THE PRODUCTION OF INDOLEACETIC ACID BY *NECTRIA PTEROSPERMI* SAW.[1]

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Abstract

The production of indoleacetic acid by *Nectria pterospermi* in the Czapek's medium containing tryptophan, as a sole nitrogen source, showed that the maximum production was obtained from 15 to 17 days old cultures with optimal initial hydrogen concentration of neutral at optimal temperatures within 25 and 31 C. Indoleacetic acid production was greatly impaired by agitation and slightly affected by illumination. No further increase in production was observed when the tryptophan was supplied more than 0.2%. Serine and aspartic acid also contributed somewhat to the production of indoleacetic acid.

Introduction

It has been known that plant pathogens and other non-pathogenic microorganisms produce indoleacetic acid in vitro. (Thimann, 1935; Wolf, 1952, 1956; Gruen, 1959; Srivastava and Shaw, 1962; Hirata, 1963; Sequeira and Williams, 1964).

Nectria pterospermi, pathogen of the canker of maple-leaved pterospermum, also produces indoleacetic acid in culture media. (Yu et al., 1967). Since it was suggested that the pathogenesis of Nectria-canker was induced by production of indoleacetic acid (Berducou, 1952; Yu et al., 1967), a better understanding of the production of the auxin by the pathogen would be meaningful. In the present work the production of indoleacetic acid in vitro under different conditions were studied. The possible relationship between the productivity of indoleacetic acid and the growth of the fungus was discussed.

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Materials and Methods

Nectria pterospermi was cultivated in 25 ml of Czapek's solution containing tryptophan as a substitute of KNO_3 in a 125 ml flask. The cultures were incubated under various conditions such as different temperatures, pH values, concentrations of tryptophan, and amino acids. Then, the productivity of indoleacetic acid (IAA) was determined by Gordon and Weber's method photometrically as already described in the previous report (Yu et al., 1967). Namely, to a 1.0 ml of culture filtrate, 2.0 ml of the Gordon and Weber's reagent (1.0 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added and optical density at 530 nm was read using Unicam SP 500 spectrophotometer.

Besides, two other methods were performed to obtain the comparative data. A standard curve of known concentrations of authentic IAA against the length of avena coleoptile was prepared to calculate the equivalent concentration of IAA in the sample solution by bioassay. The same sample was also spotted on Whatman No. 4 filter paper and developed with isopropanol: ammonia (28%): Water (8: 1: 1) or 70% aqueous ethanol solution. The chromatograms were sprayed with Salkowski's reagent and the spot area was compared with a standard chromatogram spotted with a series of quantities of authentic IAA. Thus, the concentration of IAA in a given sample was detected (Yu et al., 1967).

There was a linear relationship between the quantities of IAA in the culture filtrate and the optical density at 530 nm. It was found that color reaction was completed in 60 min. under the experimental condition rather than 25 min. as stated by Gordon and Weber (1951). Therefore, the reading of the reaction mixture was made 60 min. after addition of the color reagent. Furthermore, IAA contents of the culture filtrate and the ether extracts of the sample culture filtrate were compared to justify a proper handling of the sample solution. Certainly, a little loss occurred during extraction with ether, yet, the difference was not much. For this reason, the analysis of IAA was carried out only with the culture filtrate in the present experiments.

Results

As shown in Fig. 1, the production of IAA by the fungus in Czapek-tryptophan medium exhibited a maximum IAA accumulation around 15–17 days after incubation, thereafter, it was decreased and maintained fairly constant for a period of time.

Effect of temperature on the production of IAA was examined by incubating the cultures at the temperatures of 16, 19, 22, 25, 28, 31, and 34 C for 6 days and 13 days, respectively. The maximum production of IAA was within

the range of 28 and 31 C for 6 days old cultures, while the peak was obtained at 25 C in the case of 13 days old cultures (Fig. 2).

