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SHORT COMMUNICATION

PHENYLALANINE AND TYROSINE AMMONIA-LYASE ACTIVITY IN SOME BASIDIOMYCETES

R. J. BANDONI, K. MOORE, P. V. SUBBA RAO and G. H. N. TOWERS

Department of Botany, University of British Columbia, Vancouver, Canada

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Abstract—Ammonia-lyases for phenylalanine[1] and tyrosine,[2] once considered to be characteristic enzymes of vascular plants, were shown to be present in 12 species of Basidiomycetes.[3] In the present communication 56 other fungi, all representatives of the class Basidiomycetes were examined for the presence of these enzymes and the results are reported herewith.

MATERIALS AND METHODS

Preparation of Cell-Free Extracts

MYCELIAL mats produced by organisms grown in Roux bottle flats under static conditions at 25° were collected by filtration, washed with 100 ml distilled water and macerated in a mortar with an equal weight of aluminum oxide. Cells grown in shake cultures at 25° were harvested by centrifugation at 10,000 g for 15 min and washed thrice with distilled water. The wet cells were homogenized in a mortar with an equal weight of aluminum oxide.

The macerated tissue (10 g wet wt. for each organism) was extracted with 20 ml of 0·05 M Tris-HCl buffer, pH 8, and the resulting slurry centrifuged at 4100 g for 20 min. The supernatant, hereafter referred to as the crude enzyme, was used to test for enzyme activity.

Assay for the Non-Oxidative Deamination of Phenylalanine and Tyrosine

The reaction mixture containing 0·5 ml 0·05 M Tris-HCl buffer, pH 8·8, 0·5 ml crude enzyme and 5 μmoles substrate (L-phenylalanine or L-tyrosine) in a final volume of 2 ml was incubated at 30°. After 1 hr the reaction mixture was acidified with 1 ml of 1 N HCl and extracted with 5 ml peroxide-free ether. Suitable aliquots from the ethereal layer were evaporated to dryness under a stream of air and the amount of cinnamyl compound was determined spectrophotometrically after dissolving the residue in 3 ml 0·05 N NaOH (1 and 2). Protein was determined by the method of Lowry *et al.*[4] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

A total of 56 organisms were examined for the presence of phenyl-alanine and tyrosine ammonia-lyase activity. Cultures which produced a positive test for enzyme activity with

[1] J. KOUKOL and E. E. CONN, *J. Biol. Chem.* **236**, 2692 (1961).
[2] A. C. NEISH, *Phytochem.* **1**, 1 (1961).
[3] D. M. POWER, G. H. N. TOWERS and A. C. NEISH, *Can. J. Biochem* **43**, 1397 (1965).
[4] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).

Table 1. Phenylalanine and tyrosine ammonia-lyase activity in some *Basidiomycetes*

Organism	U.B.C. No.	Incubation time in days	Culture conditions		μM product/mg protein	
			Shake	Static	Tyrosine ammonia-lyase	Phenylalanine ammonia-lyase
Ustilago zeae (Schw.) Unger	711	3	+		0	0·19
**U. hordei* (E3) (Pers.) Lagerh.		3	+		0	0·28
U. bullata (Berk.)	675	3	+		0	0
Platygloea pustulata (Martin & Cain)	519	24		+	0	0·02
Stereum hirsutum (Willd. ex Fr.)	549	42		+	0	0·03
S. pini (Schleicher ex Fr.)	724	43		+	0	0
Steccherinum adustum (Schw.) Banker	723	7		+	0	0·05
Coprinus domesticus (Pers.)	606	3		+	0	0·03
Rhodotorula mucilaginosa (Jörg.) Harrison	873	3	+		0·10	0·13
Sporidiobolus johnsonii (Nyl.)	865	1	+		0·03	0·22
Sporobolomyces roseus (Kluyv. & v. Neil)	901	2	+		0·01	0·13
S. salmoneus (Derx.)	903	2	+		0·01	0·13
S. salmonicolor (Fischer et Brebeck) Kluyv. & v. Neil	904	4	+		0·05	0·45
Tilletiopsis washingtonensis (Nyl.)	907	3	+		0	0·42
Schizophyllum commune (Fr.)	528	12		+	0	0·07

* Culture donated by Dr. C. O. Person, of this department.

All organisms except *U. hordei* and *S. commune* were grown in a medium containing the following constituents/l. malt extract, 30 g; Bacto-soytone, 5 g; yeast extract, 1 g. *U. hordei* was grown in a modified Vogel's medium (5) containing yeast extract, 5 g; salt-free casein hydrolysate, 5 g; L-tryptophan, 50 mg; Vogel's salt solution, 20 ml and vitamin solution (6) 10 ml in 1 l. distilled water. *S. commune* was grown in a medium containing glucose, 10 g; $NaNO_3$, 5 g; KH_2PO_4, 5 g, $MgSO_4 \cdot 7H_2O$, 0·5 g; yeast extract, 0·1 g, L-phenylalanine, 1 g; distilled water, 1 l.

[5] H. J. Vogel, *Microbiol. Gen. Bul.* No. 13 (1956).

[6] R. H. Holliday, *Genet. Res. Camb.* 2, 204 (1961).

one or both substrates are depicted in Table 1. Ammonia-lyase activity for phenylalanine and/or tyrosine was not detected in representatives of the following genera: *Armillaria, Bovista, Bullera, Clavaria, Coniophora, Daedalea, Dacromyces, Exobasidium, Fomes, Ganoderma, Itersonilla, Lenzites, Merulius, Montagnea, Poria, Polyporus, Ramaria, Sirobasidium, Tremella.*

Where enzyme activity was evident the ammonia-lyase for L-phenyl-alanine predominated. This is in accordance with an earlier survey made by Power *et al.*[3] The majority of fungi examined by these workers possessed ammonia-lyases for both phenylalanine and tyrosine, but in this present study it was highly significant that this characteristic was restricted to one family, *Sporobolomycetaceae.* The presence or absence of enzyme activity was not always exhibited by all members of one genus as was evidenced from examination of 3 species of *Ustilago* (*U. Hordei, U. zeae, U. bullata*) Table 1.

The information given in this report, although of no taxonomic significance, primarily for reasons of variation in culture media, length of incubation and analysis of insufficient numbers of representatives of the different groups, was intended to bring to light those organisms which are good sources of these enzymes and offer ideal material for future study.

Ustilago hordei is a particularly useful source of phenylalanine ammonia lyase. The organism is readily available, easily cultured and offers no problems in yielding highly active preparations. Species of *Sporobolomyces, Sporidiobolus* and *Rhodotorula* provide excellent material for the study of both enzymes. Studies of these enzymes in species of *Rhodotorula* have been published recently by Ogata *et al.*[7]

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[7] K. OGATA, K. UCHIYAMA and H. YAMADA. *Agr. Biol. Chem.* **31**, 600 (1967).