INTERNATIONAL JOURNAL OF RESEARCH IN COMMERCE, IT, ENGINEERING AND SOCIAL SCIENCES (IJRCIESS) ISSN: 2349-7793
Vol. 1 No. 1 (2007): January
Double-Blind Peer Reviewed Refereed Open Access International Journal

Structure - Fungicidal Activity Relationship Of Cellulase Enzymes From *Aspergillus niger*

Dr. Arti Saxena, *Reader / Dept of Chemistry*
A.N.D.N.N.M College, CSJM University
Kanpur, 208025, India

Dr. Anurag Saxena, *Reader / Dept of Physics*
D.A-V College, CSJM University
Kanpur, 208025, India

Abstract

Antifungal potential of two potent fungicides m-dinitrobenzene (MDB) and pentachloro phenol (PCP) was studied on cell wall degrading enzymes of *Aspergillus niger*. The structure- fungicidal activity relationship was investigated by studying the effect of different nitrobenzenes and also of various chlorinated phenols and their derivatives on cellulase enzyme activity. MDB was much more effective in inhibiting the activity of enzyme than o- and p- dinitrobenzenes of similar concentrations (i.e., 1.5×10^{-3} M). On the other hand, it was observed that the fungicidal activity of chlorophenols increases as the degree of chlorination increases. Accordingly, pentachloro phenol is much more active than mono, di, tri, or tetra chlorophenols. Lauryl PCP caused the greatest inhibition of Cellulolytic activity.

Keywords: Antifungal, Nitrobenzenes, Phenols.

INTRODUCTION

Biodeterioration caused by fungi threats cultural and natural heritage worldwide. Fungi can degrade a wide array of biological compounds. Their preferences for keratin and cellulose - based material, in addition to their great dispersal capability via spores and resistance to adverse environmental conditions, make them a pest hard to deal with. Fungi cannot only corrode, color and digest organic materials, they can also cause allergies and respiratory problems. Their control demands active removal and reduction of growth rate and spore load in storerooms. Diverse chemical agents are commonly used to achieve this, but non- toxic alternatives are constantly pursued to avoid damage to biological specimens as much as possible. Wood is a rich source of cellulose and is vulnerable to biological attack due to its high carbohydrate and protein composition. *Aspergillus* sps. have been identified as the most common fungi having high cellulolytic activity and so responsible for Biodeterioration. The glucose formed in the process of cellulolysis is utilized by the fungi and they in turn consume the materials and destroy them completely. Use of suitable fungicides may inhibit the activities of enzymes produced by the fungi.

It is also proposed to study the fungicidal activity by introducing new effective functional groups in the fungicides used.

MATERIALS AND METHODS

2.1 Chemicals

All the inorganic chemicals used were of analytical grade, obtained from BDH Laboratories, Bombay, India. DEAE Sephadex A - 50 was obtained from Sigma Chemical Company (St. Louis, Mo., USA), electrophoretic materials from Biochemical center (New Delhi, India) and Sephadex G - 100 & G - 200 were the products of Pharmacia Fine Chemicals, Uppsala, Sweden. Carboxymethyl cellulose was obtained from Loba Chemie Indo Austranal.

2.2 Microorganism

The cellulolytic fungus *Aspergillus niger* was obtained from Defence Materials Stores Research and Development Establishment, Kanpur. The culture was grown on potato dextrose agar slants containing filter paper strips as cellulosic substrate at a temperature of 30± 2°C for a period of ten days and maintained at 4°C by subculturing every month.

2.3 Elaboration of Enzymes

On the 7th day of growth of the fungus, the mycelia were harvested by filtration through four layers of cheese cloth. The culture filtrates were directly centrifuged at 5,000 rpm for 20 minutes at 4°C. Simultaneously, the mycelial mat was collected on preweighed Whatman filter paper No.1., washed with distilled water and dried at 70°C until constant weight was obtained. The supernatant obtained was used as a source of crude Extracellular Enzyme preparation.

After removing the supernatant, the mycelial mat was dried between the folds of filter paper. The partially dried mycelia were crushed in a grinder with small amount of distilled water which was diluted to obtain 2.0% solution of mycelia. This suspension was used for the study of Intracellular Enzymes.

2.4 Enzyme Purification

The crude culture filtrate (225 ml) was concentrated by ultrafiltration (Diaflo - membrane, PM10) to 25 ml in an ultrafiltration system. The enzyme was precipitated by adding solid $(NH_4)_2SO_4$ to the culture filtrate and the precipitate was dissolved in a small volume of citrate buffer (50 mM, pH 5.5). The entire work of enzyme purification was performed at 0 - 4°C.

