

METABOLISM OF AROMATIC AMINO ACIDS BY *PYTHIUM APHANIDERMATUM* (EDSON) FITZP.

AROMATIC amino acids are known to be utilized by several micro-organisms for various metabolic processes. It has been well established that phenylalanine and tyrosine are the important precursors of phenolic compounds¹ and are also involved in the biosynthesis of melanin pigments². Tryptophan has been found to be utilized for the synthesis of indole acetic acid (IAA)³ and in certain cases it is also known to be involved in the biosynthesis of diphosphopyridine nucleotide through nicotinic acid⁴. As no work has been reported on the metabolism of aromatic amino acids in *Pythium aphanidermatum* (Edson) Fitzp., the fungus causing damping-off of tomato seedlings, the present investigation was taken up with L-phenylalanine, L-tyrosine and L-tryptophan and the results are presented in this paper.

The fungus was grown in Czapek's medium supplemented by 0.1% phenylalanine, tyrosine or tryptophan. The observations on the growth of the fungus and the utilization of added amino acids were made at 5-day intervals up to 25 days. The residual amino acids⁴, IAA³, phenolic⁵ and indole⁶ compounds were estimated either quantitatively or qualitatively. The presence of ammonia in the culture filtrate was detected with Nessler's reagent⁶. The acetone powder of the mycelial mats was used in assessing the activity of phenylalanine ammonia lyase and tyrosine deaminase⁷.

The results indicate that there is a high degree of correlation between the growth of fungus and percentage utilization of added amino acids (Fig. 1).

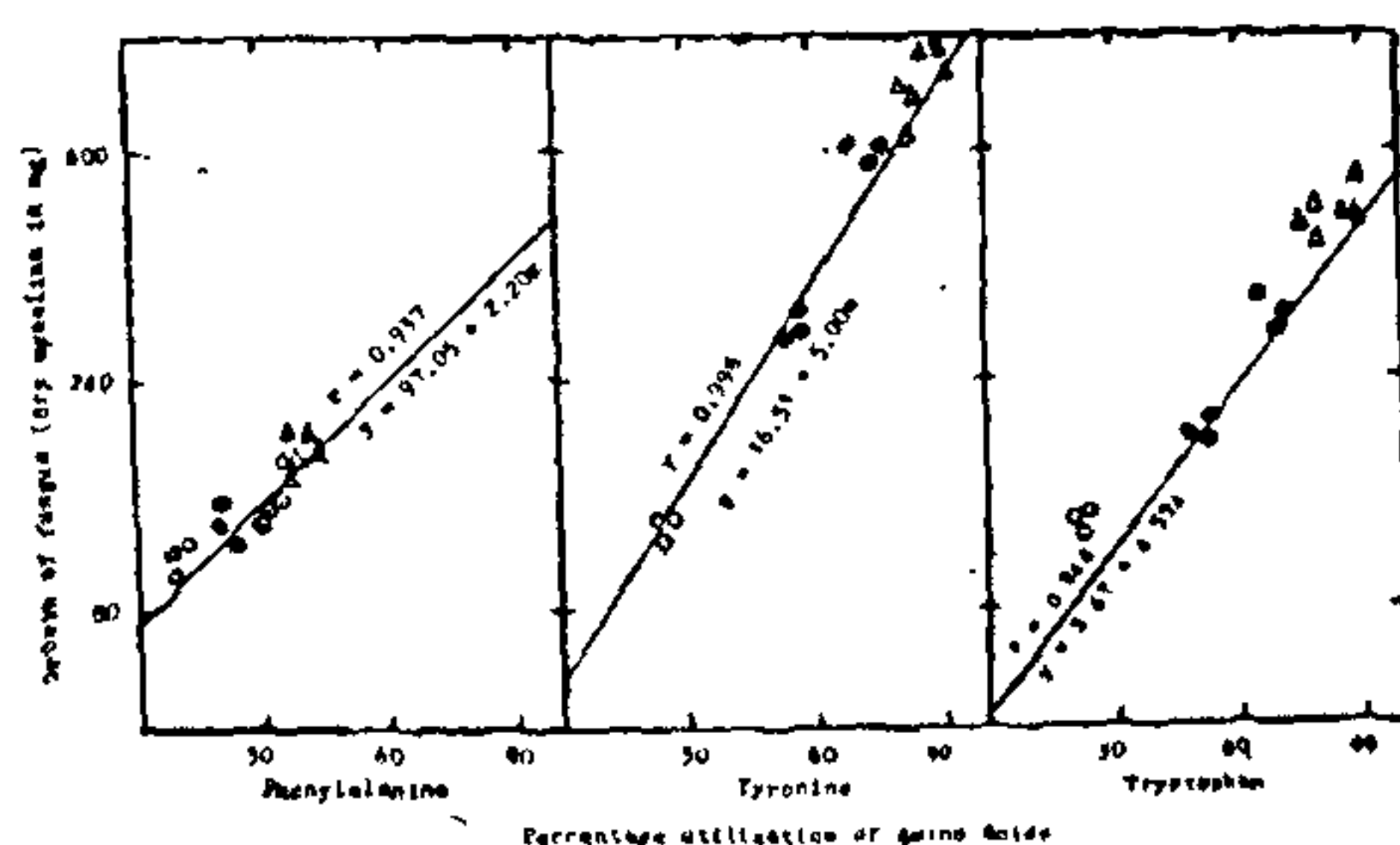


FIG. 1. Growth of *Pythium aphanidermatum* in relation to percentage utilization of amino acids. ○—5th day, ●—10th day, ⊙—15th day, △—20th day and ▲—25th day.

