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INDOLE COMPOUNDS IN *SACCHAROMYCES CEREVISIAE* AND *ASPERGILLUS NIGER*

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Abstract

The endogenous and exogenous indole compounds in yeast (*Saccharomyces cerevisiae* Hansen) and a strain of black mold (*Aspergillus niger* van Tieghem) were studied. The endogenous compounds were extracted with absolute alcohol at low temperature while the exogenous ones were extracted with peroxide free ether. Paper chromatography and color reaction of various reagents were used for identification of the indole compounds. Indoleacetic acid, indoleacetonitrile and indoleacetaldehyde were discovered in yeast extracts. Indole, indoleacetic acid, tryptophan and skatole were discovered in extracts of the black mold strain. Three unidentified compounds of yeast cell extract and two of the black mold mycelium extract were also detected.

Introduction

Indole-3-acetic acid (IAA) has been known for many years as an auxin occurred in various fungi (Epstein and Miles, 1967; Gruen, 1959a and b, 1965; and Wolf, 1952). A special strain of black mold (*Aspergillus niger* 78) was reported as capable of transforming tryptamine in respect to its amino group and indole ring (Dvornikova *et al.*, 1968). Although some other auxins have also been extracted from *Exobasidium*, however, little research has been done on the indole compounds other than IAA. According to the pathways of IAA synthesis from tryptophan in cabbage and tomato (Andersen, 1968; and Wightman, 1964), some intermediate indole compounds are evolved. Some results have also shown that there are relationships between the yeast growth and these compounds (Bau and Ng, 1975; Yanagishima, 1967; Yanagishima and Shimoda, 1968; and Yanagishima *et al.*, 1969 and 1970). In the light of these findings, the author has been interested in the possibility of other indole compounds being present in yeast and black mold.

Materials and Methods

The strain of yeast (*Saccharomyces cerevisiae* Hansen) was obtained from Mr. S. M. Sun of the University of Hong Kong and was purified by dilution method in this laboratory.

The strain of black mold (*Aspergillus niger* van Tieghem) was isolated as a spontaneous mutant from our culture collection. It is characterized by its late and slight conidia formation.

The yeast was cultured in 250 ml Erlenmeyer flasks containing 100 ml of liquid nutrient medium with the following composition: D-glucose (dextrosol), 30 g; ammonium nitrate, 1 g; di-potassium phosphate, 1 g; magnesium sulphate, 0.5 g; and distilled water, 1,000 ml. The pH of the solution medium was adjusted to 4 with 4 N hydrochloric acid before autoclaving. The flasks were fixed in a Gyrotory water-bath shaker of 120 rpm and the temperature was adjusted to 25±1°C. The cultures were grown for four days.

The black mold was inoculated in bottles of 7 cm across by 8.5 cm tall containing 80 ml of Czapek's solution medium. The cultures were grown in complete darkness for 10 days at room temperature of 25–28°C.

Separation of the yeast cells from the solution medium was made by centrifugation at 5,000 rpm for 15 minutes at 5°C. The yeast cells were washed three times with de-ionized water. The mycelial mats of black mold were separated from the solution medium by suction filtering and were washed as the yeast cells.

Endogenous indole compounds were extracted with absolute alcohol. Sonification of the yeast cells and mashed mycelial mats was carried out in absolute alcohol at 0°C for 30 minutes, and extraction was made in a refrigerator at about 5°C for three days. This procedure was repeated three times.

Extraction of the used solution media was also performed. The solution media were adjusted to pH 3.5 with 4 N hydrochloric acid. Peroxide free ether with a quarter of the volume of solution media was added. Extraction were also repeated three times in a refrigerator of 5°C for two days.

The extracts were condensed by vacuum distillation with a Buchi rotavapor to approximately 1/20 of their original volumes.

Paper chromatography was developed on Whatman's No. 1 filter paper of 3×35 cm in a Shandon Chromajar with isopropanol, 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, diazotized *p*-nitroaniline, Salkowski, and diazotized sulfanilic acid reagents. The R_f values

and the change of color in various reagents were used for qualitative identification (Stowe and Thimann, 1954; Sen and Leopold, 1954).