Further fractionation of the active material was achieved by ion - exchange chromatography on DEAE - Sephadex A - 50 medium (Pharmacia Fine Chemicals, Uppsala, Sweden). The enzyme preparation was subjected to gel filtration on Sephadex G-200, pre - equilibrated with citrate buffer.

Electrophoresis was performed as described by Davis (1964) on 7% acryl amide gels using TRIS - glycine and citrate - NaOH as electrophoretic buffers. Cellulose was assayed by using p-nitrophenyl - β - glucoside (pNPG) as substrate according to Shewale and Sadana (1978).

2.5 Preparation of stock solution of fungicides

A stock solution of 1.5×10^{-3} M (3.990 g/L) pentachloro phenol (PCP) was prepared in ethanol and subsequently diluting with distilled water to get PCP of desired concentration.

A stock solution of 1.5×10^{-3} M (2.5200 g/L) m- dinitrobenzene (MDB) was prepared in isopropanol and subsequently diluting with distilled water to get MDB of desired concentration.

2.6 Inhibitory effect of fungicides

Impregnation method was used for chemical protection of cellulose from fungal attack. It includes insolubilizing biocidal toxicants onto the surface. This is the normal method of biocidal deposition onto the cellulosic materials. Different concentrations of fungicides were added to the broth and potato - dextrose - agar media to observe the qualitative and quantitative growth of the fungal culture and the activities of the cellulolytic enzymes. Fungicidal activities were also examined by introducing new effective functional groups in MDB & PCP.

RESULTS AND DISCUSSION

In order to find out the structure - fungicidal activity relationship, the effects of different nitrobenzenes and also of various chlorinated phenols and their derivatives on the activities of carboxymethyl cellulase enzymes were investigated. The purified enzymes were incubated with definite concentrations of various nitrobenzenes and of chlorinated phenols and their derivatives at 55°C for different periods. To prevent the denaturation of the enzyme, the pH of the mixtures was adjusted to 5.0 and the mixture were allowed to react with 1.0% (w/v) solution of carboxymethyl cellulose in 100 mM citrate - phosphate buffer (pH 5.0). After 60 minutes of incubation the enzyme activity was assessed by Nelson - Somogyi method, 1944. A control experiment without any fungicide was also run simultaneously. Table (1) gives the comparative fungicidal efficacy of ortho-, meta- and para- dinitrobenzenes. The comparative fungicidal efficacy of various chlorinated phenols and their derivatives against the activity of carboxymethyl cellulases, is recorded in Table (2).

TABLE - 1

Comparative fungicidal efficacy of o-, m- & p-dinitrobenzenes

Fungicide	Final concentration of fungicide (M)	% Inhibition of enzyme activity
Control	Nil	0.0
o-Dinitrobenzene	1.5×10^{-3}	27.5
m-Dinitrobenzene	1.5×10^{-3}	94.5
p-Dinitrobenzene	1.5×10^{-3}	23.0

Number of determinations were three in each case.

Results recorded in Table (1) show that m-dinitrobenzene is much more effective in inhibiting the activity of enzyme than o-and p-dinitrobenzenes of the similar concentrations, indicating thereby that the electron withdrawing nature of -NO2 group potentiates the antifungal activity preferably at meta- position than at ortho- and para- positions. Albert et al. (1980), while describing the synthesis of some 4 - nitro - isothiazoles as potential antifungal agents, concluded that the nitro group is essential for antifungal activity.

TABLE - 2

Comparative fungicidal efficacy of chlorinated phenols and their derivatives

Fungicide	Final concentration of fungicide (M)	% Inhibition of enzyme activity
Control	Nil	0.0
p-Chlorophenol	1.5×10^{-3}	7.0
dichlorophenol	1.5×10^{-3}	16.6
2,4,5-trichlorophenol	1.5×10^{-3}	37.2
2,3,4,6-Tetrachlorophenol	1.5×10^{-3}	52.3
Pentachlorophenol (PCP)	1.5×10^{-3}	92.0
Sodium pentachlorophenate	1.5×10^{-3}	66.4
Lauryl pentachlorophenol (LPCP)	1.5×10^{-3}	95.0
Pentachloronitrobenzene	1.5×10^{-3}	60.0

Number of determinations were three in each case.

