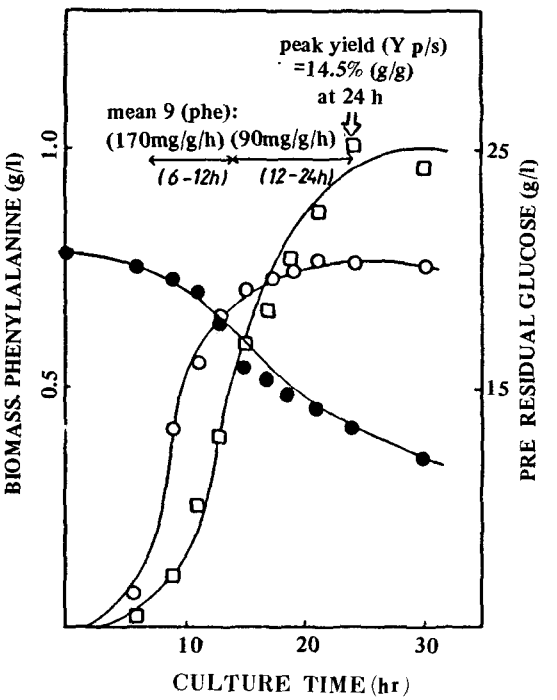

Fig. 3 Kinetics of phenylalanine production by multiple-mutation strain NST 37 in a 1 liter fermentor culture at pH 6.5 and 37°C.

Medium MMT3 was used, supplemented with glucose, thimine (2μg/l), tyrosine (18.2mg/l), and tryptophan (9.19mg/l).

Symbols used: ○ , cell mass; □ , phenylalanine; ● , residual glucose.

Table 2. Comparison of kinetic parameters obtained in batch cultures.

Strain	Source of data	av. sp. growth rate	av. q (phe)	Yp/X % W/W
NST 37	Fig 3.			
	0–10h	0.35	0.16	
	13–24h	0.014	0.09	
	0–24h		0.11	134
NST 33	Fig. 4			
	0–10 h	0.29	0.17	
	14–24 h	0.0003	0.04	
	0–24 h		0.12	125
NST 50 (arop)	Fig. 4			
	0–16 h	0.23	0.11	
	20–30h	0.06	0.02	
	0–30 h		0.08	53

Table 3. Comparison of yield of phenylalanine obtained with various mutants in shaken flask cultures.

Strain [a]	DAHP synthase iisoenzyme phenotype [b]		CM-p-PDH phenotype [c]	Yield of phenylalanine [d]
	Phe	Tyr		YP/x %w/w
Group I.				
NST47	–	+	wild type	0.1
NST43	–	FBI^R	wild type	1.7
NST44	+	FBI^R	wild type	4.5
NST54	FBI^R	+	wild type	20
NST45	FBI^R	FBI^R	wild type	25
Group II.				
NST21	–	FBI^R	deregulated	48
NST26	FBI^R	FBI^R	deregulated	72

Footnote:

a. All the strains also carry $aroH^-$ $tyrR^-$ $tyrA^-$ and $trpE^-$ mutations

b. Notation used: –, isoenzyme absent; +, wild type isoenzyme present; FBI^R, isoenzyme desensitized to endproduct inhibition.

c. Wild type indicates that both inhibition and repression are functional, deregulated indicates that both are absent.

d. Cells were grown in medium MMB-20 g/l glucose for 24 hrs. Final cell densities were limited by the tyrosine content of the medium; the final concentration of tyrosine being 10mg per liter.

To elucidate the role of the individual mutatuins in hyperproduction further experiments were carried out to compare the yields of phenylalanine by strains carrying various different combinations of mutations. Comparisons were carried out largely using shaken flask cultures, but essentially similar kinetics were obtained with both flask and fermentor cultures. Data obtained from these strain comparisons are presented in the sections that follow.

Effect of changes to common pathway. Group 1 strains of Table 3 are a series of strains which lack repression control of DAHP synthase, and which differ from one another in DAHP synthase isoenzyme content; all the strains have a wild-type phenylalanine terminal pathway. The series are arranged in order of increasing effective activity *in vivo*. It can be seen from the data on this group of strains that phenylalanine yield is significantly affected by the level of DAHP synthase present in the cells. Both a desensitized DAHP synthase Phe (compare strains NST44 and NST45;NST47 and NST54) and a desensitized DAHP synthase Tyr (compare strains NST47 and NST43;NST54 and NST45) make a significant contribution to increased yield, with the Phe isoenzyme appearing to have a larger effect.