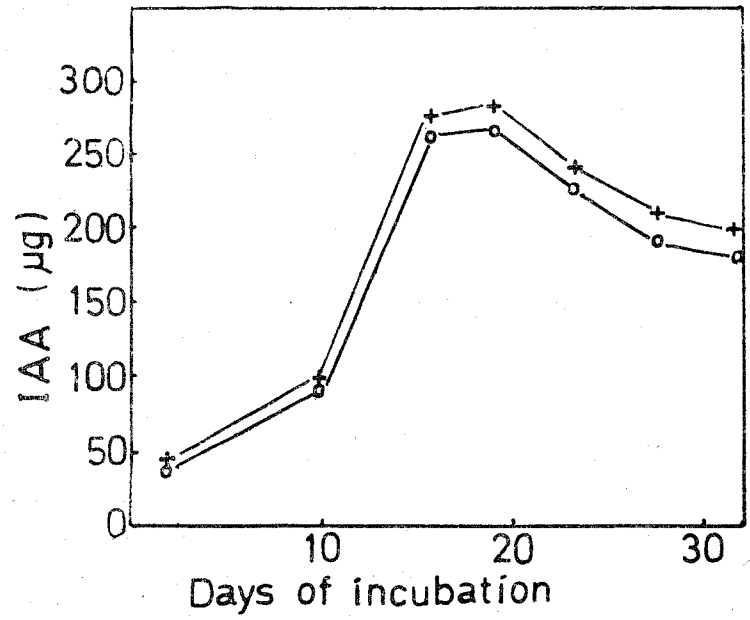

Fig. 1. The production of indoleacetic acid by *Nectria pterospermi* in Czapek-tryptophan liquid medium. They are culture filtrate (+—+) and ether extract (0—0).

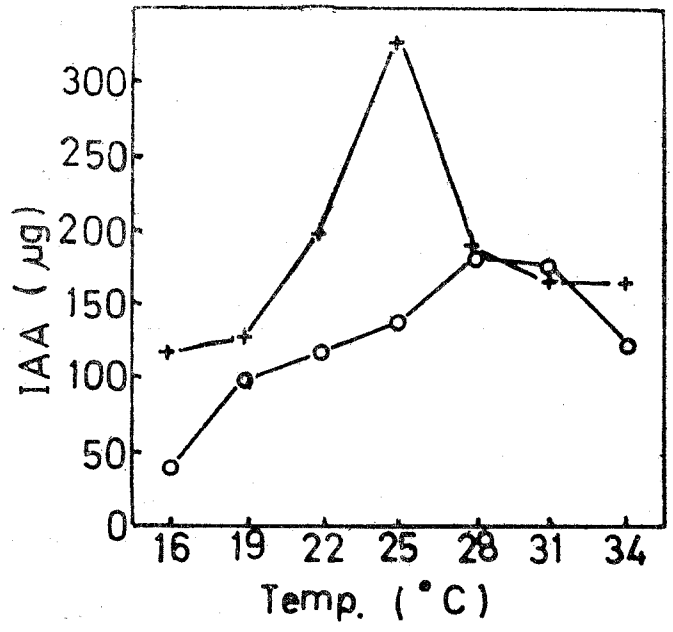

Fig. 2. The effect of temperature on the production of indoleacetic acid by *Nectria pterospermi* measured 6 days (0—0) and 13 days (+—+) after incubation.

Meanwhile, the growth of the fungus was measured in terms of its dry weight. At day 7, the optimal temperature for the growth was within 28 to 31 C which were about the same for the maximum production of IAA. On the other hand, the optimal temperature for the fungal growth (25–34 C) was wider than that of IAA production (25 C) when the measurement was made with the 13 days old cultures.

Maximum production of IAA and maximum growth of the fungus were observed at neutral condition when the cultures incubated in the medium with different pH values were compared (Fig. 3). Fortunately, the pH value of the Czapek-tryptophan medium after autoclaving was 6.9 or 7.0 without adjustment.

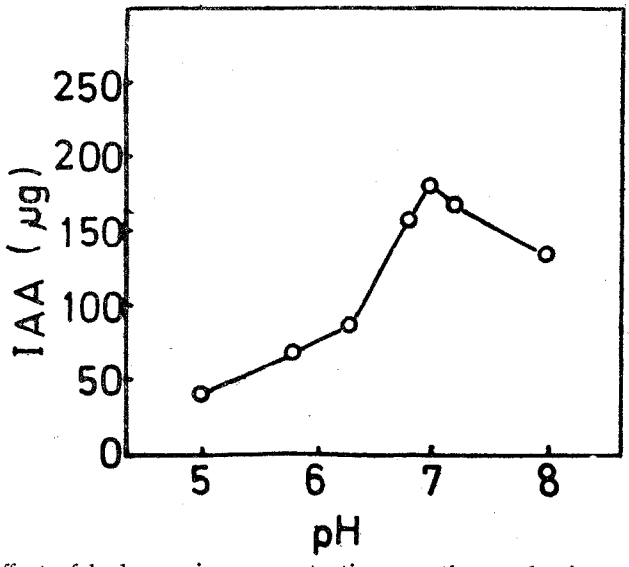

Fig. 3. The effect of hydrogen ion concentrations on the production of indoleacetic acid by *Nectria pterospermi* incubated for 14 days at 28°C.

As shown in Table 1, production of IAA was lower in shaken culture though mycelial growth was far better in stationary culture.

