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Microbial Bioproducts

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With contributions by
G. P. Agarwal, L. de Boer, H. Brandl,
L. Dijkhuizen, R. C. Fuller, R. A. Gross,
H. Höke, A. Läufer, R. W. Lenz,
R. Müller, Ch. Syldatk

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Microbial and Enzymatic Processes for L-Phenylalanine Production

L. de Boer and L. Dijkhuizen*
Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

The aromatic amino acid L-phenylalanine is one of the building blocks for the dipeptide sweetener α-aspartame. Considerable progress has been made in recent years towards the development of microbial and enzymatic processes for L-phenylalanine production. Biosynthesis of this aromatic amino acid occurs via a complex pathway and is carefully controlled. Therefore, an extensive program for strain construction generally is required in order to achieve high levels of L-phenylalanine overproduction. In addition, microorganisms may possess a variety of enzymes capable of degrading L-phenylalanine. The possible application of a number of these enzymes for the conversion of suitable precursors into L-phenylalanine has received considerable attention. This paper attempts to review the information currently available on both approaches.

1 Introduction

L-Phenylalanine is produced commercially by way of the chemical synthesis from benzaldehyde, glycine and acetic acid anhydride, through purification from protein hydrolysates, or via microbial and enzymatic processes. L-Phenylalanine is one of

* To whom correspondence should be addressed

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the building blocks of the dipeptide sweetener aspartame, L-α-aspartyl-L-phenylalanine methylester [1, 2] and the market of aspartame, and also that of L-phenylalanine, is steadily growing. The projected demand for L-phenylalanine for aspartame production is presented in Fig. 1 [3]. The amino acid is also used for pharmaceutical purposes and as a food additive. The rapidly increasing demand for L-phenylalanine and the advantage of stereospecific biosynthesis have stimulated investigations into the possible production of this amino acid via microbial as well as enzymatic processes.

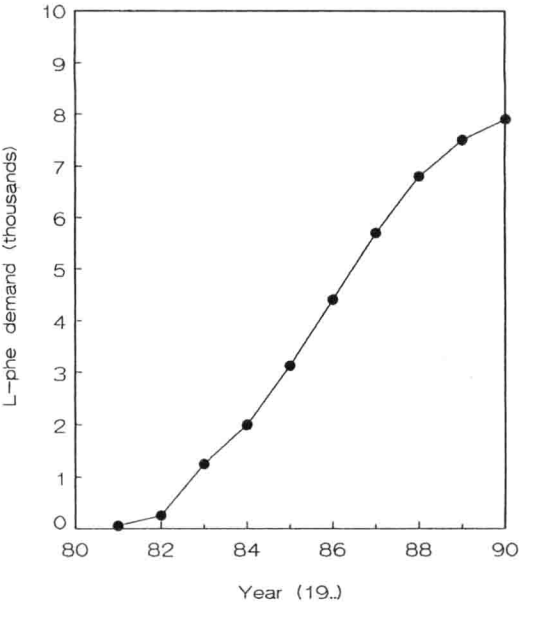

Fig. 1. Demand for L-phenylalanine (expressed in metric tons) for aspartame production [3]

2 Microbial Production of L-Phenylalanine

Synthesis of L-phenylalanine is energetically expensive [4]. Not surprisingly, this process is controlled accurately to meet the cellular demand and no phenylalanine overproducing bacterial strains have been isolated from the natural environment. The selection of a suitable putative production organism thus has to be based on alternative criteria. Conceivably, these should involve: ability of the organism to grow rapidly in mineral media without any requirement for expensive vitamins or other supplements; non-pathogenicity and absence of toxic products; inability to degrade L-phenylalanine and its precursors; sensitivity to inhibition of growth by phenylalanine analogs; availability of methods for the isolation of stable mutants and for further genetic manipulations required during strain development. The choice of the organism may also depend to some extent on its ability to use specific substrates as carbon- and energy sources for growth

[5–9]. The abundant availability, low cost and high levels of purity of methanol [8] makes this compound for instance an attractive feedstock for bioprocesses. Moreover, it may be argued that those methylotrophic bacteria employing the ribulose monophosphate (RuMP) pathway of formaldehyde assimilation offer the distinct additional advantage of possessing an unique metabolic pathway leading to erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) which are precursors for the biosynthesis of the aromatic amino acids [6, 10, 11].

