On the Ability of Taphrina Deformans to Produce Indoleacetic Acid from Tryptophan by Way of Tryptamine[1]

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Summary. The metabolism of tryptophan by *Taphrina deformans* has been studied to confirm the reported ability of this organism to produce tryptamine. Such amine production was not observed, despite use of amine oxidase inhibitors at levels which should have resulted in the accumulation of tryptamine in the medium. It has been shown that the metabolites of tryptophan include indolepyruvic acid, indolelactic acid, tryptophol, and indoleacetic acid, and that the original report of tryptamine production must be reevaluated in light of the extraction procedures employed.

While much indirect evidence, summarized in the reviews of Stowe (10) and Mahadevan (8), suggests pathways by which indoleacetic acid (IAA) is produced from the supposed precursor tryptophan, the confirming biochemical evidence has been almost entirely lacking. The absence of such evidence can be attributed in part to the difficulties encountered in obtaining enzyme preparations from plant tissues which produce only small quantities of indoles. As a result, some workers have attempted to describe these pathways via studies of the metabolism of tryptophan by microorganisms from which active enzyme preparations may more easily be prepared, in the hope that the data obtained will allow a more complete investigation of the production of IAA in the tissues of higher plants.

The position of tryptamine (TNH_2) as a metabolic intermediate in the production of IAA from tryptophan is a case in point, for the evidence available is entirely indirect. In the reports of Libbert (6, 7) of the production of tryptamine from tryptophan by higher plant tissues, the possibility of bacterial contamination must be considered real, as no antibiotics were included in the incubation medium, and amine production was noted only after a 5-hour incubation in 1 experiment, and 9 hours in another.

While there are 2 recent reports (2, 11) of organisms which are capable of producing tryptamine when grown on a broth containing tryptophan, that of Crady and Wolf (2) dealing with 2 different species of fungi has served as a basis for this work. The objective of this study was to confirm the work of these authors, and to study the properties of the enzyme(s) responsible for the production of tryptamine from tryptophan in *Taphrina deformans*, the organism responsible for the disease called peach leaf curl, and *Dibotryon morbosum*.

Materials and Methods

Freeze dried and fresh slants of *Taphrina deformans* (Berk.) Tul., A.T.C.C. No. 11124 were obtained on 3 separate occasions from the American Type Culture Collection, Washington, D. C. These cultures were obtained by the A.T.C.C. in 1952 from the Centraal Bureau voor Schimmelcultures in Baarn, Netherlands, and is the same strain as was used by Evelyn Crady and F. T. Wolf.

The organism was maintained on potato-glucose agar slants containing 1.5 % Bacto-Agar, 2 % (w/v) glucose, and an aqueous extract of Elizabeth Park Instant Mashed Potatoes, available through the distributor (S. Vogel and Sons, East Hartford, Connecticut). Twelve g of this dehydrated potato preparation were suspended in 1 liter of distilled water, and the particles removed by filtration before addition of the carbohydrate. Potato-glucose broth was prepared in an identical manner with the omission of the agar. The use of media prepared from fresh potatoes did not alter the pattern of tryptophan metabolism observed. The new medium is easily prepared and eliminates the time required in the peeling of fresh potatoes. DL-Tryptophan (Nutritional Biochemicals Corporation) was included in the liquid test media in 0.1 to 1.0 % levels (1.0–10.0 g per liter of final medium). Where stated the amine oxidase inhibitors Catron (betaphenylisopropylhydrazine), Marsilid (1-iproniazid-2-isopropylhydrazine), and Parnate, (2-

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phenylcylopropylamine) were included in the medium in concentrations of 10^{-6} to 10^{-3} M.

Liquid media were inoculated by platinum loop transfer of cells from agar slants. Growth took place in the dark on a rotary shaker at 100 rpm and at 23°. Cultures containing 125 ml of medium were grown in 500 ml Erlenmeyer flasks.

At intervals during the incubation period, colorimetric tests were made on the culture filtrates using the procedure of Gordon and Weber (3) and using 35 % $HClO_4$ (v/v). To a 2 ml aliquot of the culture filtrate, 4 ml of the reagent were added, and, after standing 25 minutes the resulting colors were read in a Klett-Summerson colorimeter using a green Corning glass filter. Uninoculated medium to which the reagent was added served as the blank control for all measurements. A modification of this reagent developed in the course of these studies and of more general use in the quantitative determination of indoles will be published elsewhere (in preparation).

