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L-Phenylalanine ammonia-lyase activity of fungi, yeasts and *Streptomyces*

Chang, Jiyoung, Yang Mo Goo and Kong-Hwan Kim*
Dept. of Pharmacy, Seoul National University, Seoul, Korea
**Dept. of Biotechnology, Ajou University, Suwon, Korea*

Fungi, 이스트, 그리고 *Streptomyces*에서 L-phenylalanine ammonia-lyase의 활성도 비교

장지영·구양모·김공환*
서울대학교 약학대학 약학과
**아주대학교 공과대학 생물공학과*

ABSTRACT: Microorganisms isolated from soil (150 strains), fungi (39 strains), yeasts (9 strains) and *Streptomyces* species (39 strains) were assayed for L-phenylalanine ammonia-lyase (PAL) activity. 17 strains of fungi and 46 strains of soil isolates were proved to produce PAL, *Aspergillus panamensis*, *Penicillium varioti* and 11 soil isolates showed comparatively large PAL activity. When PAL activity was assayed with cell-free extracts of these 13 strains and 7 strains of *Rhodotorula* and *Rhodosporidium geni*, *Rhodosporidium toruloides* (IFO 0559) showed the highest PAL activity with 0.333 units per g of the wet cell weight.

KEY WORDS□L-phenylalanine ammonia-lyase, L-phenylalanine, *trans*-cinnamic acid.

Production of L-phenylalanine was achieved mainly by selection of microorganisms which hyperproduce L-phenylalanine in the fermentation broth. The application of genetic engineering on the production of L-phenylalanine was also tried. Development of new biochemical methods for the production of L-phenylalanine reduces its cost sharply recently and the chemical synthetic process for its production is no more adequate. L-phenylalanine is one of the essential amino acids for human and animal nutrition and is a raw material for the production of aspartame (L-aspartidyl-L-phenylalanine methyl ester), an artificial sweetner which has recently become one of the most important commercial products. Economic production of L-phenylalanine have become one of the most important research fields. We are interested in the production of biological products by microbial or enzymatic transformation (Choi and Goo, 1986) and especially we thought that it should be possible to convert *trans*-cinnamic acid to L-phenylalanine since already many plants are known to transform L-phenylalanine to *trans*-cinnamic acid, which is further converted usually to coumarine, lignins and other derivatives. Examination of literature showed that several people already studied L-phenylalanine ammonia lyase (PAL, Kukol and Conn, 1961; Havir and Hanson, 1968; O'Neal and Keller, 1970). PAL was also found to be existing in some fungi (Kalghatgi and Subba Rao, 1975 and 1976) and yeasts (Ogata *et al.*, 1966 and 1967). Among the prokaryotic organisms, some *Streptomyces* were demonstrated to produce PAL (Bezanson *et al.*, 1970).

Since PAL catalyzes not only the nonoxidative deamination of L-phenylalanine but also the con-

version of *trans*-cinnamic acid into L-phenylalanine (Yamada *et al.*, 1981), enzymatic methods for the production of L-phenylalanine could be developed.

In this report, we examined fungi, yeasts, *Streptomyces* and microorganisms isolated from soil for PAL activities.

MATERIALS AND METHODS

Microorganisms and maintenance

Fungi and soil isolates examined for PAL activities in this work are listed in Table 1,2 and 3. Their origins and their maintenances are given in the legends of tables. Other fungi and *Streptomyces* spp. examined in the present study are listed more in experimental sections.

Isolation of microorganisms from soil

Microorganisms to be screened from soil for PAL activity were isolated and pure cultured by the following methods. A small amount of soil collected from various places in Korea was suspended in 10 m*l* of sterilized distilled water containing 1 μg of chloramphenicol per m*l* to reduce the number of fast growing bacteria. After the suspension was diluted 10-fold for 4 times in sequence with distilled water, 0.5 m*l* of the liquid was spreaded on a V-8 agar (20% (v/v) V-8 juice, 0.3% $CaCO_3$, 2% agar, distilled water) plate or an oat meal agar (2% oat meal, 0.1% yeast extract

