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INDOLE AUXINS CONTAINED IN *SACCHAROMYCES CEREVISIAE* AND A MUTANT OF *ASPERGILLUS NIGER*

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Abstract

The endogenous and exogenous indole compounds in *Saccharomyces cerevisiae* and *Aspergillus niger* NAC2 were studied. The endogenous auxins were extracted with absolute alcohol at low temperature while the exogenous auxins were extracted with peroxide free ether. Paper chromatography and color reaction of various reagents were used for identification of the indole compounds. Indoleacetic acid, indoleacetonitrite, and indolealdehyde were discovered in yeast extractions. Indole, indoleacetic acid, tryptophan and sakatole were discovered in extractions of *Aspergillus niger* NAC2. Three and two indole compounds were unable to be identified in the solution media extractions of yeast and *Aspergillus* respectively. Physiological roles of the indole compounds were also discussed.

Introduction

Indole-3-acetic acid (IAA) has been known for many years as an auxin fungi (Gruen, 1959a). Also, *Aspergillus niger* 78, a special strain of *A. niger*, was reported as capable of transforming tryptamine in respect to its amino group and indole ring (Dvornikova *et al*, 1968). According to the previous and recent reports, IAA is undoubtly an indole auxin present in many fungi (Gruen, 1959a and b, 1965; Epstein and Miles, 1967; and Wolf, 1952). Among these reports, it seems that the indole compounds, with the exception of IAA, do not attract the interest of the various authors. Most of them did not mention whether there is any other indole compounds present or not. Norberg (1968) has succeeded in extracting of other indole compounds and other growth stimulating substances from *Exobasidium*. According to the pathways of synthesis of indole-3-acetic acid from tryptophan in cabbage and tomato plant (Andersen, 1968; Wightman, 1964), some intermediate indole compounds are

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evolved. With the light of these, the authors are interested if they can find out other indole compounds in yeast and aspergillus.

Materials and Methods

The strain of *Saccharomyces cerevisiae* was obtained from Mr. S. M. Sun of the University of Hong Kong and was purified by dilution method at this college.

The mutant, *Aspergillus niger* NAC2, was isolated from our culture collections as a spontaneous mutant. It is characterized by its late and slight sporangium formation.

The yeast was cultured in 250 ml Erlenmeyer flasks containing culture solution of the following composition: D-glucose (Dextrosol), 30 gm; Ammonium nitrate, 1 gm; Di-potassium phosphate, 1 gm; Magnesium sulfate 0.5 gm; and distilled water, 1 liter. The pH of the medium solution was adjusted to 4 with hydrochloric acid before autoclaving. The flasks were fixed in a Gyrotory water-bath shaker of approximately 120 rpm and the temperature was adjusted to 25±1°C. The cultures were grow for four days.

The *Aspergillus niger* NAC2 was inoculated in bottles of 7 cm in diameter and 8.5 cm in height containing Czapek's solution medium of about one-third of the height. The cultures were grown in complete darkness for 10 days at room temperature of about 25–28°C.

Separation of the yeast cells from the solution medium was made by centrifugalizing in a centrifuge of 5,000 rpm for 15 minutes at 5°C. The yeast cells were washed three times with de-ionized water. The aspergillus mats were separated from the culture solution by suction filtering and washed as in yeast cells.

Endogenous indole compounds were extracted by absolute alcohol. Sonifying of the mashed aspergillus mats and the yeast cells was carried out in absolute alcohol at 0°C for 30 minutes. Extraction was carried out in a refrigerator at about 5°C for three days. This procedure was repeated three times. The extracts were condensed by vacuum distillation with a Buchi rotavapor to approximately 1/20 of their original volumes.

Paper chromatography was developed on Whitman's No. 1 filter paper of 3×35 cm in a Shandon Chromajar with iso-propanol (8): 28% ammonia (1): distilled water (1). The paper and tank were left overnight for saturation before ascending development. Broader paper strips were also used for assuring the same fraction to be tested.

Detection of the spots was made by using the Ehrlich's, Diazotized p-nitroaniline, Diazotized sulfanilic acid, and Salkowski reagents. Change of color in various reagents was used for qualitative identification (Stowe and

Table 1. *Detection of indole compounds on paper chromatography*

Origin	Rf	Ehrlich	Diazotized p-nitroaniline	Diazotized sulfanilic acid	Salkowski	compound
Yeast solution medium extract	0.76	purple	orange	—	blue	Indoleacetonitrile
	0.35	purple	light-brown	yellowish orange	rosy red	Indole-3-acetic acid
	0.15	yellow	—	—	—	Citrulline (amino acid)?
	0.95	purple to greenish blue*	—	—	—	—
Yeast cell extract	0.76	purple	orange	—	blue	Indoleacetonitrile
	0.70	purple	orange	orange	rosy red	Indole aldehyde
	0.53	purple**	—	—	—	—
	0.35	purple	light-brown	yellowish orange	rosy red	Indole-3-acetic acid
	0.33	purple***	—	—	—	—
A. niger NAC2 solution medium extract	0.80	pink	light-brown	light-brown	rosy red	Indole
	0.37	purple	light-brown	yellowish orange	rosy red	Indole-3-acetic acid
	0.20	purple	yellowish orange	—	yellow	Tryptophan
A. niger NAC2 mycelial mat extract	0.92	ash blue	light-orange	light-yellow	greyish brown	Sketole
	0.37	yellow****	colorless	yellow to light-brown	light-yellow	—
	0.05	yellow*****	colorless	orange-pink	light-yellow	—

* occurred often, insignificant with other test

** & *** seen few times

**** & ***** seen many times

Thimann, 1954; and Sen and Leopold, 1954).

Results

Eleven different indole compounds and probably one amino acid were discovered by us in this experiment. Three in yeast cell extract and two in aspergillus mycelium of them were not succeeded in identification by us. All the results are summed up in table 1.

Discussion

Production of indole compounds by various fungi is valid to many respects. According to Norberg (1968) and our experiments, we can say that IAA is not the unique indole compound that fungus can produce. Fungi, like many higher plants, can produce various indole compounds. The production of indole compounds may be different according to different species.

The difference of the indole products produced by fungi may indicate that different species of fungi may have different pathways for IAA synthesis and breaking down. Plants of different species can synthesize IAA with different paths (Anderson, 1968; Wightman, 1964). The fungus can surely do that.

Alkaloids and auxins have been reported as inhibitory substances to fungi (Hessayon, 1952; Lingappa and Lingappa, 1967). An autoinhibitory substance of alkaloid like has been reported in a green alga (Harris, 1970). The indole compounds may also be the growth regulators of the fungus.

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