Results

Six indole compounds and one amino acid were discovered in these experiments. Three other compounds in yeast cell extract and two in extract of black mold mycelial mat needed further information for their identification. All the results are summed up in Table 1.

In extract of yeast solution medium, there was quite often a yellow spot with R_f value of 0.15 on Ehrlich's reagent. It was determined as citrulline (CIT), an amino acid (Block *et al.*, 1958). However, three other types of compounds were also discovered occasionally in the extract of yeast cells. The most common of the three was the compound with R_f value of 0.95, very sensitive to Ehrlich's reagent, yielding purple color at first then changing into greenish blue gradually and persisting for months. It did not show up with other reagents. The other spots with the R_f values of 0.53 and 0.33, respectively, sometimes responded positively to Ehrlich's reagent (Fig. 1).

The alcoholic extraction of black mold mycelial mat gave quite different indole compounds as compared to its solution medium extract. Only one of the three spots in mycelial mat extract could be identified at the present time. That is skatole (SKT) with R_f value of 0.92 and giving ash blue color on Ehrlich's reagent, greyish brown on Salkowski, light orange on diazotized *p*-nitroaniline, and light yellow on diazotized sulfanilic acid (Fig. 2).

Discussion

Production of indole compound by various fungi is valid in many respects. Nevertheless 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 dissimilar in different species. In this experiment, to find any compound which was extracted by Curtis from black mold in 1958 was unsuccessful. It might due to a mutant being used in this experiment. Unidentified compounds might not be significant here, since it is well known that the Ehrlich's reagent is very sensitive to mixtures and interfering compounds which could result from indole breakdown.

The difference of the indole metabolites produced by fungi may indicate that different species of fungi have dissimilar pathways for IAA synthesis and degradation. Plants of different species can synthesize IAA via different pathways (Anderson, 1968; Wightman, 1964). The fungi can surely do so too. Nothing in the results indicates that the biosynthesis of IAA in the black mold follows any other pathway than that known from higher plants. It

Table 1. *Detection of indole compounds on paper chromatography by various reagents*

Extract	R_f[1)]	Ehrlich	Diazotized *p*-nitroaniline	Salkowski	Diazotized sulfanilic acid	Compound[2)]
yeast solution mecium	0.76	purple	orange	blue	light brown	IAN
	0.35	purple	light brown	rosy red	yellowish orange	IAA
	0.15	yellow				CIT
yeast cell	0.95	purple to greenish blue				?
	0.76	purple	orange	blue	brown	IAN
	0.70	purple	orange	rosy red	orange	IAH
	0.53	purple				?
	0.35	purple	light brown	rosy red	yellowish orange	IAA
	0.33	purple				?
black mold solution medium	0.80	pink	light brown	rosy red	light brown	IND
	0.37	purple	light brown	rosy red	yellowish orange	IAA
	0.20	purple	yellowish orange	yellow	light yellow	TRP
black mold mycelial mat	0.92	ash blue	light orange	greyish brown	light yellow	SKT
	0.37	yellow		light yellow	yellow to light brown	?
	0.05	yellow		light yellow	pinkish orange	?

1) The values are the means of ten determinations.
2) IAA = Indole-3-acetic acid; IAH = Indole aldehyde; IAN = Indoleacetonitrile; IND = Indole; CIT = Citrulline; SKT = Skatole; TRP = Tryptophan.