Table 1. *Formation of trans-cinnamic acid in PAL medium by fungi.*

strains*	UV absorbing spots**		UV absorption*** at 267 nm
	trans-cinnamic acid	others	
A. gigantus(M 1)	+	+	0.356
A. nidulans(M 3)	–	–	0.097
(Cz 57)	+	+	0.169
A. versicolor(Cz 5)	+	+	0.476
A. flavipes(Cz 7)	–	–	0.009
A. candidus(Cz 9)	–	–	0.040
A. fumigatus mui hevola(Cz 19)	+	–	0.125
A. unguis(Cz 20)	–	–	–0.92
A. oryzae(Cz 21)	+	+	0.290
(Cz 58)	+	–	0.273
P. purpurogenum(Cz 26)	+	–	0.252
By. fulva(Cz 28)	+	+	0.912
P. notatum(Cz 29)	+	–	0.850
En. apiculata(M 30)	+	+	0.177
A. panamensis(M 31)	+	–	0.317
A. rugulosus(M 32)	+	+	0.473
A. parasiticus(Cz 33)	+	+	0.203
A. avenaceus(Cz 34)	+	–	0.171
Pa. varioti(Cz 43)	+	+	0.279
(M 46)	+	–	0.019
A. clavatus(Cz 56)	+	+	0.149

**A.: Aspergillus; P.: Penicillium; By.: Byssoclamys; En.: Entomophthora; Pa.: Paecilomyces.* These fungi were those stocked in our laboratory. The M and Cz series were maintained on malt extract agar (2% malt extract, 0.1% peptone, 2% Glucose and 1.5% agar in distilled water) slants and Czapeck-Dox agar (0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.01% $FeSO_4$, 3% sucrose and 2% agar in distilled water, pH 7.0) slants, respectively.

**: Examined by TLC

***: The absorbance of the 50-fold diluted ethyl ether extract of the acidified culture(48 hrs).

Table 2. *Comparison of PAL activity of fungi and soil isolates by the cell contact method**.

Strains	Absorbance at 278 nm	Strains	Absorbance at 278 nm
A. gigantus(M 1)	0.160	S 42	0.068
A. nidulans(M 3)	0.099	S 43	0.053
A. versicolor(Cz 5)	0.107	S 45	0.106
A. candidus(Cz 9)	0.189	S 53	0.059
A. flavipes(Cz 7)	0.044	S 59	0.086
A. fumigatus mui hevola(Cz 19)	0.054	S 61	0.003
A. unguis(Cz 20)	0.074	S 62	0.057
A. oryzae(Cz 21)	-0.12	S 75	0.080
By. fulva(Cz 28)	0.124	S 76	0.418
A. panamensis(M 31)	0.194	S 77	0.178
A. parasiticus(Cz 33)	0.073	S 80	0.142
Pa. varioti(Cz 43)	0.170	S 90	-0.01
(M 46)	0.025	S 91	0.044
Conidiobolus sp. (M 48)	0.122	S 100	0.040
A. clavatus(Cz 56)	0.026	S 102	-0.17
S 18	0.043	S 104	0.118
S 25	0.044	S 107	0.065
S 26	0.058	S 108	0.077
S 28	0.032	S 109	0.085
S 29	0.027	S 110	-0.21
S 32	0.370	S 111	0.104
S 33	0.073	S 112	0.132
S 34	0.156	S 114	0.070
S 35	0.155	S 122	0.132
S 38	0.079	S 123	0.173
S 39	0.097	S 127	0.088
S 40	0.160	S 133	0.159

*: The solution of 25 mM L-phenylalanine, 25 mM Tris·HCl (pH 8.5), 0.005% cetylpyridinium chloride and 0.3 m*l* of cell suspension in a final volume of 5.0 m*l* was incubated for 30 min. The change of the UV absorption of the medium was examined.

and 2% agar in distilled water) plate. Plates were incubated at 28 °C until some colonies formed. Colonies having different growth patterns were transferred to new V-8 agar plates or oat meal agar plates, pure cultured and stocked on V-8 agar slants or nutrient agar slants.