Group II strains of Table 3 lack both inhibition and repression control of the phenylalanine terminal pathway as well as the indicated changes to the common pathway. By comparison of the two strains in this group it can be seen that introduction of a desensitized DAHP synthase Phe isoenzyme has an effect on yield with these strains as well as with group I strains.

The effects of removal of repression control on common pathway enzymes were also studied. Data relating to this are given in Table 4, which compares yields obtained with a *tyrR*+ strain and two *tyrR*$^-$ strains. This *tyrR*$^-$ mutation has been shown to abolish repression of the synthesis of common pathway enzymes DAHP synthase Phe, Tyr and shikimate kinase.[7,15] From Table 3 it can be seen that it boosts yields of phenylalanine by a factor of approximately ten.

Effects of changes to phenylalanine pathway. Table 5 shows a series of strains that were compared to determine the effect of changes to phenylalanine terminal pathway regulation on phenylalanine yield under conditions where inhibition and repression of DAHP synthase were still operative. Measurements were also made on cell extracts of these strains of the activity of phenylalanine pathway enzyme prephenate dehydratase so that these could be related to phenylalanine yield. Since two of the strains have CMP-PDH enzymes that are fully sensitive to phenylalanine inhibi-

Table 4. Effect of tyrR mutation on phenylalanine yield by multiple-mutation strains in shaken flask cultures.

Strain	Relevant genotype [a]	Yield of phenylalanine [c] at 24h. Yp/x %w/w
NST72	$tyrR^+$	6.1
NST71	$tyrR^-$	61
NST37 [b]	$tyrR^-$	65

Footnote:

a. The strains also carry aroG (FBI^R) aroF (FBI^R) pheA (FBI^R) pheO tyrA trpE mutations.

b. Strain NST37 differs from NST71 in that it lacks a Tn10 insertion, which is irrelevant to interpretation of results.

c. In all the strains tyrosine was the limiting nutrient.

Table 5. Comparison of mutants affected in thephenylalanine Pathway in shaken flask cultures.

Strain	Relative genotype	Relative Prephenate dehydratase Sp. Act. [a]		Yield of phenylalanine at 24 h. Y p/x % w/w [b]
		No Phe added	1mM Phe added	
AT2273	$pheA^+$ $pheO^+$	5.4	1.4	0.1
JP2250	$pheA^+$ pheO	87	20	6.4
NST9	pheA (FBI^R) $pheO^+$	0.15	0.15	4.7
NST16	phe (FBI^R) pheO	100	105	12.0

Footnote:

a. Specific activities found in cell extracts are expressed relative to the value obtained with strain NST16 with no phenylalanine added to the reaction mixture, which is taken as 100%.

b. Cells were grown in medium MMB-20g/e glucose supplemented with the excess amount of auxotrophic requirements. Tyrosine was used as the growth limiting factor.

tion, whereas the other two have mutant forms of the enzyme that are desensitized to phenylalanine inhibition, data are also included in the table showing relative prephenate dehydratase specific activities in the presence of phenylalanine.

As can be seen from the table the introduction of single mutatuion either desensitizing CMP-PDH to phenylalanine inhibition (*pheA*(FBI^R)) or removing repression (*pheO*) found to cause significant phenylalanine hyperproduction. Relative enzyme levels are low in the *pheA*(FBI^R) strain NST9. This presumably is due to the operation more effective repression of enzyme synthesis than occurs in the control strain AT2273, since in other strains in which repression is removed (that is in *pheO* strains) the pheA(FBI^R) mutation does not decrease enzyme levels as can be seen by comparing the data for strains JP2250 and NST16 given in Table 5. Althought the double *pheA*(FBI^R) *pheO* mutant strain NST16 produces the most phenylalanine, comparison of the last two columns of the table reveals that the increase is not in proportion to the high level of phenylalanine resistant prephenate dehydratase activity produced by the strain. One simple interpretation of this relationship is that in this strain another step in biosynthesis is limiting phenylalanine output. Most likely this would be the DAHP synthase reaction, since in this strain the reaction would be expected to be severely inhibited by intracellular phenylalanine.

Bearing this in mind, it is interesting to note that the last two strains of Table 5, namely NST21 and NST26, give higher yields than the last strain of Table 3. NST16, the relevant difference being that with strains NST21 and NST26 mutations affecting the phenylalanine terminal pathway have been combined with mutations removing controls on the first step of the common pathway, whereas with NST16 this step is still sensitive to inhibition and repression.

