STUDIES ON PHYSIOLOGY OF HELMINTHOSPORIUM SPECIES

VI. Inhibitory Effect of Different Chemicals on the Production and Activity of Cellulolytic Enzymes of *Helminthosporium apattarnae* Desh. & Desh.

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The effect of different chemicals like salts of heavy metals, oxidising and reducing compounds, phenolic compounds, antibiotics and dyes on the production and activity of cellulolytic enzymes of *Helminthosporium apattarnae* has been studied. Viscosity reducing enzyme is designated as Cx and filter paper disintegrating enzyme C_2. All chemical compounds tested, showed inhibitory effect on the production of cellulase (Cx and C_2). Maximum effect was seen in the case of $AgNO_3$, penicillin, KCN and aniline blue but it was less in the case of iodine and tetracycline. In general, all chemical compounds tested, inhibited the cellulase (Cx) activity considerably when compared with control.

Introduction

The inhibition of cellulases may help in the control of microbiological deterioration of cellulose. Apart from commercial considerations, inhibitors of cellulase can be of much help in exploiting the mechanism of cellulase action and in differentiating cellulases obtained from various sources.

Inactivation of the extracellular enzymes is one method of preventing growth of an organism on an insoluble substrate (Siu 1951). Finholt *et al.* (1952) found that an 18-carbon aliphatic amine prevents the growth of *Lentinus lepideus* on wood cellulose but does not prevent its growth on maltose. Reese and Siu (1954) found that activated carbon adsorbed cellulolytic enzymes and prevented growth of several fungi on cellulose, but it did not prevent their growth on glucose, a soluble substrate.

In the present investigation an attempt has been made to study the effect of different chemical compounds on the production and activity of cellulolytic enzymes of *Helminthosporium apattarnae*.

Materials and Methods

The pathogen isolated from leaf spot of *Cynodon dactylon* Pers. was cultivated in the medium containing CMC, 1 %; $Ca(NO_3)_2$, 0.25%; KH_2PO_4, 0.1%; $MgSO_4.7H_2O$, 0.05 % and test compounds at different concentrations. Twenty-five ml of the medium was taken in 250 ml Erlenmeyer flasks, autoclaved at 15 lb/sq inch for 20 min and inoculated with 5 drops of spore suspension. The fungus was grown for 3 days at 25 ± 1°C and contents of the flasks were filtered through Whatman filter paper No. 1. The *p*H of the culture filtrate was determined by a *p*H meter and dry mycelial weight was also recorded. The culture filtrates were

centrifuged at 5,000 rpm for 10 min and clear supernatant was used as an enzyme sample to assay Cx activity by estimating reduction in viscosity of carboxymethyl cellulose (0.5 %) and C_2 activity by loss of coherence of filter paper discs (Ghewande 1973) at optimum *p*H and temperature. The effect of inhibitors on cellulase (Cx) activity was also studied by Somogyi's micro-method (1945). The assay medium consisted of 1 ml of 1% CMC, 1.5 ml of citrate-phosphate buffer (*p*H 3.6), 0.5 ml of the solution of test compounds like metals, oxidising and reducing compounds at 2×10^{-3}M, phenolic compounds at 100 ppm, antibiotics at 0.2 %, dyes at 10^{-4} M and "kaththa" and tannic acid at 0.01 % concentrations and 1 ml of crude enzyme solution, incubated for 1 hour. The cellulase activity was assayed at optimum *p*H (3.6) and temperature 25°C. Mean titres of controls of substrate without enzyme, enzyme without substrate and also test compound were subtracted from the mean enzyme-substrate-test compound titre in order to obtain a measure of enzyme activity in the presence of test compound. Cellulase activity was expressed in terms of micro-equivalents of reducing sugar/min/mg protein.

Results and Discussion

Effect of different chemical compounds on production of cellulase

Individual test compounds like heavy metals, oxidising and reducing compounds at 2×10^{-3} M concentration, phenolic compounds at 100 ppm, antibiotics at 0.2 %, dyes at 10^{-4} M and other compounds like "kaththa" and tanic acid at 0.01 %, were added to the basal medium.

The results of Table I indicate that the final *p*H of the medium containing $AgNO_3$, iodine, KCN, "kaththa", tanic acid and erythrocine drifted towards neutrality, whereas it was changed into alkalinity in $Pb(NO_3)_2$, phenolic compounds, penicillin and aniline blue-containing media. It was changed into acidity in potassium ferricyanide medium.

Growth was increased in $AgNO_3$, $Pb(NO_3)_2$, potassium ferricyanide, Na_2S, gallic acid, phloroglucinol, "kaththa", streptomycin, tetracycline, aniline blue and erythrocine-containing media. It was interesting to note that growth in $HgCl_2$, pyrocatechol, α-naphthol, congo red, crystal violet and methylene blue containing media was completely suppressed. All chemical compounds tested on the production of cellulolytic enzymes showed inhibitory effect. Maximum effect was seen in the case of $AgNO_3$, penicillin, KCN and aniline blue, whereas in the case of iodine and tetracycline inhibitory effect was less when compared with the control.

Phenolic compounds strongly inhibited the cellulase production as also reported for *Fusarium oxysporum* f. *vasinfectum* (Reddy and Mahadevan 1967), *Alternaria tenuis* and *Pythium aphanidermatum* (Gupta and Bilgrami 1969), *F. oxysporum* f. *tuberosii* and *F. solani* var. *eumartii* (Mall 1973).

