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Identification of Indole-3-Acetic Acid in the Basidiomycete Schizophyllum commune[1]

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Summary. Indole-3-acetic acid (IAA) was detected in the ether extracts of culture filtrates of indigotin-producing strains of the basidiomycete *Schizophyllum commune*. Several solvents, known to give distinctly different R_F values for IAA, and 3 location reagents gave identical results with synthetic IAA and IAA found in the extract. Confirmation was obtained by the *Avena* straight growth test, split pea test, and ultraviolet absorption spectrum.

Numerous investigations have established the synthesis of IAA by fungi belonging to different taxonomic groups including the *Basidiomycetes* (5). Most of the reports involve pathogenic fungi which produce IAA after the addition of tryptophan to the medium. *Schizophyllum commune* Fries, on the other hand, is easy to isolate and cultivate on a chemically defined medium. A large number of biochemical and morphological mutants, many of which produce the blue pigment indigotin (8), have been isolated from this fungus which is readily amenable to genetic studies. In this investigation only these latter mutants were found to produce detectable amounts of IAA which indicates the possibility of a relationship between the formation of IAA and indigotin. For these reasons a study of the biosynthesis and the role of IAA in this organism is of interest. This paper presents the evidence that IAA is formed by the indigotin-producing strains and discusses the possible significance of the presence of IAA in these strains.

Materials and Methods

Growth Conditions. Pigment-producing strains of *Schizophyllum commune* (130 and 2456) were grown in 18 liter carboys containing 12 liters of minimal medium (8) at room temperature with constant aeration. Strains 699 and 2417, which do not produce pigment on this medium were also studied.

Extraction of the Medium. After the culture had grown for 10 days, half a liter of the medium was taken from the carboy and passed through cheese cloth to remove the mycelium. For use in paper and Celite column chromatography the clear filtrate was brought to pH 2.8 with 0.1 N HCl and extracted by shaking 3 times with 0.5 liter of peroxide-free ethyl ether. The extract was filtered and evaporated over a hot bath with constant stirring to break up lipids. The residue was taken up in 2 ml of ether (extract I). The same procedure was followed with the preparation for the *Avena* straight growth test, only, instead of ether, the residue was taken up in 2 ml of hot water (extract II). Both extracts were stored in a freezer until used.

Paper Chromatography. Chromatographic solvents were made as follows: isopropanol:ammonia:water = 20:2:1 (IprAm); isopropanol:ammonia:water = 8:1:1 (IprAm'); NaCl (8% w/v) plus 1 ml glacial acetic acid (NaClA); *n*-butanol:glacial acetic acid:water = 60:15:25 (BuA); methanol:*n*-butanol:benzene:water = 1:1:1:1 (MBBW); isopropanol:borate buffer pH 8.4 = 80:20, saturated with NaCl (IprBor); isopropanol:glacial acetic acid:water = 4:1:1 (IprA). The descending method and Whatman No. 1 paper were generally used. Exceptions were the use of the ascending method when IprAm and IprAm' were applied as solvents and No. 3 MM paper was used for elution. For 2 dimensional chromatography IprA was used as the first solvent and IprAm' as the second solvent.

Location Reagents for Paper Chromatograms. The following reagents were used: *p*-dimethylaminobenzaldehyde (reagent A) and *p*-dimethylaminocinnamaldehyde (reagent B) were prepared according to Smith (9). Salkowski reagent (reagent C) was prepared according to Tang and Bonner (12). All were used as dipping reagents.

Bioassay Techniques. The *Avena* coleoptile straight growth test (11) and split pea test (14) were employed.

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Isolation of the Compound. Extract I was fractionated on a Celite column according to the method of Hamilton et al. (6). A sample from each fraction was chromatographed with IprAm′ and treated with reagent A in order to locate the tubes that contained IAA. Under the conditions employed, this compound was found in the 30 to 50 ml fraction. Chromatography of this fraction revealed no visible spots, and only a single spot (purple) appeared after treating the chromatogram with the color reagent.