Microbial production processes for L-phenylalanine have been developed in recent years. In practice, the choice of organism often has been made on the basis of experience previously obtained in developing industrial processes for the production of other amino acids. Not surprisingly, most attention thus far has been focused on *Escherichia coli*, *Bacillus subtilis*, and various coryneform bacteria.

2.1 Regulation of L-Phenylalanine Biosynthesis

The biosynthesis of the aromatic amino acids phenylalanine, tryptophan, and tyrosine, and the control mechanisms involved in various microorganisms have been reviewed extensively [12–19]. The overall regulation of the multi-enzyme,

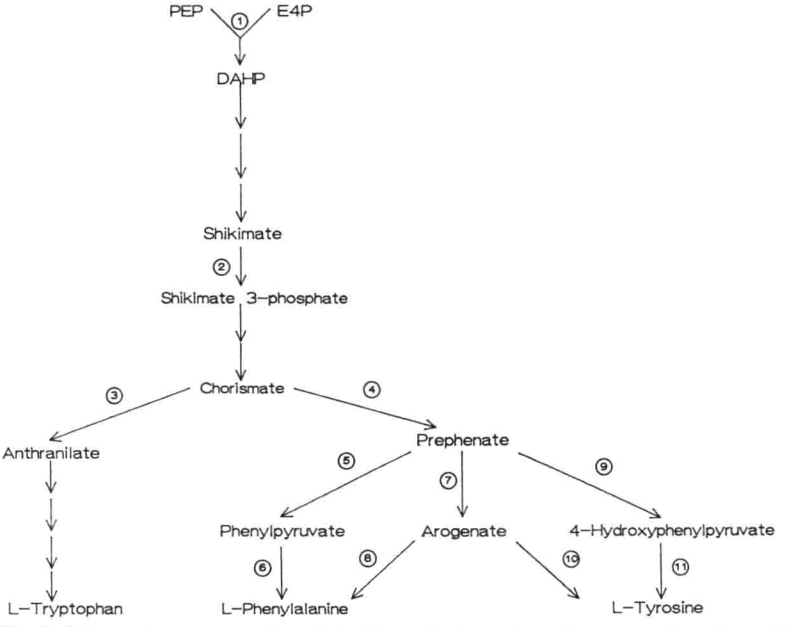

Fig. 2. Schematic representation of the biosynthetic pathway for aromatic amino acids. *1* DAHP synthase; *2* shikimate kinase; *3* anthranilate synthase; *4* chorismate mutase; *5* prephenate dehydratase; *6* L-phenylalanine aminotransferase; *7* prephenate aminotransferase; *8* arogenate dehydratase; *9* prephenate dehydrogenase; *10* arogenate dehydrogenase; *11* L-tyrosine aminotransferase

branched pathway (Fig. 2) is complex and may involve several isoenzyme systems, enzyme complexes, feedback regulation of key regulatory enzymes, both at the level of their synthesis (repression and attenuation) and activity (inhibition). Clearly, one of the main challenges in the development of bioprocesses for aromatic amino acids is the elucidation and subsequent effective deletion of the regulatory mechanisms involved in the organism under investigation.

The biosynthesis of all three aromatic amino acids starts with the formation of chorismate via shikimate in the shikimate pathway [20]. The pathway may also function in the synthesis of precursors for a variety of other aromatic compounds. The first step is the condensation of E4P and PEP, to yield 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP), which is catalyzed by DAHP syn-

Table 1. Properties of regulatory enzymes involved in L-phenylalanine biosynthesis in various bacteria

Microorganism	Regulatory enzyme	Repressor	Inhibitor	$I_{0.5}$ (μM)	Inhibitor constant (μM)
Nocardia	DS	none	phe, tyr, trp	60, 60, 2	160, 180, 3
sp. 239	CM	none	phe, tyr	60, 30	60, 35
[11]	PDT	none	phe, tyr, trp	5, 15[c], 300	10, 20[a], 600
Escherichia	DS	phe, tyr,	phe, tyr	13, 82	13, 82
coli [33, 34–37]		trp[1]	trp[1]	none[1]	none[1]
	SK	tyr, trp	none	none	none
	CM	phe, tyr[1]	phe	50	—
	PDT	phe	phe	100	150
Bacillus	DS	tyr	chor, preph	200, 25	400, 50
subtilis	SK	tyr	chor, preph	—	—
[27, 38–43]	CM	phe, tyr, trp	preph	250	250
	PDT		phe, trp	30, 30	30, 30
			met, leu	3[c], 5[c2]	3[a], 2[a2]
Corynebacterium	DS	—	phe + tyr	—	1000[3]
glutamicum	CM	phe	phe, trp	50, 5[c]	60, 9[a]
[44–47]	PDT	—	phe, tyr, trp	2, <10[c], 25	2, <10[a], 25
Brevibacterium	DS	tyr	phe + tyr	51	—
flavum	CM	tyr	phe + tyr	50–750[b]	—
[48–52]	PDT	none	phe, tyr	2.5, 1.6[c]	1.0, 2.1[a]