Estimation of PAL activity in fungi and soil isolates

L-phenylalanine ammonia-lyase screening medium (PAL medium) contained 2% L-phenylalanine, 0.2% KH_2PO_4, 0.1% K_2HPO_4, 0.01% $MgSO_4$ and 0.05% yeast extract in distilled water (pH 6.0). PAL media (5 m*l*) in test tubes were autoclaved at 15 lb/121 °C for 15 min and inoculated with fungi listed in Table 1 or soil isolates in Table 2. Other fungi, *A. versicolor* (Cz 4), *A. alliaceus* (Cz 10), *A. foetidus* (Cz 11), *A. famarii* (Cz 15), *A. ochraceus* (Cz 17), *A. sclerotiorum* (Cz 18), *P. striatum* (M 23), *P.decumbens* (Cz 24), *Pa. varioti* (Cz 27), *P. roqueforti* (Cz 37), *P. camemberti* (Cz 38), *P. digitatum* (M 44), *Thamnidium elegans* (M 49), *Botrytis spectabilis* (M 52) and *A. ustus* (Cz 54) (see the legends of Table 1 for abbreviations) were also inoculated. The inoculated media were incubated at 28 °C on a shaker rotating at 180 rpm for 7 days. After acidification of the cultured media with 0.5 m*l* of 6 N HCl, it was extracted with 5 m*l* of ethyl acetate. The ethyl acetate extract was evaporated in *vacuo*. The residue was dissolved in ethyl acetate (5 drops) and examined by TLC for *trans*-cinnamic acid. The TLC plate (silica gel) was developed with *iso*-propanol-NH_4OH-water (20:1:1, v/v). Microorganisms which produce *tran*-cinnamic acid were identified by comparison with an authentic spot by TLC.

Screening of *Streptomyces* species for PAL activity

Streptomyces strains (39 strains) employed in the present study are those purchased from IFO (IFO strains) or ATCC (ATCC strains) or provided by NRRL (NRRL strains): *S. griseus subsp. griseus* (IFO 3357), *S. fradiae* (IFO 3718, NRRL 1195, NRRL B-3357) *S. hygroscopicus var limoneus* (IFO 12703, IFO 12704), *S. kanamyceticus* (IFO 13414, NRRL 2535), *S. globisporus* (ATCC 1829), *S. aureofaciens* (ATCC 10762, NRRL 2209, NRRL B-1287, NRRL-1288), *S. rimosus* (NRRL 2234), *S. chrysomallus* (NRRL 2250), *S. venezuelae* (NRRL 2277), *S. erythraeus* (NRRL 2338), *S. rimosus* f. *paromomycinus* (NRRL 2455), *S. kitasatoensis* (NRRL 2486), *S. lincolnensis* (NRRL 2936), *S. coeruleorubidus* (NRRL 3045), *S. moderatus*

Table 3. *PAL activity of Rhodotorula and Rhodosporidium species*§

strains	Cultivation for 72 h			Cultivation for 144 h			
	turbidity (A_{610})	*trans*-cinnamic+ acid produced (A_{268})	L-Phe++ remained (A_{570})	turbidity (A_{610})	*trans*-cinnamic+ acid produced (A_{268})	L-Phe++ remained (A_{570})	pH
R. rubra(IFO 0001)	1.299	0.801*	1.846	1.932	1.567	1.614	7.9
R. glutinis(IFO 0389)	0.094	1.841	1.773	0.119	2.277	1.607	6.5
Rh. toruloides(IFO 0413)	1.533	2.270*	1.633	1.472	1.495**	0.551	7.5
R. glutinis var. dairensis (IFO 0415)	1.660	2.245	1.842	0.950	1.976	0.599	8.0
Rh. toruloides(IFO 0559)	1.834	1.674*	1.659	1.647	2.251***	1.540	7.9
R. rubra(IFO 0889)	2.078	1.522*	1.273	2.096	1.598	1.405	7.7
R. rubra(IFO 0911)	0.971	1.054*	1.956	1.727	1.528	1.245	7.5
R. minuta var. texensis (IFO 0932)	0.779	1.346	2.231	1.380	1.721**	1.795	7.5
R. aurantiaca (IFO 0951)	0.908	0.252	2.222	1.282	0.820	1.686	7.5

§: The yeasts are maintained on malt extract agar slants (see the legends in Table 1). They were purchased from the Institute for Fermentation in Osaka. *R.: Rhodotorula; Rh.: Rhodosporidium.*

+: The accumulation of *trans*-cinnamic acid was measured by the following method: The broth (5 m*l*) was centrifuged to give a supernatant, which (2 m*l*) was acidified by the addition of HCl (6 N, 2 m*l*) and extracted with ethyl ether (2 m*l*). The ethyl ether layer was separated and equilibrated with NaOH (0.1 N, 4 m*l*), the absorbance of which at 268 nm was measured.