Culture filtrates were extracted separately for basic, neutral, and acidic indoles by partitioning against reagent grade methylene chloride at pH 2.0 achieved through the addition of H_3PO_4, and at pH 8.0 achieved through the addition of a saturated solution of K_2CO_3. The extracts were taken to dryness and the residues dissolved in a minimum volume of absolute ethanol for thin layer chromatographic or electrophoretic separation.

Thin layer chromatography was carried out according to the methods of Obreiter and Stowe (9) using 200 mm × 200 mm glass plates coated with a thin layer of silicic acid: carboxymethylcellulose (28.5: 1.5, w/w). Solvents useful in resolving the *Taphrina* tryptophan metabolites were: A) 2-butanone: *n*-hexane (1: 1, v/v); B) 2-butanone: *n*-hexane (48: 52, v/v); C) Petroleum ether: active or (d) amyl alcohol (3:2, v/v) saturated with 0.5 M HCOOH; D) 1-butanol: chloroform (3: 2, v/v) saturated with 0.5 M HCOOH; E) isopropanol: 28 % NH_4OH: HOH (8: 1: 1, v/v); F) isoamyl alcohol (saturated with 2 N NH_4OH): 2-butanone (1: 1, v/v); and G) 1-butanol: glacial acetic acid: HOH (4: 1: 5, v/v). The results obtained with nonactivated plates and paper chromatograms run in the solvents used by Crady and Wolf (solvent E and 70 % ethanol) were identical.

Paper electrophoresis was performed in a Durrum-type electrophoresis apparatus (Spinco, Model-R). Two chambers, each containing 8 narrow paper strips (No. 4939-707 Paper Strips, Arthur H. Thomas Company, Philadelphia, Pa.) could be run simultaneously. Strips were saturated with 0.05 M pH 5.8 potassium phosphate buffer and runs lasted for 5.5 hours at a constant voltage of 110 volts. In such a system, authentic tryptamine travels a repeatable distance of 8.0 to 10.5 cm from the origin toward the cathode.

Indoles were detected by use of the Ehrlich's reagent, prepared by the addition of 2 % (w/v) *p*-dimethylaminobenzaldehyde to a mixture of absolute ethanol and 10 N HCl (1: 1, v/v).

Results

A typical thin layer chromatographic separation of the indoles which can be extracted from *Taphrina deformans* medium 3 days after inoculation is shown in figure 1.

The pattern of fluorescent spots seen in the chromatography of samples of aged indolepyruvic acid and described by Kaper and Veldstra (5) and Winter (12) can be seen when this compound is chromatographed in solvents A, C, and D, and an identical pattern is observed in the chromatographic behavior of the acids extracted from *Taphrina* medium. Also, a pink band migrating toward the anode is found on electrophoresis of both the fungal extract and aged indolepyruvic acid solutions.

While authentic IAA and indolelactic acid are difficult to separate in solvents C (R_F's = 0.60) and F (R_F's = 0.75), they are readily separated in solvent D (IAA = R_F 0.75, indolelactic acid = R_F 0.52). Use of solvent D (fig 1) shows that both of these compounds are present in the acids extracted from

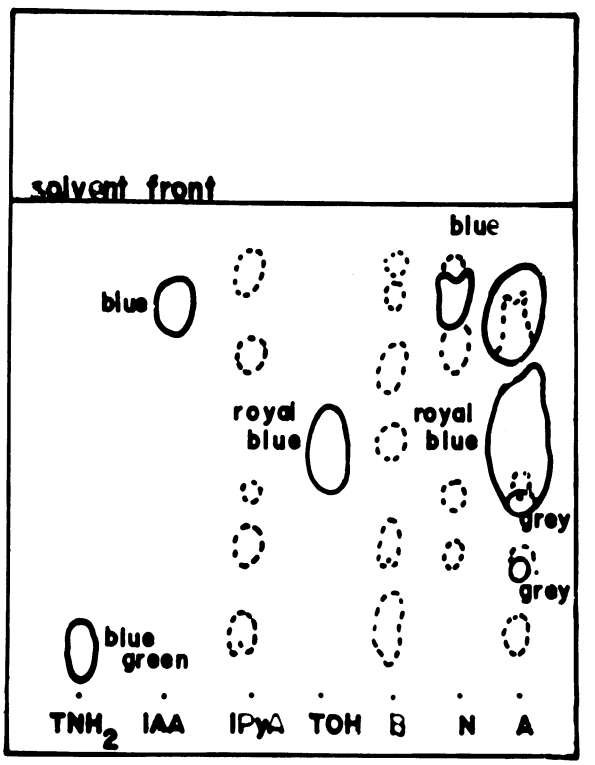

FIG. 1. Thin layer chromatographic separation of the tryptophan metabolites of *Taphrina deformans*. The solvent was butan-1-ol: chloroform (3: 2 v/v) saturated with 0.5 M HCOOH, and the detection reagent was Ehrlich's. Column: 1) 20 μg of authentic tryptamine, 2) 20 μg of authentic IAA, 3) 30 μg of an aged indolepyruvic acid solution, 4) 20 μg of authentic indolelactic acid, 5) bases extracted from *Taphrina* medium 3 days after inoculation, 6) the extracted neutral substances from the same medium, and 7) the acidic indoles from the same 3-day-old medium.