DS, DAHP synthase; SK, shikimate kinase; CM, chorismate mutase; PDT, prephenate dehydratase; chor, chorismate; preph, prephenate; tyr, tyrosine; phe, phenylalanine; trp, tryptophan

[a] activator constant, defined as the activator concentration giving a two-fold increase in enzyme activity

[b] constant determined in the presence of 10 μm tryptophan

[c] $A_{0.5}$ (i.s.o. $I_{0.5}$) for an activator is defined as the activator concentration giving 50% of the maximum activation

[1] phe, tyr and trp feedback regulated isoenzymes, respectively

[2] expressed in mM

[3] at 37 °C

— no experimental data available

thase. Various isoenzymes of DAHP synthase may be present which are subject to feedback inhibition and repression by phenylalanine, tyrosine and tryptophan, and inhibition by intermediates of the pathway such as chorismate and phenylpyruvate [15, 16, 18, 21]. Only one DAHP synthase has been detected in the antibiotic producing actinomycetes that have been investigated. In these organisms DAHP synthase activity was only feedback inhibited to a minor extent, if at all, especially by L-tryptophan (22–26). In *E. coli* and *B. subtilis* feedback inhibition also occurred at the level of shikimate kinase [27–29].

Chorismate, the end product of the shikimate pathway, is converted into phenylalanine via the phenylpyruvate pathway, which is present for instance in *E. coli* and *B. subtilis*, or via dual pathways involving either phenylpyruvate or arogenate, as is the case in *Pseudomonas aeruginosa* [21, 30] (Fig. 2). In cyanobacteria, coryneform bacteria [14, 21] (e.g., *Corynebacterium* and *Brevibacterium* strains) and some sporeforming actinomycetes [31, 32], phenylalanine is synthesized exclusively via phenylpyruvate and tyrosine via arogenate. Especially chorismate mutase and prephenate dehydratase are targets for further control by phenylalanine and tyrosine [14, 16, 21]. In streptomycetes the carbon flow in the phenylalanine-specific branch is generally found to be feedback inhibited by phenylalanine at the level of prephenate dehydratase and tyrosine was shown to be an inhibitor or arogenate dehydrogenase in the tyrosine-specific branch [32].

The effectiveness and in vivo importance of the various control steps in aromatic amino acid biosynthesis can only be fully appreciated when considering the overall pattern of regulation of carbon flow over this branched biosynthetic pathway in a specific organism. This situation has only been achieved in a limited number of bacterial strains (Table 1). A comprehensive discussion of the regulation of a balanced synthesis of the three aromatic amino acids in *B. flavum* has been presented by Shiio [14]. The data provide a firm basis for the development of a suitable strategy for the construction of L-phenylalanine overproducing strains.

2.2 Strain Construction

Various examples of strain construction with the aim to develop microbial processes for phenylalanine overproduction are listed in Table 2. Feedback repression and/or inhibition control of enzymes in phenylalanine biosynthesis may be circumvented at least partially by using tyrosine auxotrophic mutants and supplying limiting amounts of this amino acid in the medium [53, 57, 58, 60, 62]. This offers the additional advantage of preventing undesirable accumulation of tyrosine from the common precursors. The remaining control mechanisms for phenylalanine biosynthesis may be eliminated by the stepwise isolation of mutants resistant to various amino acid analogs.

