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Synthesis of IAA by Some Hyphomyceteous Fungi

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IAA synthesis by *Alternaria tenuis*, *Helminthosporium gramineum*, *H. hawaiiense*, *H. holmii*, *H. monoceras*, *H. pedicellatum*, *H. rostratum*, *H. spiciferum*, *Hendersonula toruloidea*, *Myrothecium roridum*, and *Phaeotrichoconis crotalariae*, under different cultural conditions, was investigated. The species synthesized IAA in the presence of the precursor tryptophan. However, *M. roridum* synthesized IAA even in the absence of tryptophan. For maximum production of IAA by *M. roridum*, *p*H 5.0–7.0, potassium nitrate and darkness were necessary.

Key Words: IAA, Tryptophan, Hyphomycetes, *Helminthosporium*, *Myrothecium roridum*

Introduction

Since the report of synthesis of IAA by *Rhizopus suinus* by Thimann (1935), several fungi are reported to produce this auxin (Mahadevan & Chandramohan 1966, Sridhar 1967, Govindarajulu & Raghunathan 1975). Gruen (1959) reported that both pathogens and non-pathogens have the ability to produce IAA in culture media supplemented with tryptophan or other IAA precursors. The growth stimulating effect of culture filtrates of various fungi, has often been attributed to the production of IAA. In the present investigation 11 species of hyphomycetes were screened for their ability to produce IAA from tryptophan.

Materials and Methods

The test fungi were grown in 25 ml of modified Asthana and Hawker's medium 'A' (tryptophan substituted for potassium nitrate) at 28±2 °C. After 4, 8, 12 and 16 days incubation, the mycelium was removed and the filtrate was centrifuged at 1,800×g for 30 min to remove the debris. IAA content in the filtrate was estimated quantitatively (Bentley 1962). To 8 ml of IAA extract, 2 ml of Salkowski's reagent was added and incubated for 30 min in darkness. The intensity of colour developed was read at 530 nm. Un-inoculated culture broth

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containing the reagent served as blank. In *p*H studies, the *p*H of the culture broth was adjusted with the help of 6N HCl or 6N NaOH as the case may be. The effect of different nitrogen sources on IAA synthesis was studied by substituting the tryptophan of the medium with different nitrogen sources so as to supply 483 mg of nitrogen per liter.

Results and Discussion

Though all the fungi were capable of synthesizing IAA in tryptophan media, they differed significantly in quantity (table 1). *Hendersonula toruloidea* and *Helminthosporium pedicellatum* synthesized maximum amount, while *H. hawaiiense* and *H. monoceras* and *Myrothecium roridum* were poor producers of IAA. Other species produced moderate amout of IAA. Fungi also differed in the optimum age for the production of IAA. The maximum amount of IAA was produced only after four days of incubation. Similarly, Mahadevan (1965) recorded increasing quantity of IAA with the increasing age of *Fusarium vasinfectum.*

Table 1 *In vitro synthesis of IAA by species of hyphomycetes on tryptophan substituted Asthana and Hawker's medium 'A'*

Organism	Incubation (day)			
	4	8	12	16
Alternaria tenuis	137.0*	287.0	305.0	295.0
Helminthosporium gramineum	100.0	205.0	402.0	370.0
H. hawaiiense	282.0	325.0	367.0	401.0
H. holmii	25.0	134.0	162.0	250.0
H. monoceras	—	52.5	30.0	20.0
H. pedicellatum	80.0	378.0	428.0	402.0
H. rostratum	110.0	325.0	270.0	189.0
H. spiciferum	100.0	275.0	340.0	395.0
Hendersonula toruloidea	92.5	530.0	362.0	312.0
Myrothecium roridum	46.0	67.5	45.5	45.0
Phaeotrichoconis crotalariae	12.5	282.5	118.0	60.0

*Concentration of IAA in μg/ml

Except *M. roridum*, which synthesized IAA even in the absence of tryptophan, other fungi produced IAA only in the presence of tryptophan. Sequeira (1963) pointed out that different fungi may have evolved different pathways of IAA synthesis. The various factors influencing IAA synthesis in *M. roridum* were, therefore, investigated.

Influence of pH: Table 2 clearly shows that *p*H 5.0–7.0 was quite favourable for IAA synthesis. *M. roridum* failed to synthesize IAA both at *p*H 4.0 and 9.0. At *p*H 7.0

Table 2 *Effect of* p*H of the medium on the synthesis of IAA by* M. roridum

*p*H	Incubation (day)			
	4	8	12	16
4.0	—	—	—	—
5.0	27.5	50.0	97.5	2.0
6.0	17.5	37.5	55.5	87.5
7.0	—	25.0	45.0	117.5
8.0	—	55.0	37.5	—
9.0	—	—	—	—

and 8.0, IAA synthesis was delayed till 4th day by which time, the *p*H was reduced to favourable level.

Influence of light: Keeping inoculated flasks in darkness (after wrapping them in a black paper) markedly increased the IAA production clearly indicates the prevention of photo-oxidation of IAA (Galston 1949). No IAA was detected on 16th day in cultures exposed to daylight while in dark-grown cultures traces of IAA were present.

Influence of nitrogen sources: Of the various nitrogen sources used, *M. roridum* failed to synthesize IAA on peptone, while on ammonium chloride and L-asparagine, it produced only traces of IAA. Potassium nitrate was superior to tryptophan in IAA induction, on the 12th day of analysis. Similarly, Charya et al. (1979) reported that *Phoma exigua* and *Graphium penicillioides* produced IAA in the presence of potassium nitrate.

Table 3 *Effect of light on the synthesis of IAA by* M. roridum *during the growth on Asthana and Hawker's medium 'A'*

	Incubation (day)			
	4	8	12	16
Dark	50.0*	100.0	75.0	15.0
Light**	4.5	25.0	32.5	–

*Concentration of IAA in μg/ml
**Natural daylight (10 hr/day)

Table 4 *Effect of different nitrogen sources on synthesis of IAA by* M. roridum

Nitrogen source	Incubation (day)			
	4	8	12	16
Potassium nitrate	27.5	50.0	97.5	20.0
Ammonium chloride	32.5	25.0	–	–
L-asparagine	—	–	37.5	–
L-tryptophan	46.0	67.5	45.5	45.0
Urea	–	35.0	45.0	30.0
Peptone	–	–	–	–

In view of the incomplete evidence for IAA biosynthesis and optimal conditions, *M. roridum* may be an ideal tool to extricate the left-over gaps in our knowledge. It may also be used in the commercial manufacture of IAA, as it requires no tryptophan and produces copious amounts of IAA from potassium nitrate.

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