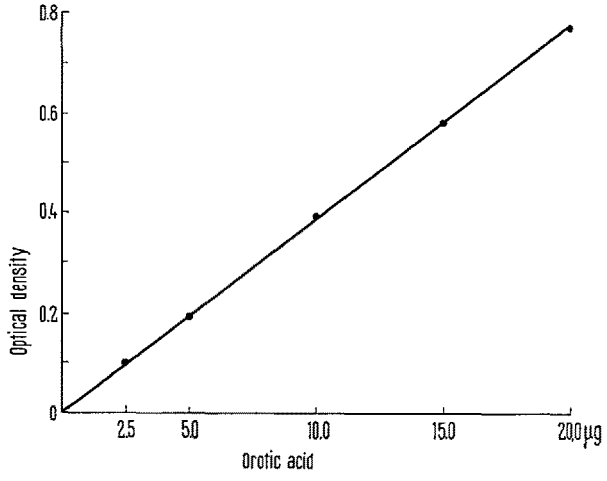
mixture is not generally influenced and therefore the preliminary adjustation of the sample need not be performed.

The question of whether or not uranyl acetate influences the course of the reaction, was answered by the determination of various amounts of orotic acid (Na salt) in the presence or absence of uranyl acetate. Table I shows that uranyl acetate does not influence the course of the reaction.

The dependence of the extinction on the amount is linear only up to the 20 μg concentration of orotic acid (Figure). If we suppose that the amount of orotic acid will be higher, we can use less supernatant after the deproteination, or even dilute the serum to a certain degree with water. Table II presents a good agreement between the doses of orotic acid (Na salt) added to the blood serum and the observed values.



Dependence of extinction on the amount of the determined orotic acid.

The method described is also very successful for the following of orotic acid in the blood serum and in some organs of rats, which was proved in several of our model experiments.

The usefulness of this method was proved also in healthy persons as well as in patients with higher levels of bilirubin, biliary acids, lipidic substances, uric acid etc. No falsely increased values were observed in any cases.

In experiments performed in vitro it was found out that other substances, namely 4-aminoantipyrine, antipyrine, amidopyrine, indol derivates, β-naphtyl-amine as well as barbituric acid, thiobarbituric acid and tryptophan react under the same experimental conditions.

Zusammenfassung. Das Prinzip des Orotsäurenachweises beruht auf der Deproteination des Serums mittels Uranylessigester und der Verwandlung der Orotsäure durch Bromation in 5, 5-Dibrombarbitursäure und nachfolgende Debromation in Barbitursäure. Letztere ergibt mit *p*-Dimethylaminobenzaldehyd ein intensiv gefärbtes Produkt, 5-(*p*-dimethylaminobenzyliden)-Barbitursäure.

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Department of Pharmacology, Medical Faculty Charles University, Plzen (Czechoslovakia), 10 July 1967.

Amylase and Polyphenol Oxidase Production by Germinating Conidia of *Colletotrichum falcatum* Went

In an earlier publication Singh[1] has reported the secretion and location of sucrase during germination of conidia of *Colletotrichum falcatum* Went, the red rot organism of sugarcane. The present investigations deal with the production of amylase and polyphenol oxidase by the germinating conidia of a light type highly sporing virulent isolate and a dark type sparsely sporing less virulent one. The presence of amylase is presumed in view of the utilization of starch by different isolates of *C. falcatum* during germination (Singh[2]). However, the existence of polyphenol oxidase is presumed on the basis of the reddening of canes infected with the red rot fungus and which is apparently comparable to the reddening or darkening of several mushrooms (Agarics and Polypores, etc.), apples, potatoes and other iron- or copper-containing plant parts on injury. This sort of reddening is known to be due to the oxidation of mono- and O-di-hydric phenols.