2.2.1 Aromatic Amino Acid Pathway

A methionine and tyrosine auxotrophic strain of *Brevibacterium lactofermentum* accumulated phenylalanine to a concentration of 4.2 g l^{-1} [62]. The successive

Table 2. Microbial production processes for L-phenylalanine

Microorganism	Main carbon source	L-phenylalanine production (g l^{-1})	Yield %	Cultivation time (h)	Ref.
Methylomonas methanolophila 6R E431 (β-2-TA^r 5-MT^r 3-AT^r)	Methanol	4	—	66	[8]
Bacillus polymyxa BT^r-7 (PFP^r β-2-TA^r)	Starch	0.5	5	72	[9]
Brevibacterium flavum 485-21 (MFP^r)	Glucose	2.2	2.2	72	[53]
Bacillus subtilis FF-25 (5-FT^r)	Glucose	6.0	7.5	48	[54]
Brevibacterium lactofermentum AJ3437 (PFP^r 5-MT^r Tyr$^-$, Met$^-$)	Glucose	22	17	72	[55]
Brevibacterium lactofermentum AJ11475 (PFP^r 5-MT^r DEC^s Tyr$^-$ Met$^-$)	Glucose	24.8	19	72	[56]
Brevibacterium flavum M-87 (PFP^r DS^r PDT^r Tyr$^-$, Met$^-$)	Glucose	23.4	18	72	[57]
Corynebacterium glutamicum 31-PAP-20-22 (PFP^r PAP^r Tyr$^-$)	Molasses	9.5	9.5	96	[58]
Corynebacterium glutamicum K38 (PFP^r MFP^r; genetically manipulated)	Molasses	19	19	100	[59]
Escherichia coli TA-6-7 Tyr134-7 (β-2-TA^r PFP^r Tyr$^-$)	Glucose	15	8.5	49	[60]
Corynebacterium sp. KY 7146 (Tyr$^-$)	n-Alkanes	10	15	68	[61]
Brevibacterium lactofermentum No. 123 (PFP^r β-3-TA^r adenine^r 5-MT^r Tyr$^-$ Met$^-$)	Glucose	21.7	16.6	72	[62]
Escherichia coli NST 74 (genetically manipulated)	Glucose	8.7	19	38	[63]

X^r, resistant to X; X$^-$, X$^-$ auxotroph; X^s, sensitive to X, MFP, M-fluorophenylalanine; PFP, P-fluorophenylalanine; 5-FT, 5-fluorotryptophan; 5-MT, 5-methyltryptophan; 3-AT, 3-aminotyrosine; PAP, P-aminotyrosine; β-2-TA, β-2-thienylalanine; β-3-TA, β-3-thienylalanine; DEC, decoyinine; DS^r, feedback inhibition resistant DAHP synthase; PDT^r, feedback inhibition resistant prephenate dehydratase

introduction of resistance to *p*-fluoro-DL-phenylalanine (PEP), β-3-thienyl-D,L-alanine (β-3-TA), adenine and 5-methyl-DL-tryptophan (5-MT) resulted in the isolation of mutants producing 5.7, 8.8, 9.3, and 12.5 g l^{-1} phenylalanine, respectively. Optimization of the culture medium, especially by the addition of fumaric acid, allowed a further enhancement of phenylalanine production to 21.7 g l^{-1} after 72 h of cultivation. Using a L-phenylalanine overproducing mutant of *B. lactofermentum*, Akashi et al. demonstrated that maximum production of the amino acid occurred under conditions of a limited supply of oxygen [64]. The authors speculate that under these growth conditions less PEP is used for energy generation, resulting in an enhanced availability of this precursor for amino acid synthesis.

Following similar approaches, tyrosine auxotrophic and analog resistant mutants of *Corynebacterium glutamicum* were isolated producing 9.5 and 10 g l^{-1} of L-phenylalanine from cane molasses and n-alkanes (58, 61). The chorismate mutase and prephenate dehydratase genes (coding for feedback inhibition insensitive enzymes) of *C. glutamicum* K38, a PFP and *m*-fluoro-DL-phenylalanine (MFP) resistant strain have been cloned. Re-introduction of these genes on a recombinant plasmid into strain K38 via protoplast transformation further enhanced L-phenylalanine production from 13 to 19 g l^{-1} [59]. A PFP and β-2-TA resistant strain of *Bacillus polymyxa* was found to produce 0.5 g l^{-1} phenylalanine from starch [9]. Enzyme measurements revealed that this strain possessed a considerably increased level of prephenate dehydratase. This enzyme also had become insensitive to feedback inhibition by phenylalanine. Significant loss of the phenylalanine produced was observed towards the end of the production phase as the result of induction of the catabolic enzyme phenylalanine ammonia-lyase by phenylalanine.

