STUDIES ON CELLULOLYTIC ACTIVITY OF SOME PYRENOMYCETOUS FUNGI

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DURING the tests of antibiotic activity of submerged cultures of 25 pyrenomycetes species[1], their cellulolytic activity was also tested. All tested species of pyrenomycetes cultures belong to the group of stromatic lignicolous pyrenomycetes, which occur on the branches of forest trees. Hence it was thought that, they should have some cellulolytic activity which enable them to grow on the substratum of woody trees.

The cellulolytic activity was measured by viscosimetric method and by mycelial growth method. For the determination of cellulolytic activity the Hoppler viscosimeter was used[2]. Twenty five-fungal species were tested (Table I).

The cultures were grown on 3 different media, Kern medium[3], common wort medium and glucose corn-

TABLE I

Celluloytic activity in some pyrenomycetous fungi

Name of the Fungus Sp.	Percentage loss in viscosity in different media			Dry weight of mycelium grown on carbcxy cellulose mg/ml.	Release of glucose from cellulose due to the enzyme complex mg/ml
	Synthetic (Kern)	Wort	Glucose corn steep		
Leucostoma auerswaldii (Nit.) Hohn.	13·0	4·5	4·5	0·59	0·397
Valsa salicina Tul.	12·5	15·4	0·0	0·83	0·0
Valsa ambiens (Pers.) Fr.	16·1	9·1	23·3	1·18	0·198
Diatrype stigma (Hoffm. ex Fr.) Fr.	15·2	37·1	30·3	1·03	0·198
Anthostoma turgidum (Pers. ex Fr.) Nitschke	29·5	31·6	28·8	1·53	0·595
Diaporthe impulsa (Cke. et Peck) Sacc.	0·0	18·9	12·5	1·37	0·397
Melanconium dimorphum Peck	8·7	15·4	8·7	0·50	0·0
Diatrypella favacea. (Fr.) Ces. et de Not.	35·3	38·9	31·3	0·90	0·0
Melanconium sphaeroideum Link	13·0	12·5	17·2	1·11	0·0
Malanconis flavovirens (Otth) Wehm.	6·7	6·3	14·8	0·65	0·397
Ophiovalsa suffusa (Fr.) Petrak	28·0	36·1	27·3	0·30	0·0
Diatrypella tocciaena de Not.	27·3	32·3	32·3	0·0	0·0
Camarops microspora (Karst.) Shear	21·4	37·1	18·5	0·92	0·595
Ophiovalsa betulae (Tul.) Petrak	17·1	15·2	13·3	0·64	0·595
Rosellinia mammiformis (Pers.) Ces. et de Not.	26·7	30·3	15·4	0·51	0·0
Diaporthe pustulata (Desm.) Sacc.	19·4	28·9	21·4	1·70	0·198
Leucocytospora massariana (Sacc.) Urban	12·5	28·5	30·0	1·16	0·0
Eutypa acharii Tul.	6·9	32·4	23·3	0·90	0·0
Pseudovalsa lanciformis (Fr.) Ces. et de Not.	30·0	26·7	28·0	0·0	0·397
Leucocytospora kunzei (Sacc.)	4·5	4·5	5·0	1·46	0·595
Diaporthe impulsa (Cke. et Peck) Sacc.	29·0	5·8	7·1	1·16	0·397
Hypoxylon fuscum Pers. ex Fr.	15·6	39·4	25·0	1·44	0·397
Valseutypella tristicha (de Not.)	19·2	26·7	16·7	0·76	0·595
Eutypa flavovirens (Hoffm. ex Fr.)	27·8	39·5	25·8	0·82	0·397
Cryptodiaporthe salicina (Curr.) Wehm.	0·0	0·0	3·7	0·0	0·0

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steep medium. The tests were carried out after 12 days of cultivation. As a substrate 1% carboxy methyl cellulose solution was used. Ten ml of the filtrate of culture media were mixed with 20 ml of carboxy methyl cellulose solution and immediately, the viscosity was measured. Similar procedure was carried out after 24 hours of incubation at 27° C.