Effects of removal of phenylalanine terminal controls are also apparent from other comparisons within Table3. This can be seen by comparing the yields obtained with *pheA*+*pheO*+strain NST47 AND *pheA*(FBI^R) *pheO* strain NST21, and also by comparing the *pheA*+pheO+ strain NST45 with the *pheA* (FBR^R) *pheO* strain NST26.

Tyrosine and tryptophan pathways. The phenylalanine is one of several pathways diverging from chorismate, and it seemed likely that diversion of chorismate down these other pathways might, under certain conditions, affect phenylalanine yields. To test for such effects the following experiment was carried out. Derivatives of strain NST21 which is blocked at the first reactions of the tyrosine and tryptophan pathways, were prepared in which these pathways are unblocked, and the resulting strains were compared with one another for phenylalanine Production in flask experiments. As a further test, the effect of supplementing the growth medium of the unblocked strain with the particular amino acid was also examined, on the assumption that this would tend to block flow of chorismate down the pathway by enhancing the operation of feedback controls. The results of these tests are shown in Table 6, from which it can be seen that although large differences in yield were not observed there is a consistent trend, with the unblocked *tyrA*$^+$ *trpE*$^+$ strain NST30 in the absence of added tyrosine and tryptophan giving the lowest and the *tryA*$^-$*trpE*$^-$ blocked strain NST21 giving the highest yield.

Effect of *arop* mutation. Data were also obtained implicating the common aromatic transport system (specified by the *aroP* gene) in having an effect on phenylalanine yield.

Table 6. Relationship between tyrosine and tyrptophan pathway metabolism and yield of phenylalanine in shaken flask cultures.

Strain	Relevant genotype [a]	Supplement to medium [b]		Yield of phenylalanine at 24 h YP/X % w/w
		Tyrosine	Tryptophan	
NST30	$tyrA^+$ $trpE^+$	–[c]	–	30
		+	+	38
NST29	$tyrA^+$ $trpE^-$	–	–	39
		+	+	41
NST22	$tyrA^-$ $trpE^+$	+	–	34
		+	+	38
NST21	$tyrA^-$ $trpE^-$	+	+	47

Footnotes:

a. All the strains also carry *aroF* (FBI^R) *arog*$^-$ *pheA* (FBI^R) *pheO* and *tyrR* mutations.

b. Medium MMB-20g/1 glucore was used and was supplemented with 42mg of tyrosine per liter, and 20mg of tryptophan per liter. In all strains histidine was the limiting nutrient and added 17.5 mg to 1 liter of the medium ; the other nutrient requirements were in excess.

c. Notation used: –, absence of supplement, and +, present of supplement.

The results are shown in Fig. 4. which compares the kinetics of phenylalanine production in fer-mentor experiments of two strains, an *aroP*+ reference strain NST33 and an *aroP:* Tn10 mutant NST50. Both strains also carry *aroG* (FBI^R) *aroF*(FBI^R) *tryR pheA*(FBI^R) *pheO tyrA trpE* mutations. Parameters calculated from this data are included in Table 2, and it can be seen by comparing vlaues for these two strains that the phenylalanine formation is repressed in the *aroP:* tn10, both during exponential and stationary phase. Interestingly, although the medium for both strains is the same, and formulated to give phosphate limitation of these strains at approximately 1.0g dry weight/1, the *aroP:* Tn10 strain grows to a higher cell density. This observation (and several others which are not presented here) suggest that these auxotrophic stranins do not efficiently utilize phosphate. In other experiments, when *aroP:Tn10* was introduced into phenylalanine hyperproducing strains that differed from NST33 in being *tyrA*$^+$ *trpE*$^+$ and the resulting pairs of *aroP*$^+$ and *aroP*$^-$ strains compare (under conditions final cell densities were limited by histidine), no differences in phenylalanine yield were observed, either in the presence of tyrosine and tryptophan additions to the medium or in their absence (details not presented). Thus the *aroP* effect is dependant on the presence of *tyrA*$^-$ and/or *trpE*$^-$ mutations.

DISCUSSION

These results have defined the main genetic alterations required to obtain phenylalanine hyperproduction from glucose and ammonium salts using *E. coli.* Changes to the common pathway of aromatic biosynthesis and the phenylalanine terminal pathway have both been shown to be important.

The role of the common pathway reactions is illustrated by the effects on phenylalanine yield of cellular DAHP synthase isoenzyme content (Table 3), ans also by the ten-fold increase in yield observed with introduction of the *tyrR*$^-$ mutation which causes derepression of the synthesis of these enzymes. The previous report concerning trytophan hyperproducing mutants of *E. coli* contains findings that are quiet pertinent to interpretation of these effects[15]. In that study, DAHP synthase activity was shown to be markedly unstabel during stationary phase of growth, and this instability was indicated as having a major effect on the kinetics of tryptophan production.