In *Brevibacterium flavum* chorismate mutase and DAHP synthase form a bifunctional enzyme complex with common regulatory sites for phenylalanine and tyrosine. In MFP resistant *B. flavum* strains (possessing a feedback inhibition insensitive prephenate dehydratase), synergistic inhibition of DAHP synthase by MFP and tyrosine (formed intracellularly from the dipeptide Tyr-Glu in the medium) still occurred [53, 57]. The inhibitory effects on both DAHP synthase and chorismate mutase activities were simultaneously removed by inducing resistancy to MFP and Tyr-Glu, which resulted in an increase in phenylalanine production from 2.1 to 6.0 g l^{-1}. When following the same approach, phenylalanine production by strain No. 239, a PFP-resistant tyrosine/methionine double auxotrophic mutant, was enhanced from 18.1 to 23.4 g l^{-1} in strain M-87 [57].

Introduction of β-2-TA, 5-MT, and 3-aminotyrosine (3-AT) resistancies in *Methylomonas methanolophila* 6R, a methanol-utilizing bacterium [8], resulted in accumulation of all three aromatic amino acids, necessitating the isolation of tryptophan and tyrosine auxotrophic mutants in further work.

Overproduction of L-phenylalanine by strains of *E. coli* has been described by Choi and Tribe [63] and Park et al. [65]. A β-2-TA resistant strain of *E. coli* W3110 (strain TA-6–7) produced 5.7 g l^{-1} L-phenylalanine and a trace amount of tyrosine. Since tyrosine inhibited L-phenylalanine formation, a tyrosine auxotrophic mutant was isolated and this strain (TA-6–7 Tyr134) produced L-

phenylalanine up to 11.4 g l^{-1}, with low tyrosine concentrations in the medium [65]. A PFP-resistant derivative strain was subsequently isolated and found to accumulate L-phenylalanine to a concentration of 15 g l^{-1} [60]. This strain was used to study optimal production conditions in a 500-liter pilot reactor [66].

Förberg and Häggström [67, 68] studied phenylalanine production by *E. coli* strains containing the recombinant plasmid pJN6, carrying genes for DAHP synthase (*aroF*) and feedback inhibition insensitive chorismate mutase/prephenate dehydratase (*pheA*). The effects of various regimes of glucose, tyrosine, sulphate and phosphate addition in the feed on phenylalanine production were investigated in (fed-)batch and continuous cultures. Exhaustion of phosphate in batch culture resulted in an immediate decrease in phenylalanine production, followed by a phase of slow product formation. The decrease of phenylalanine production was not so dramatic following depletion of sulphate. In the chemostat experiments phenylalanine production continued during phosphate limitation while sulphate and glucose limitation caused a collapse in the specific rate of product formation. It remains to be established whether these differences are purely due to physiological factors or to variations in plasmid stability with varying growth conditions.

In a similar approach, using a temperature-controllable expression vector carrying *aroF* and *pheA* genes encoding feedback inhibition insensitive enzymes, Sugimoto et al. [69] studied the temperature dependency of gene expression and phenylalanine production in *E. coli*. The concentration of phenylalanine was temperature dependent and highest production (18 g l^{-1}) was obtained at 38.5 °C in a 2.5–1 reactor.

2.2.2 Intermediary Metabolism

A relatively novel aspect that draws increasing attention in studies with especially *B. flavum* and *C. glutamicum* is the possible enhancement of intracellular precursor concentrations to enlarge the carbon flow towards desired amino acids [70–77]. PEP is an important intermediate in central metabolic pathways. It is a precursor for the biosynthesis of various amino acids (lysine, aromatic amino acids) and intracellular PEP consumption other than for production of desired metabolites therefore should be minimized. Sugar metabolism in the amino acid producing bacterium *B. flavum* has been investigated in detail [70, 73]. Growth on several sugars involved a PEP dependent sugar phosphotransferase system (PTS). The presence of other PEP consuming enzymes, e.g., oxaloacetate decarboxylase, malic enzyme, and pyruvate kinase was investigated but only the latter enzyme, together with the PTS system, was detectable at growth supporting levels. A pyruvate kinase minus mutant was still able to grow under conditions where the PTS system was operative, thus generating the pyruvate required. Interestingly, mutational inactivation of pyruvate kinase in lysine [71] and aspartate [72] overproducing strains of *B. flavum* resulted in increased productivity. As outlined above, PEP is also a precursor for aromatic amino acid biosynthesis. Conceivably, similar approaches might also be applicable for further enhancing L-phenylalanine biosynthesis.