++: The supernatant of the broth after centrifugation was adjusted to pH 9, added with ninhydrine solution (0.1% in ethanol, 2 m*l*) and boiled for 10 min. The absorbance of the solution at 570 nm was measured.

*: 5-fold diluted

**: 10-fold diluted

***: 20-fold diluted

(NRRL 3150), *S. griseus* (NRRL 3383, NRRL 3851, NRRL B-2682), *S. bifurcus* (NRRL 3539), *S. clavuligerus* (NRRL 3585), *S. vellosus* (NRRL 8037), *S. espinosus* (NRRL 11439), *S. venezuelae* (NRRL B-902), *S. alborgriseolus* (NRRL B-1305), *S. parvulus* (NRRL B-1628), *S. viridifaciens* (NRRL B-1679), *S. galbus* (NRRL B-2283), *S. murinus* (NRRL B-2286), *S. ribosidificus* (NRRL B-11466), *S. lividans* (1326, 3131), *S. coelicolor* (A3(2)). All the NRRL strains were kindly provided by Agricultural Research Culture Collection, International Depository authority (Peoria, U.S.A.). *S. coelicolor* A3(2) were kindly granted by professor Schrempf at Universität München, Institut für Genetik and Mikrobiologie. *S. lividans* (3131 and 1326) were kindly donated by Dr. Thompson at Biogen Company (Geneva, Switzerland). All the *Streptomyces* spp. were either stocked on ISP medium No 4 agar (Difco) slants or YM agar (0.4% yeast extract, 1% malt extract, 0.4% glucose and 2% agar) slants, or lyophilized after suspending in the YM media (without agar) with 24% sucrose solution. Their PAL activity was assayed by the following method. S media (1 m*l*; 1% glucose, 0.4% peptone, 0.4% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.2% KH_2PO_4 and 0.4% K_2HPO_4 in distilled water) were inoculated with 39 *Streptomyces* spp. They were cultured at 28 °C for 40 h. The seed cultures were mixed to 10 m*l* of the S media containing 0.1% L-phenylalanine. After the inoculated media were cultivated at 28 °C for 24 h, cells were harvested by centrifugation and washed twice with 0.9% saline. The weighed wet cells were resuspended in 5 m*l* of 50 mM Tris·HCl (pH 8.5), sonicated for 10 min at maximum power in ice-salt water bath. Cell free extracts were obtained by centrifugation of the sonicated media at 16,000 × g for 30 min at 4 °C. The cell free extracts (0.2 m*l*) were added to 2.8 m*l* of 0.833 mM L-phenylalanine in 0.1 M Tris·HCl (pH 8.5) at 30 °C. The increase in optical density at 290 nm was monitored for 10 min.

Comparison of PAL activity of fungi and soil isolates

PAL activities were compared by two methods. In the first method the production of *trans*-cinnamic acid in the broth was examined. Soil isolates were inoculated in 30 m*l* of PAL media in 300 m*l* flasks. After they were cultured at 28 °C for 72 h and 144 h, the cultured media (5 m*l*) were centrifuged. The supernatant (2 m*l*) was acidified with 0.2 m*l* of 6 N HCl and extracted with 2 m*l* of ethyl ether. The ethyl ether extract was equilibrated with 4 m*l* of 0.1 N NaOH solution. The absorbance of the aqueous layer was examined at 268 nm by UV spectrophotometer. Fungi were inoculated in PAL media (5 m*l*) and cultured at 28 °C on a rotary shaker for 48 h. The cultured media were acidified with 1 m*l* of 2 N HCl and extracted with 5 m*l* of ethyl ether. The ethyl ether extract was diluted 50-fold with ethyl ether and was measured the absorbance at 267 nm.