Table 1. *Effect of agitation on the production of indoleacetic acid by* ***Nectria pterospermi***

Condition	6 days		13 days	
	IAA (μg)	Growth (mg)	IAA (μg)	Growth (mg)
Shaken culture	127	897	84	777
Stationary culture	145	473	300	826

Table 2. *Effect of illumination on the production of indoleacetic acid by Nectria pterospermi*

Condition	6 days		13 days	
	IAA (μg)	Growth (mg)	IAA (μg)	Growth (mg)
In the light	184	505	198	721
In the dark	160	441	207	865

The effect of light on the growth and IAA production was not very great under the experimental condition (Table 2), though it was essential for the sporulation of the fungus (Yu, 1967). However, IAA production in the dark was a litlle higher than that in the light when the 13 days old cultures was compared.

Increased concentrations of tryptophan in the medium brought higher concentratrations of IAA in the culture filtrates up to the 0.2% of tryptophan. Thereafter, the response of the fungus to the concentrations of tryptophan was remained fairly flat up to 0.5% of tryptophan (Fig. 4).

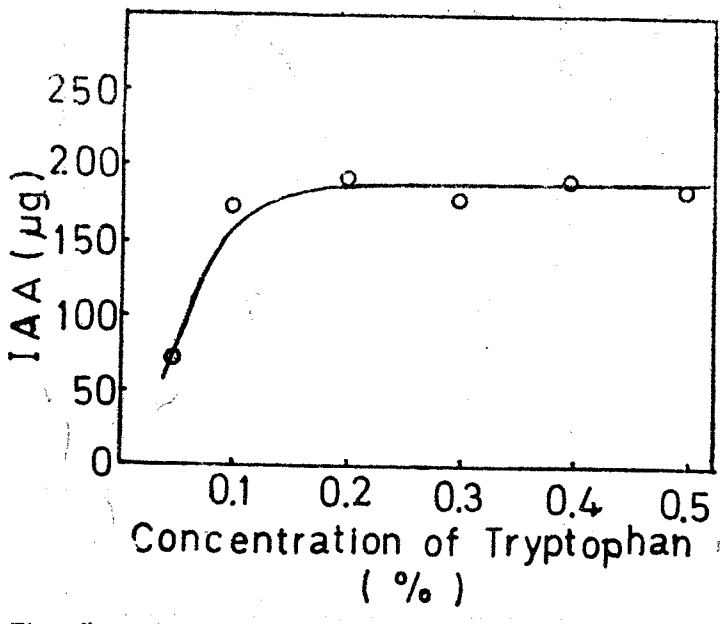

Fig. 4. The effect of the concentrations of tryptophan on the production of indoleacetic acid by *Nectria pterospermi* incubated for 14 days at 28°C.

A few nitrogen sources other than tryptophan were chosen for the studies of the production of IAA (Table 3). Serine and aspartic acid seemed to be incorporated into IAA but they were not so effective comparing with tryptophan at the same concentration.

Table 3. *The effect of different nitrogen sources on the production of indoleacetic acid by* ***Nectria pterospermi*** *incubated for 14 days in the dark.*

Nitrogen sources	IAA (μg)	Mycelial growth (mg)
$NaNO_3$	0	820
NH_4NO_3	0	777
Tryptophan	257	784
Serine	13	828
Indole	0	0
Indole+Serine	0	0
Aspartic acid	10	843
Leucine	0	780
Glycine	0	768
Histidine	0	756
Threonine	0	730
Proline	0	715
Asparagine	0	768

Discussion

The productivity of IAA by the fungus in culture seemed to depend on two factors, i.e. the rates of biosynthesis and the destruction. When the rate of IAA synthesis exceeded the destruction rate, IAA accumulated in the culture. The production of IAA by *Nectria pterospermi* reached a maximum yield around 15 to 17 days after incubation, however, it was decreased thereafter. The destruction rate might be higher than the synthesis rate for prolonged incubation, so that the quantities of IAA in the culture filtrates decreased accordingly. Temperature also affected the IAA production. The maximum yield of IAA was obtained with 13 days old culture at 25 C and 6 days old culture at 28–31 C. Obviously, longer incubation at higher temperatures caused the synthesis rate below the destruction rate. Since aeration accelerated the oxidation of IAA (Ray, 1958; Hare, 1964), agitation of the culture increased the destruction of IAA in the present experiments. It was evident that the yield of IAA was not matched with the vegetative growth of the fungus under the experimental condition since the increased growth of the fungus did not always increase the IAA concentration in the culture fluid.

白桐癌腫病菌植物生長素之產生

于 浩 陳其昌 吳龍溪

白桐病原菌 (*Nectria pterospermi*) 於含有胰化氨基酸之 Czapek 培養液中可產生植物生長素吲哚醋酸 (Indoleacetic acid)。其產量於培養後15至17日間達最高峯，最適溫在 25° 至31°C 之間，最適酸鹼度爲中性。振動培養減少其產量，光線之影響不顯着，胰化氨基酸超過 0.2% 濃度並不增多吲哚醋酸產量。絲氨酸與天門冬酸可爲吲哚醋酸之前驅物。

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