Actively growing (12–16 days old), single-conidium cultures of 2 *C. falcatum* isolates (Nos.[3] 78 and 244, the dark and the light type, respectively) were used in the present investigations. Amylase: Secretion of amylase by germinating conidia has been detected both qualitatively and quantitatively by the following 2 methods (Bernfeld[4]): (1) Change of the Iodine-staining properties of the substrate. (2) Increase in the reducing power of soluble starch. Both these methods are characteristic for the action of α-amylases. However, the second method can only be used for the assay of β-amylase.

(1) Change of iodine-staining property of the substrate: Qualitative detection of amylase was achieved by germinating conidia, in suspension drops, on oat-meal agar plates containing 1.0% soluble starch (B.D.H.) at 27 °C[5]. After 18 h[5], the plates were flooded with 0.5% iodine solution. A central unstained area became evident in the deep violet agar plates in both the isolates. The unstained area marked a zone of amylase activity as a result of the germination of the conidia and utilization of starch in the medium. This area was slightly bigger in the light type isolate (Figure I 1, 2) than in the dark type one (Figure I 3, 4), thereby indicating greater enzyme activity of the former.

(2) Increase in the reducing power of soluble starch: This method of assaying the amylase activity is based on the increase in the reducing power of soluble starch, and is applicable to both α- and β-amylases. The activity was measured through an enzyme extraction, obtained by crushing to fineness 0.2 g of germinated conidia of each

[1] P. Singh, Phytopath. Z. *54*, 79 (1965).
[2] P. Singh, Indian Phytopath. *19*, 30 (1966).
[3] Indian Type Culture Collections, I.A.R.I., New Delhi, India.
[4] P. Bernfeld, in *Methods in Enzymology* (Eds S. P. Colowick and N. O. Kaplan Academic Press Inc., New York 1955), vol. 1, p. 149.
[5] Optimum temperature and incubation period (Singh[2]).

isolate, which have been washed and dried, with 0.5 g of purified sea sand. Further 0.2 g of this powdered spore material was suspended in 4 ml of distilled water for 2 h and then centrifuged (2800 rpm for 5–7 min). The enzyme extract, obtained as semiclear supernatant, was filtered through a sintered-glass bacterial filter. One ml of such extract was added to 10 ml of 0.5% soluble starch (B.D.H.), buffered at pH 5.5 with 0.01 *M* acetate buffer.

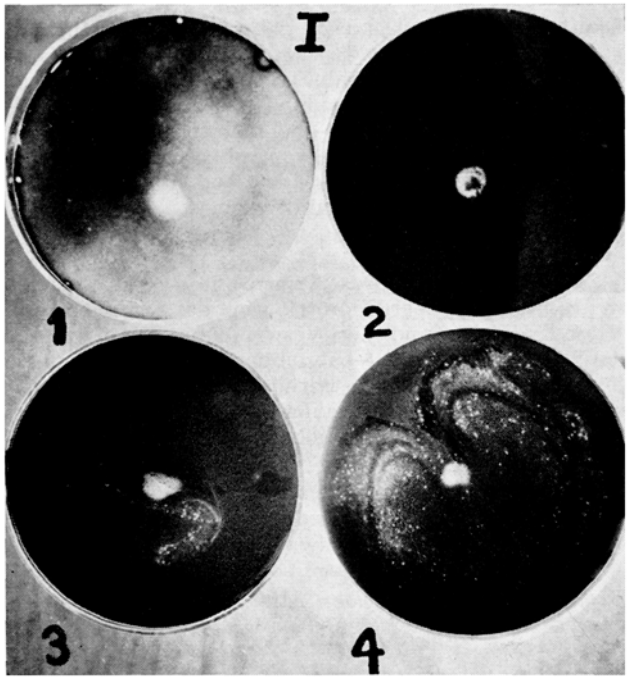

Fig. I. Zones of amylase activity seen as central unstained areas in deep violet agar plates as a result of iodine-staining reaction of the substrate. Plates 1 and 2 showing slightly bigger zones of the enzyme activity than plates 3 and 4.