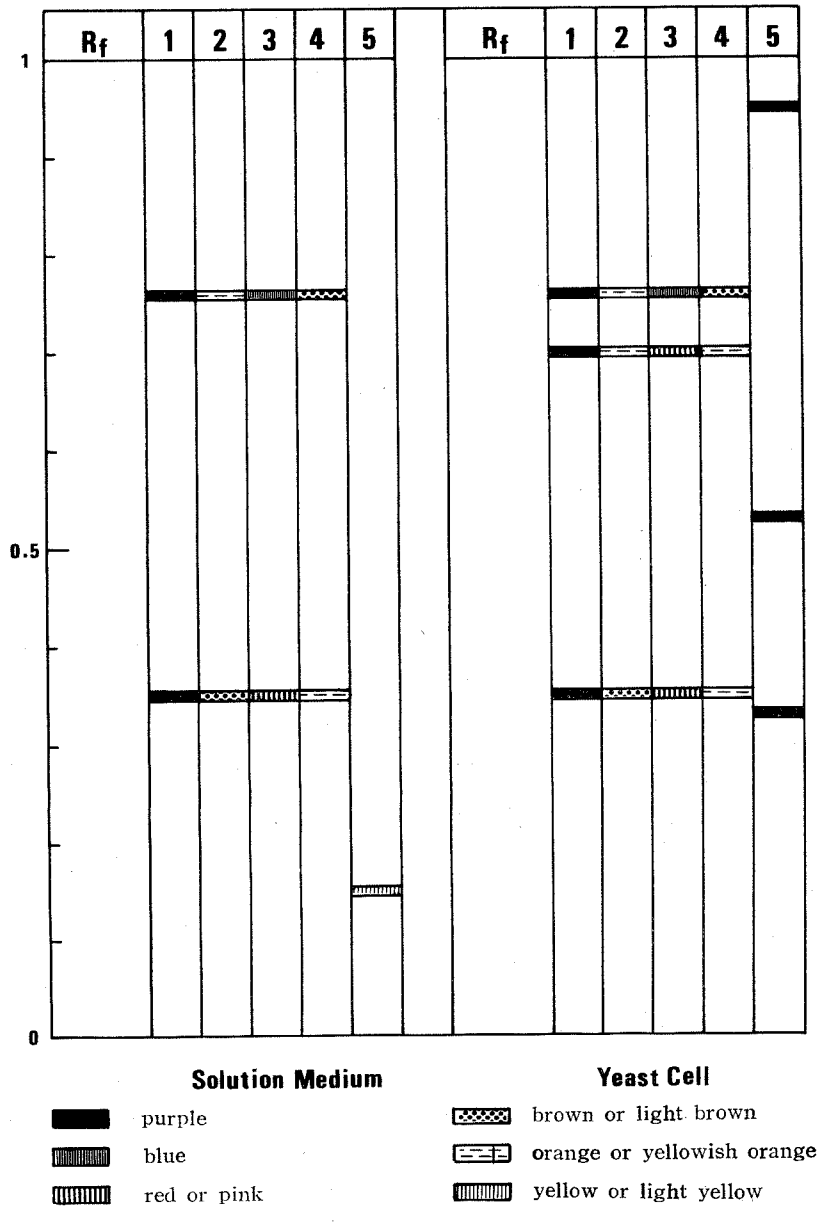

Fig. 1. Diagramatic sketch of indole compounds detected by paper chromatography with yeast extract. 1=Ehrlich reagent; 2=Diazotized *p*-nitroaniline reagent; 3=Salkowski reagent; 4=Diazotized sulfanilic acid; 5=Unidentified compound or compound of occassionally present in Ehrlich reagent.