In the second method, fungi were inoculated in 20 m*l* of PAL media in flasks (50 m*l*) and cultured at 28 °C on a rotary shaker for 2 to 3 days. The cells harvested by centrifugation were washed with 0.9% saline and resuspended in 0.9% saline (10 m*l*/g of wet weight). The cell-suspended solution (0.3 m*l*) was mixed with the solution containing 25 mM L-phenylalanine, 25 mM Tris·HCl (pH 8.5) and 0.005% cetylpyridinium chloride to give a final volume of 5.0 m*l* (Yamada *et al.*, 1981). The mixture was incubated at 30 °C for 10 min and filtered on a glass filter with a layer of celite. The absorbance of the filtrate at 278 nm was measured.

Comparison of PAL activity of Rhodotorula and Rhodosporidium species

The PAL activities of 9 strains belonging to *Rhodotorula* and *Rhodosporidium* listed in Table were investigated. Yeasts were inoculated in 30 m*l* of PAL medium and cultured at 28 °C on a shaker rotating at 180 rpm for 3 days. Cell growth was determined turbidimetrically at 610 nm. To measure the accumulation of *trans*-cinnamic acid the culture (5 m*l*) was centrifugated. The supernatant (2 m*l*) was acidified with 6 N HCl (0.2 m*l*) and extracted with 2 m*l* of ethyl ether. The ether extract was reextracted with 4 m*l* of 0.1 N NaOH. The absorbance of the alkaline extract at 268 nm was determined (Ogata *et al.*, 1967). To measure the amount of L-phenylalanine left in the culture, 5 m*l* of the cultured medium was centrifugated. The supernatant (2 m*l*) was adjusted to pH 9 with 1 N NaOH and added with 2 m*l* of 0.1% ninhydrine solution in ethanol. The mixture was heated for 10 min in boiling water bath and was measured the absorbance at 570 nm. Cell growth, the amount of *trans*-cinnamic acid and L-phenylalanine, and the pH of the media after 6 days' incubation were also determined by the same method.

Preparation of cell-free extracts for PAL assay of 20 strains selected

The strains which proved to possess high PAL activity in the previous experiments were seed-cultured in a media (3 m*l*) containing 1% malt extract and 0.1% yeast extract in distilled water. The inoculated media were incubated at 28 °C for 24 h on a rotary shaker (180 rpm). The seed cultures (2 m*l*) were transferred to media (100 m*l*) composed of 1% malt extract, 0.1% yeast extract and 0.1% L-phenylalanine in distilled water. The inoculated-media were incubated at 28 °C for 14 to 16 h on a rotary shaker. Cells were harvested by centrifugation for 15 min at 8,500 rpm and washed twice with 0.9% NaCl solution. The weighed cells were either frozen at −70 °C and maintained at −20 °C or immediately disrupted by sonic oscillation. The cells were resuspended in 50 mM Tris·HCl (pH 8.5) buffer (2 m*l* per g of wet weight). The suspension was kept cold by submerging the container in ice-salt water and disrupted by sonication for 10 min at maximum power (sonication for 10 seconds, followed by 5 secs' break). The debris was removed by centrifugation at 16,000 × g for 20 min at 4 °C and the supernatant was used as a crude enzyme preparation.

Assays of PAL activity with cell-free extracts

PAL was assayed by the method of Hodgins (1971). The standard assay mixture contained 2.8 m*l* of 0.833 mM L-phenylalanine in 0.1 M

Tris·HCl (pH 8.5) and 0.2 m*l* of enzyme at 30 °C. The PAL catalyzed reaction was started by the addition of the enzyme preparation and the formation of *trans*-cinnamic acid was monitored at 290 nm (Zucker, 1956). The change in optical density was recorded for 10 min at 1 min's interval. The enzyme preparation was diluted with cold 50 mM Tris·HCl (pH 8.5) buffer when required. The activity was determined from the linear protion of the optical density increase over a time period of 0 to 5 min. One unit of enzyme was defined as the amount of protein that catalyzed the appearance of 1 μ mole of *trans*-cinnamic acid per min at 30 °C.