The contents of the 10 tubes that contained the compound were combined, concentrated to 5 ml by distillation of the solvent and 3 ml were used for recording of the spectrum.

Results

Chromatography of extract I resulted in the detection of a number of spots, some of which reacted with indole indicator reagents. The properties of one of these spots (spot 1) coincided with those of synthetic IAA (table I). The purple color of this spot was accompanied by a yellow color, which developed first. Two dimensional chromatography showed that the yellow color was due to anthranilic acid which is also synthesized by the pigmented strains (2).

The biological activity of extract II was tested with the *Avena* straight growth test and with the split pea test. Both the *Avena* coleoptile and the split pea were floated in a solution containing either the ether extract or the piece of the chromatography paper containing the unknown compound. Both tests showed the presence of an auxin. The concentration of the auxin was calculated from the *Avena* straight growth test by interpolation on the IAA curve of the controls and was found to be approximately 0.97 mg/l (table II).

Since pure peroxidase plus H_2O_2 destroys IAA (4) and the loss of activity with this enzyme is specific for IAA (13), the *Avena* straight growth test was performed also with the unknown compound after treatment with horseradish peroxidase. One tenth mg of the enzyme was diluted with 3 ml of 8 mM citric acid buffer at pH 3.7 plus 1 drop of 0.3 % H_2O_2. After 0.2 ml of the ether extract was added, the mixture was incubated at 30° for 2 hours after which it was placed in a boiling water bath

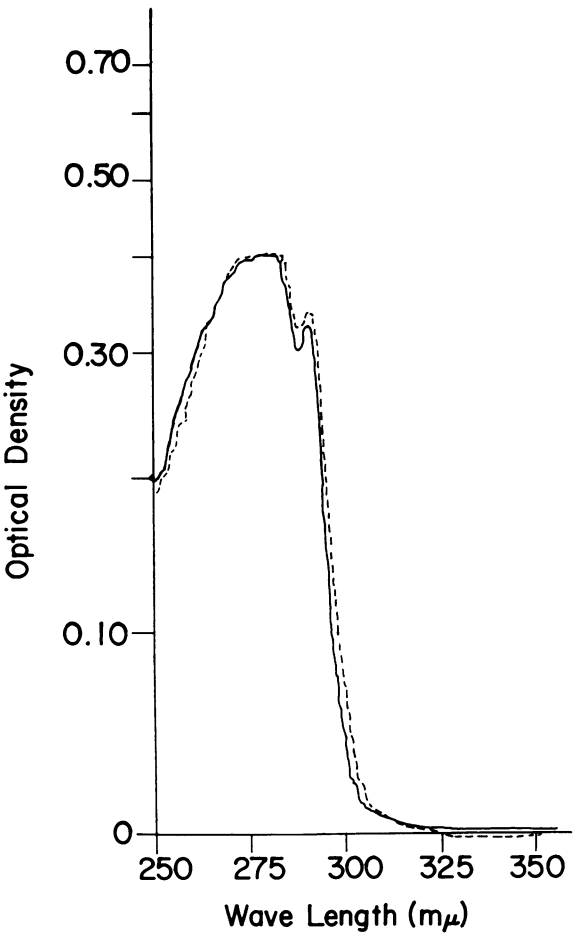

FIG. 1. Ultraviolet absorption spectrum of synthetic IAA (- - -) and IAA extracted from the culture medium of pigmented strain 130 by column chromatography (———). The Celite effluent was placed in a stoppered quartz cuvette and scanned at a rate of 50 mμ/min with a Beckman DB spectrophotometer.

