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FURTHER OBSERVATIONS ON PHENYLALANINE AMMONIA-LYASE IN FUNGI

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Key Word Index—*Nectria cinnabarina*; *Ascomycetes*; Basidiomycetes; fungi; phenylalanine ammonia-lyase; effect of light.

Abstract—Phenylalanine ammonia-lyase (PAL) has been detected in an Ascomycete, *Nectria cinnabarina*. Growth in light increases levels of PAL in some but not all Basidiomycetes.

INTRODUCTION

The enzyme, phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5), which is ubiquitous in higher plants (see reviews by Camm and Towers [1] and Zucker [2]), also occurs in Basidiomycetous fungi [3,4] and even *Streptomyces* sp. [5]. Although flavonoids have been isolated from *Aspergillus candidus* [6] as yet there are no published reports on the presence of PAL in Ascomycetes. According to J. B. Pridham (personal communication), however, *Alternaria* sp., contains this enzyme and we have now made a short survey of Ascomycetous fungi for PAL and TAL (tyrosine ammonia-lyase). We have also examined a number of Basidiomycetes to discover whether light has an effect on the levels of this enzyme, as has been reported for cultures of the Basidiomycete, *Polyporus hispidus* [7,8].

RESULTS AND DISCUSSION

The following Ascomycetes tested showed no detectable PAL activity under conditions of growth and assay conditions described in the Experimental: *Chaetomium globosum*, *Morchella* sp., *Hansenula anomala*, *Peziza anthracophila*, *Cephaloascus fragrans*, *Talaromyces* sp., *Ceratocystis fimbriata*. Only *Nectria cinnabarina* showed activity for PAL and this fungus was also able to deaminate tyrosine to *p*-coumaric acid. Cinnamic acid, a product of PAL activity, was identified by PC, UV spectrum, color reactions with diazotized *p*-nitroaniline or sulfanilic acid and recrystallization to constant sp. act. (7×) in experiments using phenylalanine-[^{14}C]. *p*-Coumaric acid was identified in the same way, in cell-free preparations with tyrosine-[^{14}C] as substrate. *In vivo* studies in which either cold or radioactive phenylalanine or tyrosine was administered to *N. cinnabarina* showed many compounds which gave positive reactions with diazotized *p*-nitroaniline and

Table 1. The effect of light on PAL activity in a number of fungi

Organism	UBC no.	Conditions of growth: Light Sp act. (nmol/hr/mg protein)	Conditions of growth: Dark Sp act. (nmol/hr/mg protein)
Armillaria mellea	688		27
Bovista sp.	535	2	2
Clavaria cristata	502	27	15
Coniphora puteana	504	0	0
Exobasidium vaccinii	588–2	0	0
Fomes subroseus	704	125	105
Lenzites saepiaria	516	0	0
Merulius tremellosus	719	51	52
Montagnea sp.	538	0	0
Nectria cinnabarina	230	27	29
Polyporus adustus	728	44	47
Poria subacidia	725	0	0
Ramaria secunda	525	25	17
Schizophyllum commune	528	19	12
Sporobolomyces roseus	901	50	31
Steccherrinum adustum	723	33	34
Tricladium splendens	8142		21

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sulfanilic acid. Preliminary data (PC, color with spray reagents and radioautography) indicated that these were *p*-hydroxybenzoic, *p*-hydroxyphenylacetic and protocatechuic acids. In addition, there were several unidentified phenols.

The effect of light on PAL is shown in Table 1. Of the 18 organisms tested *Sporobolomyces*, *Clavaria*, *Schizophyllum*, *Ramaria* and *Fomes* showed increases in PAL which varied from 20 to 80% on exposure to light.

In a previous study blue light was shown to stimulate PAL and styrylpyrone biosynthesis in *Polyporus hispidus* [12], the action spectrum for styrylpyrone biosynthesis being similar to that of a flavin. In higher plants light stimulation of PAL activity is perhaps mediated by phytochrome [13–16] but so far phytochrome has not been found in fungi and nothing can be said about the fungal photoreceptor.

EXPERIMENTAL

All organisms were cultured at 25° and prepared as described earlier [4]. The light-grown mycelium was grown in bottles exposed to light for 1 hr/day; the dark-grown mycelium was totally unexposed to light. The mycelium was harvested from day 5 to day 8, homogenized and transferred to fresh media and maintained in light or dark. After 5 consecutive transfers, cultures were harvested after 5 and 10 days' growth. Harvesting and preparation of cell free extracts were carried out according to Bandoni *et al.* [4].

PAL was assayed according to the procedure of Ref. [1]. For radioactive assays the reaction mixture contained 1 ml 50 mM Tris–HCl, pH 8·8, 0·5 ml enzyme preparation, 5 μmol substrate (L-phenylalanine or L-tyrosine) and 2·5 μCi of phenylalanine-[2-^{14}C] (sp. act. 4·4 mCi/mmol) or tyrosine-[2-^{14}C] (sp. act. 3 mCi/mmol) in a final vol. of 2·5 ml. The reaction mixture was acidified with 0·5 ml 6 N HCl and extracted 3× with 5 ml of peroxide free Et_2O. The ethereal layer was evaporated to dryness and counted as described in Ref. [15]. Protein was determined according to Ref. [16].

Extraction and identification of products. Incubation mixtures and culture medium were acidified with 6 N HCl and extracted 3× with Et_2O. The Et_2O was evaporated to dryness and analyzed by two-dimensional PC as previously described [15]. Spots were visualized with long wave UV light, spraying with diazotized *p*-nitroaniline or sulfanilic acid. Comparison of R_f vs color variations with authentic samples were used for identification. Radioactive compounds were detected by radioautography. Cinnamic acid-[^{14}C] and *p*-coumaric acid -[^{14}C] were recrystallized to a constant sp. act. with 25 mg cold material from EtOH–H_2O (2:10).

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