Although in *tyrR* mutants during exponential phase of growth levels of DAHP synthase Tyr isoenzyme were greater than DAHP synthase Phe isoenzyme levels, the Phe isoenzyme was found to be significantly more stable than the Tyr isoenzyme during stationary phase. This may explain the observations reported here (Table 3) showing that the Phe isoenzyme apparently has greater effects on phenylalanine yield than the Tyr isoenzyme. The unstability of DAHP synthase is also relevant to the observation reported here (Fig. 3 and4, Table 3) that rate of phenylalanine output is much lower during stationary phase than during exponential growth.

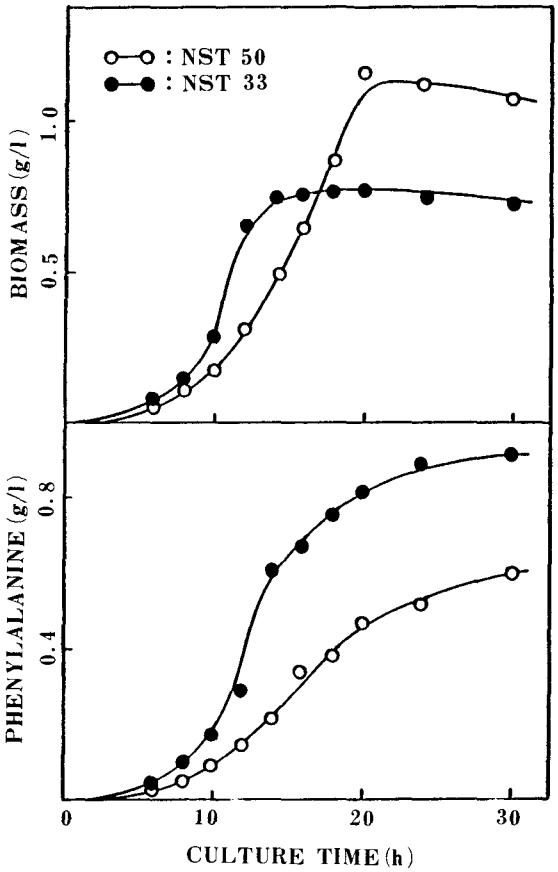

Fig. 4 Comparison of arop$^+$ strain NST 33 with arop: Tn 10 strain NST 50.
The cells were grown in medium MMT4-20g/1 glucose. The phosphate content of the medium was calculated to limit cell density at approximately 1 g/1 dry weight of cells.

The other major effect on yield found here was not surprisingly the removal of phenylalanine terminal pathway controls (Tables 3 & 5), with the best yields being obtained in strains with both common and terminal pathway controls abolished. Wastage of chorismate down pathways other than the phenylalanine pathway was of lesser quantitative significance (Table 6). This last fact is fortunate since in an industrial process it would be better if relatively expensive nutrient requirements of *tyrA*$^-$ *trpE*$^-$ auxotrophs do not have to be added to the medium. More investigation is reguired before a mechanism for the *aroP* effect can be established.

In the work on tryptophan hyperproducing mutants of *E. coli*[15] high specific rates of product formation were noted. In the present study even higher rates of product formation per mass of cells were obtained (see Table 2). Further work is in progress to try and take advantage of these high specific rates by culture of cells to higher cell densities. This envolving is the use of fermentors with better oxygen transfer than available for the work reported here so that oxygen deplation can be avoided.

요 약

여러가지 *Escherchia coli* 변이주의, glucose 와 ammonium염과 같은 간단한 기질로부터 방향족 아미노산 특히 phenylanine을 생합성하는 능력을 비교 검토한 결과 방향족아미노산 생합성과정중 common pathway의 첫번째반응이 phenylanine생합성에 가장 큰 영향을 준다는 것을 확인하였다.

따라서 관계효소인 DAHP synthase의 효소활성과 생합성에 관련된 각종 대사 제어작용을 효과적으로 제거시킴으로서 phenylalanine생산량을 크게 높일 수 있었으며 더욱이 phenylalanine terminal pathway의 첫단계 반응을 촉매하는 prephenate dehydratase의 효소활성과 효소생합성에 관련된 제어작용도 동시에 제거하면 phenylalanine생산이 상승적으로 증가됨을 보였다.

한편 방향족아미노산의 transport system에 관계하는 *arop* 유전자의 변이는 phenylalanine생산을 크게 저하시키는 효과를 나타내었다.

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