Table I. *Comparison of the Properties of the Extracted Unknown Compound with those of IAA*

Compound	IprAm	NaClA	BuA	MBBW	IprBor	Color reagents*** A	B	C
	R_F	R_F	R_F	R_F	R_F			
Spot 1	0.33	0.56	0.88	0.92	0.80	yellow→ purple	brown/pink→ purple	pink
IAA*	0.33	0.56	0.84	0.92	0.80	yellow→ purple	brown/pink→ purple	pink
IAA**	0.33	0.56	0.89	0.92	0.80	purple	purple	pink

* Synthetic IAA was co-spotted so as to overlap the spot of the extract.
** Synthetic IAA only.
*** A = *p*-dimethylaminobenzaldehyde; B = *p*-dimethylaminocinnamaldehyde; C = Salkowski reagent.

Table II. *Auxin Content in Culture Medium of Mutant Strain No. 130 of* S. Commune

In a total volume of 40 ml, the following were present in the final concentration indicated: sucrose, 2%, penicillin G 0.1 mM, KH_2PO_4 5 mM. In the reference solutions IAA was added to give the full concentration shown.

	Straight growth	Equivalent
	% elongation	mg/l
Extract*	92.7	0.97
Chromatogram**	79.0	0.48
Chromatogram***	53.7	
Extract + peroxidase	22.8	
Reference solutions		
IAA (10.0 mg/l)	104.0	
IAA (3.0 mg/l)	96.0	
IAA (1.0 mg/l)	82.1	
IAA (0.3 mg/l)	67.3	
No IAA	57.6	

* 0.2 ml Of extract II was diluted to one third of its concentration and 2 ml of the final solution was used for this test.

** 25 µl Of extract II was applied to Whatman No. 3MM paper. The paper was developed with IprAm and the spot corresponding to the R_F of IAA was cut and immersed in the solution.

*** A piece of the chromatography paper that showed no reaction with Ehrlich reagent.

for 25 minutes to inactivate the enzyme. The pH was then adjusted to 5.5 with 1 N NaOH. The enzyme caused the complete loss of activity of the compound, a result which is consistent with the hypothesis that the auxin in the filtrate culture is IAA.

After the IprAm 8:1:1 solvent and No. 3 MM Whatman paper were used, the spot with the R_F value corresponding to that of IAA was eluted with 50% ethanol. The ethanol solution was concentrated and chromatographed again with NaClA as the solvent. Synthetic IAA was similarly treated and chromatographed. Both the IAA and the unknown spots stained pink with Salkowski reagent and gave the same R_F values.

The ultraviolet absorption spectrum of the isolated compound was compared with that of IAA (fig 1). Both show a shoulder at 290 mµ and a peak at 280 mµ, which is characteristic of IAA.

Discussion

Besides IAA, the only natural auxins which give a pink color with the Salkowski reagent are indolepyruvic acid and indoleacetamide (10). The latter 2 compounds, however, differ from IAA in their auxin activity and behavior on paper chromatograms. Indolepyruvic acid decomposes readily and gives a specific chromatographic pattern (7), and the R_F value of indoleacetamide is much higher than that of IAA (0.81 compared with 0.40 in IprAm'). Indolecarboxylic acid also gives a pink color with Salkowski reagent, but it has no auxin activity, as was demonstrated by Clarke et al. (1).

Thus the evidence presented clearly demonstrates the presence of IAA in the culture filtrate of indigotin-producing strains of *S. commune*. It was not present in detectable amounts in the 2 non-indigotin-producing strains studied.

The metabolic significance of IAA in this fungus is uncertain. The fact that IAA is produced in excess favors the view that it does not have a growth regulating role. Experiments with labeled indole and IAA (3) showed that when supplied in a small concentration the fungus was able to metabolize indole to IAA and indigotin, and IAA to indole and indigotin. This raises the possibility that IAA, as well as indigotin, is produced by the fungus as a means of detoxication of indole, which is toxic to the fungus in a very small concentration (P. G. Miles, unpublished observations). Accumulation of IAA to toxic levels might be prevented either by further breakdown or by polymerization and formation of insoluble compounds.

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