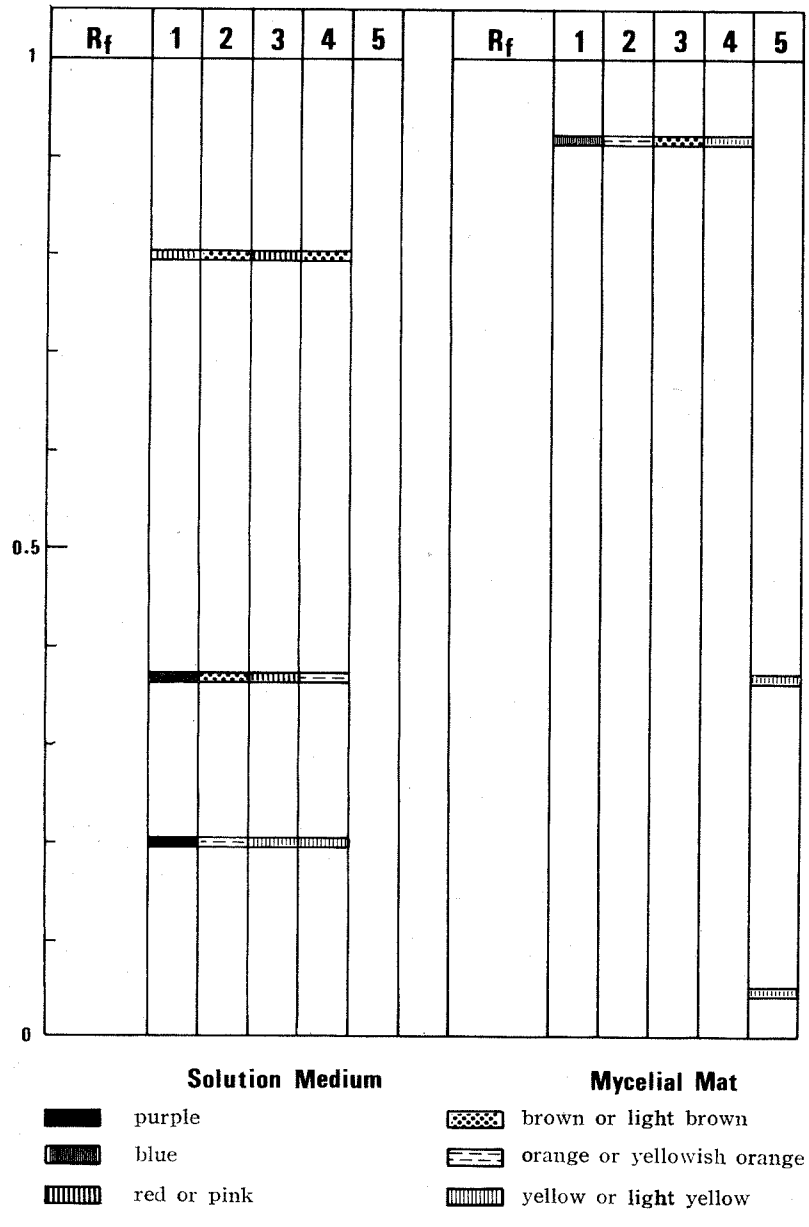

Fig. 2. Diagramatic sketch of indole compounds detected by paper chromatography with black mold extract. 1=Ehrlich reagent; 2=Diazotized *p*-nitroaniline reagent; 3=Salkowski reagent; 4=Diazotized sulfanilic acid; 5=Unidentified compound or compound of occassionally present in all four reagents.

might throw some light in this case by borrowing the pathway of amino acid synthesis, anthranilic acid reacts with 5-phosphoribosyl-1-pyrophosphate to form indole-3-glycerol phosphate. By action of the enzyme indoleglycerophosphate aldolase, indole-3-glycerol phosphate is broken down into indole and D-glyceraldehyde-3-phosphate. The action of enzyme tryptophan synthase gives rise to tryptophan by addition of L-serine to the indole ring. Oxidation of tryptophan gives indole-3-acetic acid as the end-product. Skatole may be formed as a result of the breakdown of tryptophan.

It is noteworthy that yeast cells were reddened in the inhibited cultures. The possible indole metabolite effect on growth of yeast in some detail has been examined. Elucidation of the pathways of indole compounds should be connected with the autoinhibitory substances in yeast.

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酵母菌及黑麴霉之吲哚化合物

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本實驗在研究釀酒酵母菌和一種變體黑麴霉之細胞內與細胞外吲哚化合物。細胞內化合物在低溫下可用純酒精萃取，而細胞外化合物則需用無過氧化物之乙醚萃取。鑑定各種吲哚化合物係採用濾紙色層分析法及對不同試劑之呈色反應。釀酒酵母菌萃取物內含有吲哚乙酸、吲哚乙腈、及吲哚醛。變體黑麴霉萃取物內則含有吲哚、吲哚乙酸、色胺酸、及3-甲吲哚。此外釀酒酵母菌細胞萃取物有三種及變種黑麴霉菌絲體萃取物有二種尙未鑑定之化合物存在，均有待進一步之研究。