Individual results were calculated according to the formula :

$$\frac{TO - TE}{TO} \times 100$$

where

TO—Initial reading

TE—Final reading after 24 hours.

The fungi were cultivated on the synthetic medium with cellulose or carboxy methyl cellulose as alternative carbon sources. Concentration of cellulose was 10 g per litre of media. The mycelial growth was measured by dry weight estimation, in cultures grown on medium with carboxy methyl cellulose and in the case of cultures with cellulose, mycelial growth was determined by estimating the quantity of cell protein. Protein estimation was done by Follin's reagent method[1] in the culture which was treated overnight in 1 N NaOH. One per cent cellulose suspension in acetate buffer (pH 4·0) was overnight incubated with the filtrate of the fermentation medium at 37° C and reducing sugars were estimated according to method of Miller[5] (Table I).

From 25 species tested, 22 cultures grew well on the medium with carboxy methyl cellulose and 18 on the medium with cellulose. The quantitative difference in growth was compared with the cellulose decomposing activity of the cultures grown on medium with cellulose as carbon source. The results obtained in the studies of cellulolytic activity of Pyrenomycetes by different methods are not well correlated. However, most of the fungal species tested exhibit cellulolytic activity. *Anthostoma turgidum* was the most active species, because it showed the highest activity, determined by all the mothods used.

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INHIBITION OF MONOAMINE OXIDATION OF GUINEA PIG LIVER MITOCHONDRIA BY EXTRACT OF *LATHYRUS SATIVUS* SEEDS

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THE consumption of *Lathyrus sativus* seeds as the principal dietary constituent causes a neuropathological disease called 'lathyrism' among the rural population in certain parts of Central India[1]. *β-n*-oxalyl-L-*a*, *β*-diaminopropionic acid, the toxin present in these seeds, is believed to be the cause of neurolathyrism in adult human[2-4]. The amino acid is neurotoxic to many experimental animals[5-7] and causes certain biochemical changes[8,9]. As yet there is no report regarding the effect of this toxin on amine metabolism. Biogenic amines in the brain, heart, kidney, liver, lungs and intestine are metabolized by the action of monoamine oxidase (MAO)[10-12] and also by the action of amine tetrazolium reductase (MADH)[13,14]. *In vitro* effects of *Lathyrus sativus* extract on MAO and MADH systems of guinea pig liver mitochondria were studied, an account of which is presented in this communication.

Materials and Methods

Lathyrus sativus seed concentrate (L.S.C.) was prepared according to the method of Adiga *et al.*[15]. The seed meal was refluxed with 75% aqueous ethanol (400 g seed/000 ml ethanol) for 90 minutes and the extraction repeated thrice with fresh portions of the solvent. The pooled extract (crude) was concentrated *in vacuo* (40–45° C) to one-sixth the volume and shaken with equal volume of chloroform to remove lipids and pigments. L.S.C. after treatment with chloroform is described as partially purified. Guinea pig liver mitochondria was prepared according to the method of Schneider and Hogeboom[16].

The standard assay system for MAO contained 0·02 M PO_4 buffer, pH 7·0, 0·01 M tyramine, 0·0125 M semicarbazide, 20 mg mitochondria (1 mg protein) and 0·4 ml of different L.S.C. preparations (crude–7·8 protein/ml, partially purified 4·5 mg protein/ml) in a total volume of 2 ml. The incubation mixture was preincubated for 10 min followed by 15 min incubation period at 37° C. The aldehyde formed was measured at 420 nm[17].

The standard assay system for MADH contained 0·025 M PO_4 buffer, pH 7·5, 0·01 M tryptamine, 0·5 mg neo-tetrazolium chloride (NTC), 50 mg mitochondria (2·5 mg protein) and 0·4 ml of different L.S.C. preparations in a total volume of 2 ml. The assay mixture was preincubated for 10 min followed by 8 min incubation period. Diformazan produced