Among the three aromatic amino acids tyrosine has supported the maximum growth of the fungus with 88% utilization in 25 days. Phenylalanine has resulted in poor growth of fungus with 39% utilisation within the same period. On the other

hand the fungus has utilized most of the tryptophan within 15 days and thereafter the utilization rate was slowed down.

IAA and two other indole compounds were present only in tryptophan medium with more intense reaction for ammonia with Nessler's reagent. The production of IAA in the medium was found to be increased with increase in incubation period (Fig. 2). The increase in IAA content could be

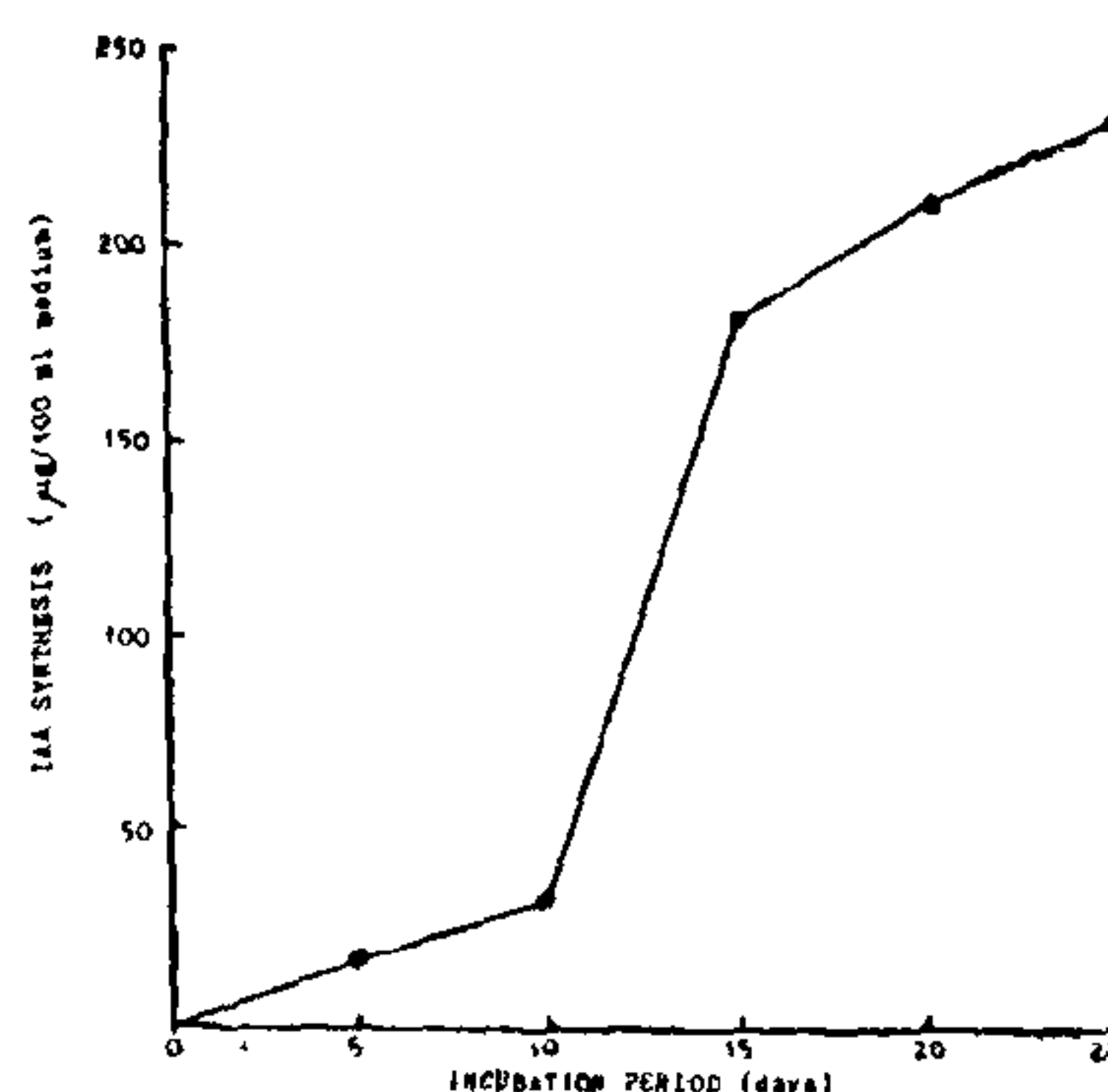


FIG. 2. IAA synthesis by *Pythium aphanidermatum* in tryptophan supplemented Czapek's medium.

