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Utilization of Aromatic Amino Acids By *Rhizoctonia bataticola*

By

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ABSTRACT

Rhizoctonia bataticola, the root-rot pathogen of groundnut readily utilized L-phenylalanine, L-tyrosine and DL-tryptophan when either substituted as nitrogen source or supplimented in Czapek's medium. In the tryptophan supplemented medium indoleacetic acid was measured; the quantity of IAA synthesised considerably varied with incubation time and also with the amount of precursor fortified in the medium. Several phenolic substances were detected in the culture filtrate. Like IAA, ammonia in the culture filtrate also varied with incubation time. Phenylalanine ammonialyase (PAL-ase) activity was detected in the culture filtrate of the medium which received phenylalanine. But, no tyrosine-deaminase was detected in tyrosine fortified medium. The role of these amino acids in the biosynthesis of IAA and phenols by *R. bataticola* is discussed.

INTRODUCTION

Microorganisms utilize several amino acids like phenylalanine, tyrosine and tryptophan for their growth and metabolic activities (van Andel, 1966). Tryptophan is utilized as a sole source of nitrogen by *Fusarium oxysporum* f. *vasinfectum* (Mahadevan, 1966). Tryptophan is used by many fungi for the synthesis of indoleacetic acid (IAA) (Mahadevan, 1965; Sridhar, 1967; Bhaskaran, 1972); phenylalanine and tyrosine serve as important precursors for a variety of phenolic substances (Neish, 1964). Considerable work has been done on the metabolism of these aromatic amino acids in higher plants and in a few species of bacteria, but the information on the utilization and metabolism of these amino acids by phytopathogenic fungi is relatively meagre. In this paper, we report on the utilisation of L-phenylalanine, L-tyrosine and DL-tryptophan by *Rhizoctonia bataticola* (Taub). Butler, the root-rot pathogen isolated from groundnut (*Arachis hypogaea* L.).

MATERIALS AND METHODS

The fungus was grown in Czapek's medium supplemented with 0.1 per cent of the respective amino acids. In another experiment, the nitrogen source in Czapek's medium was substituted with 0.5 per cent phenylalanine, tyrosine or tryptophan. Growth of the fungus was recorded at 10 days interval up to 50th day.

The culture filtrate obtained from each treatment was adjusted to pH 3.0 with 2N HCl and extracted with equal volumes of peroxide-free ether for 24 hr at 2 ± 1°C with solvent changes at 8

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and 16 hr. The ether fractions were pooled, evaporated under a stream of air and the residue dissolved in 2 ml of distilled methanol.

An aliquot of 100 µl of the final methanol extracts was spotted on Whatman No. 1 filter paper and developed ascendingly in a solvent system of *iso*-propanol : ammonia : water :: 10 : 1 : 1 (v/v). The developed papers were air-dried and sprayed with (i) Salkowski spray reagent, (ii) alcoholic bromophenol blue 0.1 per cent (BPB) and (iii) diazotized sulfanilic acid (DSA).

IAA in the methanol extract was also estimated quantitatively employing Salper's reagent (Gordon and Paleg, 1957). Presence of ammonia in the culture filtrate was detected by Nessler's reagent (Dawson *et al.*, 1969). Residual amino acids in the culture filtrate were estimated by paper chromatography and colorimetry (Mahadevan, 1966). Activities of phenylalanine ammonialyase and tyrosine deaminase were tested in the acetone powder of the mycelial mats (Higuchi and Kawamura, 1964).