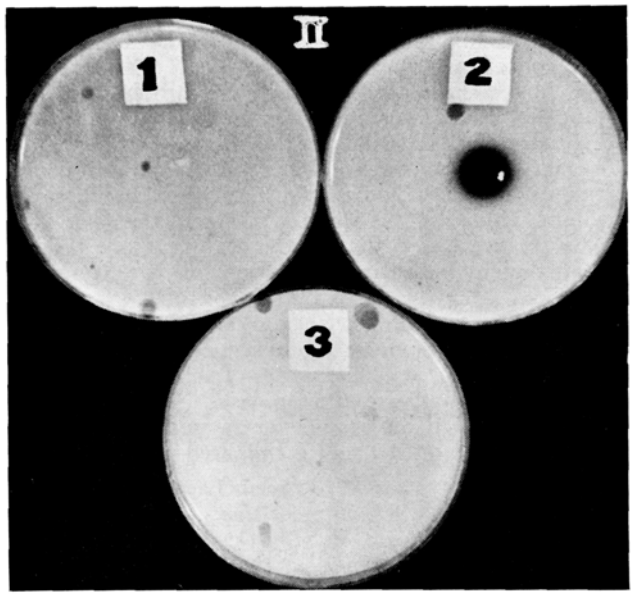

Fig. II. Production of polyphenol oxidase as observed by BAVENDAMM reaction, as dark brown halo (seen as black area) in the centre of plate 2. Small faint dark spots scattered in plates 2 and 3 are ordinary water drops.

Control for each isolate was run with boiled enzyme extract. The incubated reaction mixtures were acidified with HCl and boiled on a waterbath for half an hour. After cooling, these mixtures were made slightly alkaline with $CaCo_3$, until the effervescence ceased. The alkaline solutions were titrated against Fehling solution (A.O.A.C.[6]) and the percentage hydrolysis of starch calculated.

The results (Table) show that the light type isolate (No. 244) hydrolysed more starch than the dark type one (No. 78), indicating greater amylase activity of the former. There was no hydrolysis in the controls. Though, apparently, the difference in the amylase activity of the 2 isolates is low, the results are statistically significant.

Polyphenol oxidase. Qualitative detection of polyphenol oxidase has only been done through BAVENDAMM reaction[7], which involve oxidation of tannic acid. Washed conidia were germinated, in suspension drops, on oatmeal agar plates containing 0.25% and 0.5% tannic acid at 27 °C. After 18 h, a dark brown halo appeared in the agar plates containing 0.25% tannic acid in the light type isolate (Figure II 2). No such halo developed in the control (without tannic acid, Figure II 3) and in 0.5% tannic acid (Figure II 1). The latter was due to no germination because of toxicity of higher concentration. No such halo was seen in the dark type isolate treatments. Conidia of the dark type did germinate in the plates containing 0.25% tannic acid as observed under the microscope, but the enzyme produced was in undetectable quantity. After 2–3 days when small hyphae have been formed, sufficient quantity of the enzyme was produced in such plates, and the halo appeared. No such halo, however, developed in the control as well as in the treatment containing 0.5% tannic acid. In the present studies, no attempt has been made to specify the oxidase and this is to be done in greater details in the near future.

Amylase activity of germinated conidia of *C. falcatum* isolates as shown by percentage hydrolysis of starch

Enzyme extract of isolate used	Percentage hydrolysis of starch
244 A	0.137
B	Nil
78 A	0.108
B	Nil

A, treated; B, control; Nil, not detectable.

Zusammenfassung. Keimende Konidien von *Colletotrichum falcatum* bilden Amylase und Polyphenoloxydase. Die Enzymproduktion ist bei einem «Lichtstamm» höher als bei einem «Dunkelstamm».

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20 July 1967.

[6] Association of Official Agricultural Chemists. Official Methods of Analysis, 8th Edition, Washington (1955).
[7] W. Z. BAVENDAMM, PflKrankh. PflPath. PflSchutz. *38*, 257 (1928).
[8] Present address: Department of Forestry, P.O. Box-5213, St. John's, Newfoundland, Canada.