2.2.3 Application of Recombinant DNA Technology

The rate of amino acid biosynthesis may be increased considerably by the introduction of structural genes on multi-copy plasmids [5, 78–80]. At the moment there is limited information only in the literature about the application of recombinant DNA technology for the production of L-phenylalanine [59, 63, 67–69]. Detailed studies on the molecular biology and genetic regulation of aromatic amino acid synthesis thus far mainly have been restricted to *E. coli* (reviewed in Refs. [15, 16, 81–83]). The phenylalanine operon in *E. coli*, encoding chorismate mutase and prephenate dehydratase (*pheA*; [84]) and the genes encoding the three DAHP synthase isoenzymes (*aroF*, *aroG*, *aroH*; for a comparison, see Ref. [85]) have been cloned and sequenced. In view of the well-developed *E. coli* genetics it is not surprising that application of recombinant DNA technology for the construction of strains overproducing amino acids (L-phenylalanine [69] and several other amino acids [86, 87]) has been successful especially with this organism. Nevertheless, rapid progress also has been made in recent years towards the development of recombinant DNA technology for coryneform bacteria, including transformation, transfection methods, the development of suitable host-vector systems, and protoplast fusion techniques (for reviews, see Refs. [86, 88–91]). Moreover, genes coding for enzymes of the common aromatic amino acid biosynthetic pathway in *B. lactofermentum* [92], chorismate mutase [59] and prephenate dehydratase of *C. glutamicum* [59, 93] already have been cloned by complementation of auxotrophic mutants of the same organisms.

The analysis of the molecular basis for mutations in structural genes and regulatory sequences, causing desensitization of enzymes to feedback inhibition and increased levels of gene expression, respectively, is just beginning [85, 94, 95]. Conceivably, this is another area where recombinant DNA technology may significantly contribute to a further enhancement of amino acid production.

3 Degradation of L-Phenylalanine

Many bacteria are versatile organisms, amongst others able to grow on a variety of amino acids as carbon-, energy- and/or nitrogen source. Synthesis of catabolic enzymes during microbial amino acid production may result in a considerable decrease in yield and productivity. In many production strains synthesis of catabolic enzymes is inducible and repressed in the presence of alternative carbon sources such as glucose. Degradation of the amino acid to be overproduced may nevertheless still occur following depletion of alternative substrates towards the end of the process. It thus follows that the early identification and efficient deletion of catabolic enzymes via mutation clearly is of importance during strain construction.

Metabolic pathways for L-phenylalanine degradation are diverse. Whereas the last step in the biosynthesis of phenylalanine is generally catalyzed by aromatic amino acid aminotransferases [13], the initial step in the catabolic pathways may involve either phenylalanine hydroxylase [96–98], phenylalanine

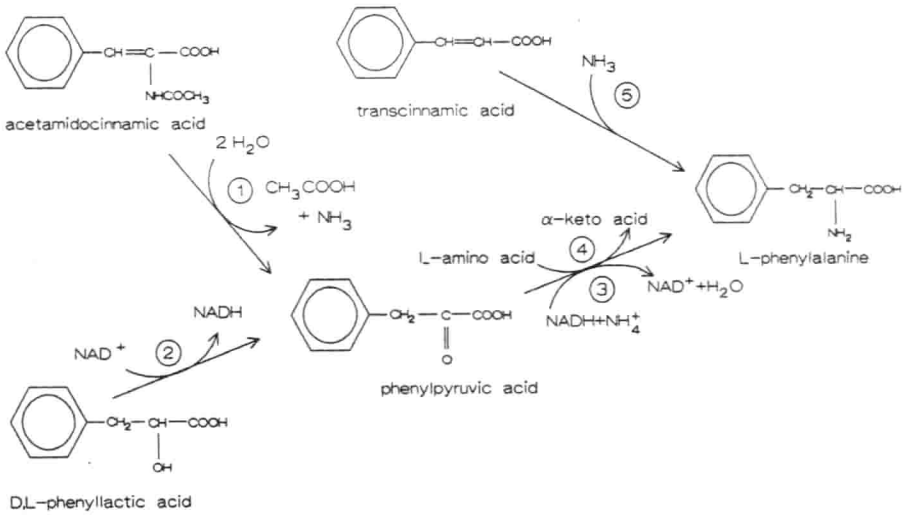

Fig. 3. Enzymatic reactions leading to L-phenylalanine production from phenylpyruvate, acetamidocinnamic acid and D,L-phenyllactic acid. *1* ACA acylase; *2* D- and L-hydroxyisocaproate dehydrogenase; *3* L-phenylalanine dehydrogenase; *4* aromatic amino acid aminotransferase; *5* phenylalanine ammonia-lyase