RESULTS

R. bataticola utilised all the three amino acids as the sole nitrogen source. When the amino acids were added at 0.1 per cent level, most of them were utilized within 20 days, whereas at 0.5 per cent concentration, the utilization was slow (Fig. 1a). The growth of the fungus in the different amino acids differed considerably. Maximum growth was observed on the 30th day in Czapek's, 0.1 per cent tyrosine and 0.1

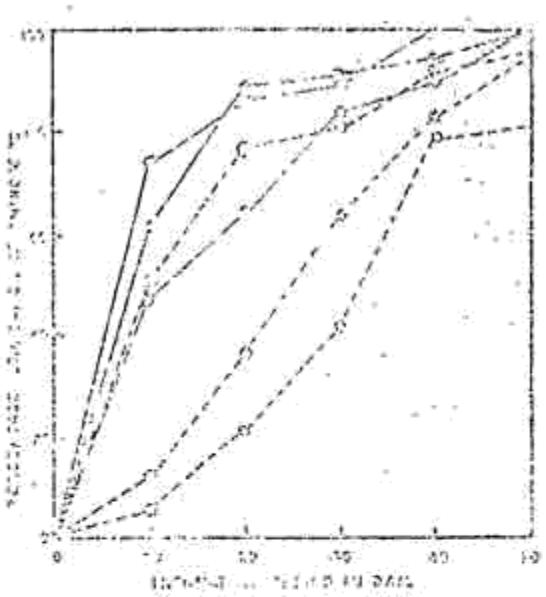

Fig. 1a. Percentage utilization of amino acids by *Rhizoctonia bataticola*

per cent phenylalanine media; in 0.1 per cent tryptophan and in 0.5 per cent concentrations of all the three amino acids, maximum mycelial growth occurred on 20th day. At 0.1 per cent, all the three amino acids promoted growth while at 0·5 per cent they were inhibitory; this was more so with phenylalanine than with tyrosine or tryptophan (Fig. 1b).

IAA was detected only in the tryptophan medium. The amount of IAA synthesis increased up to 20 days in 0.1 per cent tryptophan supplemented medium, and up to 30 days in 0.5 per cent tryptophan medium (Fig. 2).

Besides IAA, the tryptophan medium contained five phenolic substances as indicated by DSA positive spots. One of them (Rf : 0.773) was positive to BPB spray. Phenylalanine and tyrosine media had no indole compounds. DSA spray revealed the presence of four phenolic spots in these

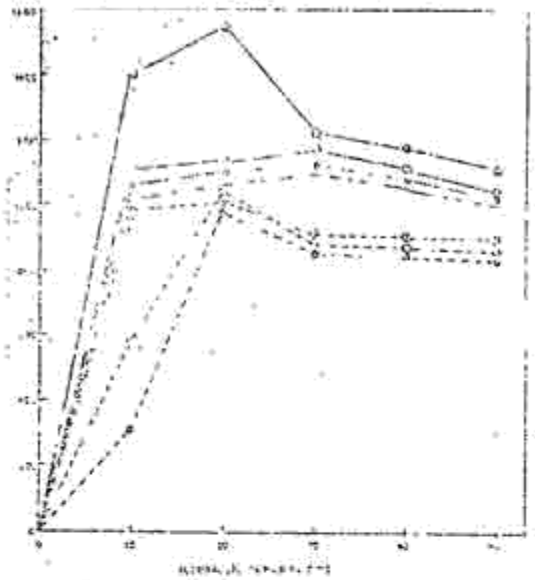

Fig. 1b. Growth of *R. bataticola* in different media

Control
0.1% phenylalanine
0.5% phenylalanine
0.1% tyrosine
0.5% tyrosine
0.1% tryptophan
0.5% tryptophan

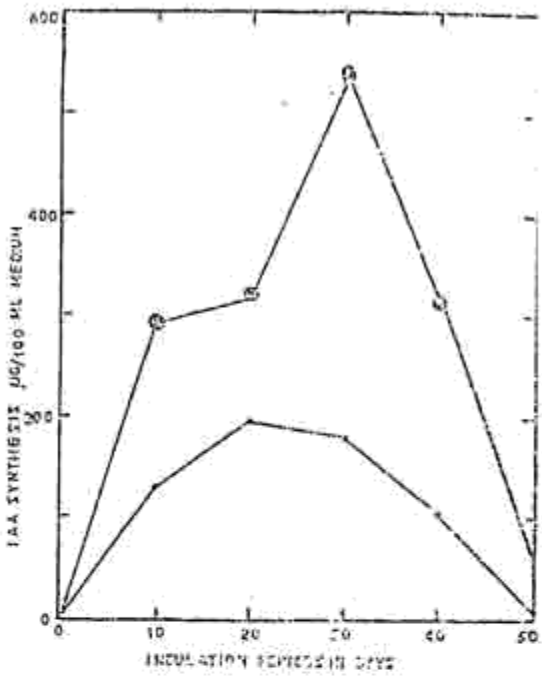

Fig. 2. IAA synthesis (µg/100 ml medium) b *R. bataticola* in tryptophan—Czapek' media