It is clear from the results recorded in Table (2) that the fungicidal activity of chlorophenols increases as the degree of chlorination increases. Results indicate that the trichloro phenols are more active than the dichlorophenols which in turn are more active than the monochloro phenols. The tetra and pentachloro phenols are much more active than the mono, di and trichloro phenols. The sodium salt of pentachloro phenol has also been found to be an effective biocide but to a lesser extent than that of pentachloro phenol itself. But when phenolic group of PCP is replaced by a nitro group, the fungicidal efficacy of the resultant pentachloro nitrobenzene decreases than that of the former. Thus, it can be concluded that PCP is a much more effective fungicide in controlling the activity of carboxymethyl cellulase enzymes. If the phenolic group of PCP is replaced by some fatty acid, the resultant compound called the Lauryl PCP (or LPCP) caused the greatest inhibition of cellulolytic activity. In general, phenolic compounds reduce the enzyme activity firstly by reducing the solubility of the enzyme proteins by forming insoluble protein - phenolics complexes (Williams, 1963) and secondly by direct inhibition of the enzymes by forming a soluble but inactive enzyme - inhibitor complex (Hulme and Jones 1970; Loomis and Battaile, 1966; Zanobini et al., 1967; Firenzuol et al., 1969). The inhibitory activity of PCP appears to be of the second type since enzyme protein was not precipitated by the addition of PCP. Leclercq (1977) studied the fungicidal efficacy of pentachloro phenol PCP and tetrachloro phenol TCP in wood protection against Basidiomycetes and concluded that a greater amount of TCP was required to inhibit the activities of enzymes than PCP. Soni and Bhatia (1979) studied the inhibition of cellulase enzymes from Fusarium oxysporum by phenols and related compounds. Introduction of a second hydroxyl group at ortho position in phenol led to a great increase in inhibitory capacity. As such, catechol was the best inhibitor. Addition of a third group (-OH) in benzene ring led to significant changes in the inhibiting ability of phenolic compounds. Phloroglucinol had negligible inhibiting capacity, whereas its isomer, pyrogallol, was a more potent Inhibitor. Francoise et al. (1992) studied the relationship between the biodegradative capability of soil micromycetes for pentachloro phenol (PCP) and for pentachloro nitrobenzene (PCNB) and observed that PCNB was less accessible to fungal degradation than PCP.

Moharram et al. (2004) studied that the fungicides Kocide and Ridomil plus exerted a depressive effect on the total counts and on the individual cellulose decomposing fungal species associated with roots and shoots of tomato. When fungicides were incorporated in the liquid culture medium specified for growth and extracellular enzyme production, there was a significant reduction in mycelial growth as well as in cellulase production.

REFERENCES

1. Davis, B. (1964). *Ann NY Acad Sci*, 121, 404.
2. Shewale, J.G. and Sadana, J. C. (1978). *Can. J. Microbiol*. 24, 1204.
3. Nelson, S. (1944). *J. Biol. Chem*., 153, 375.
4. Albert, Anthony, H.; O' Brien, D. E.; Robins, Roland, K. (1980). *J. Heterocycl. Chem*., 17(2), 385.
5. Williams, A. H. (1963). *Enzyme Chemistry of Phenolic Compounds* (Pridham, J. B., ed.) p.87, Pergamon Press, London.
6. Hulme, A. C. and Jones, J. D. (1970). *Enzyme- Chemistry of Phenolic Compounds*. pp.97.
7. Loomis, W. D. and Battaile, J. (1966). *Phytochemistry*, 5, 429.
8. Zanobini, A.; Vanni, P.; Firenzuoli, A. M. (1967). *Experimentia*, 23, 1015.
9. Firenzuoli, A. M.; Vanni, P. and Mastronuzzi, E. (1969). *Phytochemistry*, 8, 61.
10. Laclercq, A. (1977). *Bull. Rech. Agron Gembloux*, 12(3), 215.
11. Soni, G. L. and Bhatia, I. S. (1979). *Indian J. Biochem. Biophys*., 16(4), 223.
12. Francoise, Seigle - Murandi; Regine, Steiman; Jean Louis, Benoit - Guyod; Bartisetiani; Muntalif; Lucile Sage (1992). *Sci. Total Environ*. ,123 - 124, 291.
13. Moharram, A. M.; Abdel - Hafez, S. I. I.; El - Said, A. H. M. and Salee, A. (2004). *Acta Microbiologica et Immunologica Hungarica*. 51(4).