ammonia-lyase (PAL; Sect. 4.1), NAD-dependent phenylalanine dehydrogenase (PheDH; Sect. 4.2), or an aromatic amino acid aminotransferase (AAT; Sect. 4.3). The reactions catalyzed by some of these enzymes are shown in Fig. 3. It should be realized that phenylpyruvate, produced from phenylalanine by PheDH and AAT activities, also is an intermediate in phenylalanine biosynthesis (Fig. 2). The possible utilization of this compound as a growth substrate by the production strain therefore should be excluded as well. This will necessitate further mutational inactivation of phenylpyruvate catabolic enzymes, e.g., phenylpyruvate decarboxylase [99].

4 Enzymatic Production of L-Phenylalanine

Enzymatic synthesis of L-phenylalanine has been described starting out with racemic mixtures of D,L-phenylalanine (chemically produced) or by conversion of achiral precursors. Resolution of racemic mixtures may involve aminoacylases (from *N*-acetyl-DL-phenylalanine), hydantoinases (from D,L-hydantoins), esterases (from D,L-phenylalanine esters) or aminopeptidases (from D,L-phenylalanine amide). Various aspects of the stereospecific resolution of amino acids from racemic mixtures have been reviewed previously [100–102].

The conversion of chemically synthesized achiral precursors into L-phenylalanine, in single or in several enzymatic steps, has been studied in detail in the last three decades [103]. Several enzymatic processes, either with whole cells or

purified enzymes, have been described which at least potentially may find an application (see below). In general, research focusses on the identification of cheap, novel substrates for biotransformations, the isolation of organisms which produce highest levels of the enzymes of choice, and most suitable reaction conditions. The enzymatic approach requires that process conditions are carefully controlled, in order to ensure prolonged stability of substrates and biocatalysts, and may necessitate cofactor regeneration. In the following the properties of phenylalanine ammonia-lyase, phenylalanine dehydrogenase and aromatic amino acid amino-transferase, enzymes which have been studied in most detail for phenylalanine production, are reviewed. A general reaction scheme is presented in Fig. 3.

4.1 L-Phenylalanine Ammonia-Lyase (PAL)

The first report concerning the discovery of PAL, which catalyzes the non-oxidative deamination of L-phenylalanine into ammonia and transcinnamic acid under physiological conditions, was published in 1961 [104]. Subsequent work demonstrated PAL activity in plants [104, 105], yeasts [106–110], fungi [111, 112], and bacteria [9, 113, 114]. Instead of pyridoxal 5′-phosphate, the cofactor in many amino acid transforming enzymes, PAL contains dihydroalanine as a prosthetic group. In plants PAL especially functions in secondary metabolism; it constitutes the first step of a highly branched pathway for the synthesis of lignins and related polyphenols. In various yeasts, fungi and bacteria the enzyme catalyzes the first step in phenylalanine catabolism, to generate carbon, energy and nitrogen

Table 3. Enzymatic production of L-phenylalanine from transcinnamic acid using phenylalanine ammonia-lyase as a biocatalyst

Microorganism	Cinnamic acid conc. (%)	L-Phenylalanine production (g l^{-1})	Maximum yield (%)	Processing time (h)	Ref.
Endomyces lindneri	4	32	71	48	[112]
Rhodotorula glutinis	2.2	18	70	25	[118]
Rhodotorula rubra FP10M6	1.75	17.8	91	37	[125]
Rhodotorula rubra SPA10	2	17.7	89	30	[126]
Rhodotorula rubra SPA10	1	9.4	84	24	[127]
Rhodotorula rubra SPA10	2	>17	88	30	[128]
Rhodotorula rubra FP10M6	4	50	83	120	[129]
Rhodotorula graminis	1.5	50.8	86	88	[130]
Rhodotorula rubra	5.5	59	90	—	[131]