Effect of different chemicals on cellulase activity

It is clear from Table II that the salts of heavy metals inhibited cellulase activity to a great extent followed by oxidising and reducing compounds. In general, all the

TABLE I

Effect of different chemical compounds on cellulase production at 25°C

Compounds	pH		Mean dry wt. (mg)	Cellulase activity				
	Initial	Final		% Viscosity loss after (min) at pH 2.6				R.T. (min) at pH 7.6
				5	10	20	30	
$AgNO_3$	5.8	6.1	150*	7	13	14	18	43
$CuCl_2.2H_2O$	5.3	5.3	80**	26	36	36	39	44
$HgCl_2$	5.8	—	—	—	—	—	—	—
$Pb(NO_3)_2$	5.8	7.6	155**	29	38	38	39	40
Iodine	6.1	6.8	112	44	47	49	50	39
$K_3[Fe(CN)_6]$	6.4	5.5	162**	28	33	35	35	40
KCN	6.4	6.7	67**	19	26	29	29	49
Na_2S	8.8	8.5	150*	26	31	32	33	54
Pyrocatechol	6.1	—	—	—	—	—	—	—
Catechol	6.7	7.3	85**	6	32	33	33	47
Gallic acid	6.1	7.6	144	36	42	43	43	49
Phloroglucinol	6.4	7.6	125	35	40	40	40	44
α-naphthol	5.5	—	—	—	—	—	—	—
Kaththa	5.5	6.4	134	26	32	33	35	46
Tannic acid	5.2	6.4	111	26	31	32	32	45
Penicillin	6.4	7.3	104*	17	29	29	29	50
Streptomycin	6.4	6.4	150*	32	35	35	35	41
Tetracycline	5.1	4.9	165**	43	47	47	47	34
Aniline blue	5.6	7.6	140	10	26	26	26	48
Congo red	—	—	—	—	—	—	—	—
Erythrocine	5.8	6.1	134	39	40	42	42	38
Crystal violet	—	—	—	—	—	—	—	—
Methylene blue	—	—	—	—	—	—	—	—
Control	6.1	6.8	113	43	57	68	78	28
Mean			126.722					
S.Em.			± 6.855					
C.D. at 5%*			20.452					
1%**			28.090					

— = No growth, No activity.

compounds tested, showed inhibitory action on cellulase activity when compared with the control.

As regards the effect of metallic compounds, similar results were also obtained by Basu and Whitaker (1953), Ghosh (1964), Eriksson and Pettersson (1968), Singh (1968), Mandels and Reese (1963, 1965) and Sison *et al.* (1958) for *Myrothecium verrucaria, Aspergillus terreus* and *Penicillium verriabile, P. notatum, Fusarium lateritium* f.

TABLE II

Effect of different chemical compounds on cellulase activity at pH 3.6 *and at* 25°C.

Compound	Cellulase (Cx) activity (micro equivalents of reducing sugar/min/ 2.565 mg protein)	Compound	Cellulase (Cx) activity (micro equivalents of reducing sugar/min/ 2.565 mg protein)
$AgNO_3$	0.225	α-naphthol	3.375
$CuCl_2.2H_2O$	0.675	Tannic acid	2.025
$HgCl_2$	1.575	Kaththa	3.150
$Pb(NO_3)_2$	0.450	Penicillin	3.825
KCN	2.475	Streptomycin	3.825
Na_2S	1.350	Tetracycline	2.475
Iodine	2.475	Aniline blue	4.275
$K_3[Fe(CN)_6]$	1.575	Congo red	2.025
Pyrocatechol	1.350	Erythrocine	2.025
Catechol	2.475	Crystal violet	2.025
Gallic acid	2.700	Methylene blue	4.500
Phloroglucinol	3.375	Control	11.925

cajani, many fungi and *Poria vaillantii* respectively. However, Thomas (1956) showed that of the compounds tested, mercuric acetate alone completely inhibited the activity of *Stachybotrys atra* cellulase although partial inhibition was also produced by copper sulphate and lead acetate at 0.02 % concentration. Husain and Rich (1958) found that out of Hg^{++}, Zn^{++}, Cu^{+++}, Fe^{++} and Ag^{+} tested at 10^{-3} M concentration against the cellulase of *Cladosporium cucumerianum*, only Hg^{++} was inhibitory. Hakan Bjorndal (1968) found that Cu^{++}, Zn^{++} and Sn^{++} did not cause any inhibition at 10^{-3} M concentration but Hg^{++} had an inhibitory effect at concentration as low as about 10^{-5} M.

Results regarding oxidising and reducing compounds in the case of *Helminthosporium apattarnae* agree with the results reported for *Myrothecium verrucaria* (Basu and Whitaker 1953), *Stachybotrys atra* (Thomas 1956), *Pestalotiopsis westerdijikii* (Reese and Mandels 1957), *Aspergillus terreus* and *Penicillium verriabile*(Ghosh 1964). However, Sison *et al.* (1958) reported that potassium cyanide stimulated the cellulase activity of *Poria vaillantii.*

All phenolic compounds exerted strong inhibitory effect on *H. apattarnae* cellulase. These results are in conformity with those reported for *Trichoderma viride*, *Myrothecium verrucaria* and *Pestalotiopsis westerdijikii* (Reese and Mandels 1957), *Fomes marginatus* (Lyr 1961), *Fusarium oxysporium* f. *vasinfectum* (Reddy and Mahadevan 1967), *Alternaria tennuis* and *Pythium aphanidermatum* (Gupta and Bilgrami 1969) *Fusarium oxysporum* f. *tuberosii* and *F. solani* var. *eumartii* (Mall 1973).

Dyes at 10^{-4} M concentration strongly inhibited the cellulase activity of *H. apattarnae*. These results are similar to those obtained for *Myrothecium verrucaria*

(Basu and Whitaker 1953), *Pestalotiopsis westerdijikii* (Reese and Mandels 1957) and many other fungi (Pal and Basu 1961).

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