Taphrina medium. The indole 3 carbon alcohol tryptophol has characteristic R_F's in solvents A (R_F = 0.5), B (R_F = 0.70), and C (R_F = 0.46). A spot showing identical R_F values in these 3 solvents can readily be shown to be present in the neutral indoles extracted from *Taphrina* medium. Thin layer chromatograms prepared from 3, 12, 24, 48 and 96-hour-old cultures show steadily increasing quantities of these substances, IAA and indolelactic acid being present even in the first extract.

Under any growth conditions or length of culture it was impossible to obtain any basic Ehrlich positive compounds by extraction of *Taphrina* growth medium at pH 8.0 with methylene chloride whether TLC or electrophoresis was used. To insure against the possibility that the amine was being metabolized as rapidly as it was formed, and hence not accumulating in the growth medium, marsilid (1), catron (4), and parnate (13), which are known to result in 100% inhibition of the oxidation of monoamines at 10^{-6} M to 10^{-5} M, were included in the media over a concentration range of 10^{-6} M to 10^{-2} M. Where growth was not totally inhibited (at 10^{-3} M or lower) the inhibitors could not be shown to cause the appearance of any accumulated amine on thin layer chromatograms nor could the production of indoleacetic acid under these conditions be shown to be markedly inhibited.

Conventional Warburg respirometry indicated approximately a 10% promotion of respiration by tryptophan added to washed whole cells of *Taphrina*. RQ values obtained from these experiments do not indicate decarboxylation, being in every case less than one and consistently decreasing with time.

Discussion

Of the ability of *Taphrina deformans* to form indoleacetic acid when cultured on a complex medium supplemented with tryptophan, there is little doubt. In contrast to the findings of Crady and Wolf, in which tryptamine was the only reported intermediate between tryptophan and IAA, this work shows that while tryptamine is absent from the growth medium of this organism, indolelactic acid, indolepyruvic acid, and tryptophol are present. In view of the findings of Crady and Wolf that this organism is capable of forming IAA when incubated on a broth containing tryptamine, and the failure in this work to show accumulation of the amine when *Taphrina* is grown on medium containing the amine oxidase inhibitors Marsilid, Parnate, and Catron, it is suggested that production of IAA by this organism proceeds through the more commonly observed intermediate indolepyruvic acid, and not through TNH_2. This suggestion is supported by the inability of this work to show inhibition of IAA production in this organism when levels of amine oxidase inhibitors are used which totally inhibit such production if the sole pathway of conversion were through the amine, as suggested by Crady and Wolf.

The failure to find tryptamine as a metabolic intermediate on the pathway to IAA in *Taphrina deformans* becomes clear if the original publication of Crady and Wolf (2) is examined. The information given the reader concerning the extraction procedure used is that "culture filtrates were acidified with HCl, extracted twice with ether in a separatory funnel, and the ether extracts were then evaporated almost to dryness. Strips of Whatman No. 1 filter paper were spotted with the extract . . ." No mention is made of further solvent partitioning, and it must be assumed that none was performed. The compound extracted and chromatographically identified as tryptamine by these authors could only have been an acidic or a neutral indole. The amine, under the extraction conditions used, would have been retained in the aqueous layer. One can find a component of the acid and neutral extract which in some solvents chromatographs similarly to authentic tryptamine. This compound is, however, nonindolic, and on spraying with Ehrlich's reagent does not change color, but instead only becomes a more intense yellow-brown, not the characteristic purple to blue-green of indoles. Moreover, it does not show the fluorescent properties of tryptamine and does not migrate on electrophoresis. The techniques used by these authors in the study of the tryptophan metabolism of *Taphrina* were also used in the study of the metabolism of the same amino acid of *Dibotryon morbosum* and the same criticisms are relevant.

Given the results presented, it is suggested that the report of tryptamine production by both *Taphrina deformans* and *Dibotryon morbosum* be discounted. This leaves only the study of Weissbach et al. (11). Their report, as well as work to be published elsewhere (in preparation) indicates that tryptamine formation by bacteria is a reality.

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