RESULTS AND DISCUSSION

Microorganisms in soil, which formed colonies in a medium with chlormaphenicol which was added to remove fast growing bacteria were isolated and pure cultured and about 150 strains were stocked. These soil isolates and fungi listed in Table 1 and in the experimental sections were cultured in PAL media and formation of *trans*-cinnamic acid was investigated. *A. gigantus, A. versicolor, A. fumigatus mui hevola, P. purpurogenum, By. fulva, P. notatum, En. apiculata, A. panamensis, A. rugulosus, A. parasiticus, A. avenaceus, Pa. varioti A. clavatus, A. nidulans, A. oryzae* (Cz 21 and Cz 58) and 46 soil isolates were found to convert L-phenylalanine to *trans*-cinnamic acid as shown in Table 1. When the soil isolates were examined for their growth appearances in liquid media and on solid media, among 46 PAL producing soil isolates, 16 strains (S 18, S 25, S 27, S 28, S 39, S 40, S 43, S 45, S 53, S 59, S 61, S 62, S 90, S 91, S 93, S 100) were thought to be fungi and 28 strains (S 29, S 32, S 33, S 34, S 35, S 38, S 42, S 106, S 107, S 108, S 109, S 111, S 112, S 114, S 116, S 122, S 123, S 127, S 130, S 133, S 134) were thought to be bacteria or yeasts. S 26 and S 100 were resumed to be belong to *Streptomyces* genus. They were not further identified. Though two soil isolates look *Streptomyces* spp. when 39 *Streptomyces* spp. were assayed for PAL, none showed PAL activity when *trans*-cinnamic acid formation from L-phenylalanine was determined either by TLC or by increase in UV absorption at 290 nm. We concluded that all the 39 *Streptomyces* strains do not have PAL activities. Recently *Streptomyces verticillatus* was reported to produce PAL.

Table 2 shows the formation of *trans*-cinnamic acid by the absorbance values at 278 nm when harvested fungi and soil isolates were incubated with L-phenylalanine in the presence of cetylpyridinium chloride which allows to pass L-Phe and its metabolites through membrane. The media incubated with *A. panamensis, P. varioti,* soil isolates S 32, S 34, S 35, S 40, S 45, S 77, S 104, S 111, S 112, S 122 and S 123 showed high UV absorption. It was presumed that if the microorganism produce PAL, it will convert L-phenylalanine to *trans*-cinnamic acid which will be extractable in ethyl ether from the acidified medium and which will be further extractable from ether into the alkaline aqueous solution. However, the data in Table 2 and 3 do not well represent the PAL activity. The reason should be that some microorganisms metabolize L-Phe to phenolic compounds. When two different screening methods, the method of whole cell incubation and the cell contact method were compared, fungi showed similar relative PAL activities in two methods, but soil isolates did not. This difference might be due to the presence of special carrier systems of the substrate and product. L-Phenylalanine and *trans*-cinnamic acid seem to move across the cell membrane by special carrier systems in intact cells. If cells are treated with cetylpyridinium chloride, small compounds can move freely through the membrane. Thus its effect would be very similar to that which the cell free extract might show.

Table 3 shows growth and PAL activities of *Rhodotorula* and *Rhodospridium* species. IFO 0559 showed the largest accumulation of *trans*-cinnamic acid in the medium among 5 strains tested. But PAL activity does not seem to be related to the growth in PAL medium or to the amount of L-phenylalanine that left over in the medium.

The strains selected from the results given from Table 2 and yeasts in Table 3 were disrupted to give cell free extract preparations which were