due to deaminating tryptophan to indole-3-pyruvic acid followed by decarboxylation to indole acetaldehyde which is subsequently oxidised to IAA as has been suggested in other fungi⁸⁻⁹. Besides these indole compounds, the tryptophan medium had six phenolic compounds, having the R_f values 0.671, 0.721, 0.790, 0.863, 0.868, and 0.929, and one of them (R_f : 0.868) was identified as anthranilic acid. The presence of anthranilic acid suggests that a portion of tryptophan might have been utilized via kynurenine pathway as indicated by Sequeira and Williams¹⁰ in *Pseudomonas solanacearum*. The culture filtrate of phenylalanine and tyrosine media had only four phenolic compounds with R_f values 0.721, 0.812, 0.851 and 0.884 and with negative colour reaction to IAA and other indole compounds and less intense reaction to Nessler's reagent. One of the phenolic compounds (R_f : 0.851) has been identified as *p*-coumaric acid. The nature and identity of rest of the phenolic compounds in all the media detected await further study.

The mycelium grown on the medium supplemented with phenylalanine had traces of phenylalanine ammonia lyase activity with negative reaction to tyrosine deaminase. It has been suggested that phenolic compounds have been formed

through deamination of phenylalanine by phenylalanine ammonia lyase and tyrosine by tyrosine deaminase¹. However the latter could not be traced out in the present study. The metabolic intermediaries of phenylalanine and tyrosine detected in the present study indicate that the metabolism of phenylalanine and tyrosine follows the same pathway in *Pythium aphanidermatum*.

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INHIBITORY ACTIVITY OF THE PARASPORAL CRYSTAL OF *BACILLUS THURINGIENSIS* VAR. *THURINGIENSIS* ON YOSHIDA ASCITES SARCOMA

Bacillus thuringiensis produces two insecticidal components during its growth phase¹⁻², the β -exotoxin and the δ -endotoxin or the proteinaceous crystal. The β -exotoxin was isolated, characterised and found to be toxic for mammals by inhibiting the ribosomal RNA synthesis³⁻⁵. The δ -endotoxin was reported to have a high insecticidal activity without any chronic or acute toxicity for warm blooded animals and fishes⁶. In the insect system it changes the membrane permeability and inhibits the active transport mechanism of the cell⁷⁻⁸.

During the process of screening for anti-tumour agents of bacterial origin, we found that the proteinaceous crystal of *B. thuringiensis* has anti-Yoshida ascites sarcoma activity, which is being reported herein.

MATERIALS AND METHODS

Tumour.—Yoshida ascites sarcoma (YAS), a rapidly developing, chemically induced tumour⁹ was maintained in isogenic Wistar rats (A/IISe)

by serial intraperitoneal (i.p.) transfer of 2×10^7 tumour cells, once in 4 days.

Purification of the crystal of *B. thuringiensis*.—The strain of the test organism *B. thuringiensis* var. *thuringiensis* serotype I was kindly supplied by Dr. De Barjac of Institut Pasteur, Paris.

The toxins of *B. thuringiensis* were produced by growing the organism as described by Delafield *et al.*¹⁰. The crude spore crystal complex and pure crystal preparations were obtained by following the procedures of Dulmage *et al.*¹¹ and Pendelton and Morrison¹².

The crystal suspensions were prepared freshly by mixing the freeze-dried materials in sterile saline such that the required concentrations were contained in 0.2 ml of the suspension. Toxicity and evaluation of anti-tumour activities of the crystal preparations were carried out in healthy isogenic Wistar rats of 100–120 gm weight and in Swiss mice of 20–25 gm weight. These animals were provided with 'pellet' diet supplied by Hindustan Lever Ltd., Bombay, and water *ad libitum*. Experimental rats were injected intraperitoneally with 20 million actively dividing YAS cells. Treatment consisted of a single i.p. dose of the crystal preparation given 24 hours after tumour transplantation. Regular observations were made on weights and general behaviour of all the animals.

RESULTS AND DISCUSSION

The acute toxicity data with crude and pure crystal preparations on the experimental animals (Table I) indicate that in contradiction to earlier reports⁶, high doses of the crudespore-crystal complex were lethal to mice and rats.

TABLE I
Toxicity of crystal preparations of *B. thuringiensis* in experimental animals

Drug	Animal	Route	Maximum Lethal	
			tolerated dose mg/kg	dose mg/kg
1. Spore-crystal complex	Mice	i.p.	30	35
	Rat	i.p.	35	40
2. Pure crystal	Mice	i.p.	60	70
	Rat	i.p.	120	—

An interesting observation is that with the purification of the crystal preparation, the toxicity was reduced as seen in the increase of the maximum tolerated dose.