0.1% tryptophan—Czapek's
0.5% tryptophan—Czapek's

media and one of them (Rf: 0.839) gave a positive colour with BPB and was identified as p-coumaric acid by the colour reaction and Rf value with DSA on chromatograms (Table 1).

Presence of ammonia in the culture filtrate varied quantitatively in the different media and also with differen incubation periods. In tryptophar media ammonia was detected up to 30

TABLE 1. Phenolic compounds on the chromatograms sprayed with DSA

Spot No.	Phenylalanine and tyrosine media		Tryptophan media	
	Rf value	Colour	Rf value	Colour
1.	0.893	Yellow	0.941	Light yellow
2.	0.839	Deep orange	0.891	Deep yellow
3.	0.811	Light yellow	0.773	Light brown
4.	0.704	Light brown	0.701	Light brown
5.	...		0.674	Light brown

days, whereas in phenylalanine and tyrosine media ammonia was present up to 50 days (Table 2). Phenylalanine-ammonialyase activity was detected in phenylalanine media (Table 3) but not tyrosine deaminase.

DISCUSSION

R. bataticola utilized phenylalanine, tyrosine and tryptophan for growth. IAA was detected only in tryptophan media. *Pyricularia oryzae*, *Fusarium oxysporum* f. *vasinfectum*, *F. oxysporum* f. *melonis* and *Verticillium dahliae* have been reported to produce IAA only when tryptophan is supplemented (Sridhar, 1967; Mahadevan, 1965; Bhaskaran and Prasad, 1972; Bhaskaran, 1972). A major portion of the added tryptophan was converted to IAA within 30 days with the release of ammonia in the medium suggesting that tryptophan is deaminated during IAA synthesis (Greenberg, 1961). In the later stages of growth, the quantity of IAA in the medium decreased. This may possibly be due to the fungal metabolism of IAA leading to the formation of indoleaspartic acid followed by decarboxylation to indoleacetamide (Andreae and Good, 1957). However, this requires investigation.

TABLE II. Detection of ammonia in the culture filtrate of *R. bataticola* grown in different media

Media	Incubation time (days)				
	10	20	30	40	50
0.1% tyrosine	+	+	+	+	++
0.5% tyrosine	—	—	—	+	+
0.1% phenylalanine	+++	+++	++	+	+
0.5% phenylalanine	—	—	+	+	++
0.1% tryptophan	+	+	+	—	—
0.5% tryptophan	—	++	+++	—	—
Control (Czapek's medium)	—	—	—	—	—

+, ++ and +++ indicate the intensity of colour with Nessler's reagent
— indicates absence of ammonia in the culture filtrate

TABLE III. Phenylalanine ammonialyase activity* in mycelial mats of *R. bataticola*

Media	10th day	50th day
0.1% phenylalanine	7.1778	9.3216
0.5% phenylalanine	33.8382	21.6325

* μg cinnamic acid formed with 100 mg acetone powder of the mycelial mats in 6 hr incubation

Besides IAA, five phenolic compounds were detected in tryptophan media, the nature and identity of which await further studies.

Phenylalanine and tyrosine serve as the precursors of a number of phenolic compounds (Neish, 1964). Phenylalanine-ammonialyase and tyrosine deaminase are known to mediate this conversion (Neish, 1964). *R. bataticola* produced phenylalanine-ammonia lyase. Tyrosine deaminase activity could not be detected in the cultures. It is probable that the pronounced activity of phenylalanine-ammonia lyase of the fungus leads to the cleavage of the benzene ring from the amino group of phenylalanine serving as the nucleus for the synthesis of various phenolics.

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