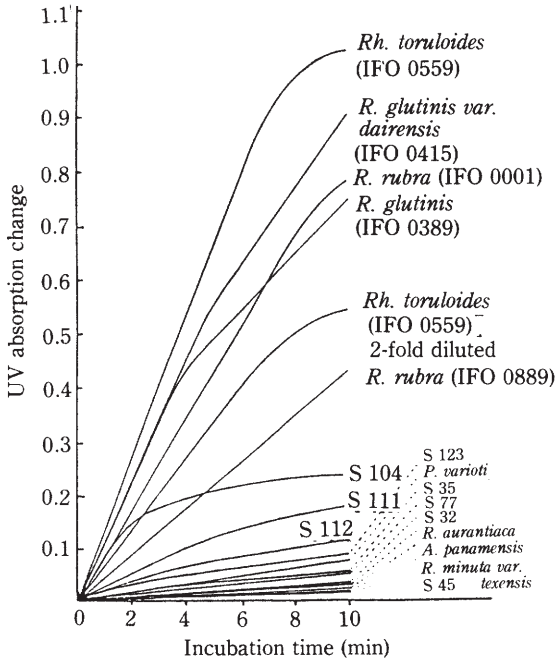

Fig. 1. *Increase of UV absorption at 290 nm. The cell-free extract (0.2 ml) was added to the standard assay mixture and the UV absorption was observed at 30°C.*

assayed for PAL activity. As cell free extracts were incubated with L-phenylalanine the increase of absorbance at 290 nm was recorded at 1 min's interval. The results are shown in Figure 1. Table 4 lists PAL activities of the strains in terms of units per g of wet cell weight. It was calculated by employing the extinction coefficient (10,000 $M^{-1}cm^{-1}$) of *trans*-cinnamic acid. The activity was determined from the linear portion of optical density change. *Rhodosporidium toruloides* (IFO 0559) showed the highest PAL activity. Fungi and soil microorganisms also had PAL activity, but much less than that of *Rhodotorula* and *Rhodosporidium* species. We isolated PAL from *Rhodosporidium* species and examined for their characterization and for their usefulness as the enzyme for the production of L-phenylalanine from *trans*-cinnamic acid (Chang, 1988).

Table 4. *PAL activities of 20 selected microorganisms*

strains		organisms	units/g(wet weight)
IFO	0001	*R. rubra*	0.198
IFO	0389	*R. glutinis*	0.265
IFO	0415	*R. glutinis var. dairensis*	0.274
IFO	0559	*Rh. toruloides*	0.333
IFO	0889	*R. rubra*	0.062
IFO	0932	*R. minuta var. texensis*	0.010
IFO	0951	*R. aurantiaca*	0.006
M	31	*A. panamensis*	0.006
Cz	43	*Pa. varioti*	0.025
S	32	soil isolates	0.010
S	34	"	negligible
S	35	"	0.017
S	40	"	negligible
S	45	"	0.005
S	77	"	0.018
S	104	"	0.122
S	111	"	0.072
S	112	"	0.042
S	122	"	negligible
S	123	"	0.027

*; Assays were performed by adding a cell-free extract (0.2 m*l*) to the standard assay mixture (2.8 m*l*) consisted of L-phenylalanine (0.833 mM) in Tris·HCl buffer (0.1 M, pH 8.5). The UV absorption increase was monitored at 290 nm by maintaining the reaction mixtures in a UV cell at 30 °C.

CONCLUSION

Assay of PAL activity in various microorganisms indicated that PAL is limited to the eukaryotic organisms mostly. Our screening results, showed that none of the *Streptomyces* species had PAL activities. Soil isolates showed good PAL activities but their taxanomical studies should be explored further. We found that *Rhodosporidium toruloides* (IFO 0559) showed the highest PAL activity. Other fungi and soil isolates seem to have limited PAL activities.

적 요

150종의 토양균에서 분리한 39종의 fungi 9종의 yeasts 그리고 *Streptomyces* spp.에서 L-phenylalanine ammonia lyase (PAL) 활성을 screen 하였다. 17종의 Fungi와 46종의 토양균이 PAL의 활성을 보였다. *Aspergillus panamensis,*

*Penicillium varioti*와 11종의 토양균들이 비교적 큰 PAL 활성을 보였다. 이들 균주들 중에서 선택한 13종과 *Rhodotorula*와 *Rhodosporidium geni*에 속하는 9종의 yeast들의 cell free extracts를 준비하여 PAL의 활성을 assay 하였을 때에 *Rhodosporidium toruloides* (IFO 0559)가 wet cell의 g당 0.333 units의 활성을 보여 가장 강한 PAL 활성을 갖는 것으로 나타났다.

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