PRODUCTION OF EXTRA-CELLULAR ENZYMES BY JUTE PATHOGENIC FUNGUS *MACROPHOMINA PHASEOLINA*

Cellulolytic enzymes are known for a long time and the involvement of the cellulolytic enzymes in plant pathogenesis is well documented[1-3]. In the present investigation we report the production of both extracellular and intracellular CMCase (C_x-cellulase) activities by the jute pathogenic fungus *Macrophomina phaseolina* (strain JARI 25). In addition, this pathogenic fungus can also produce extracellular amylase, protease, β-D-glucosidase, acid phosphatase and lipase.

M. phaseolina, a well-known jute pathogen grows well in malt medium (2·5%). The pathogenic fungus can also grow in a medium as described by Capellini and Peterson[4] containing either micro-crystalline cellulose or carboxymethyl cellulose or jute powder as the carbon source. However the growth rate is much higher in malt medium as determined by the basis of wet weight of the fungus. We have determined the pH and reducing sugar content of the culture filtrate for different days after the growth of *M. phaseolina* in media containing CMC or sucrose as the carbon source and also in malt medium. The results given in Fig. 1 indicate that the pH of the culture filtrate decreases sharply when sucrose is used as the carbon source. The decrease in pH has also been observed when the pathogen was grown in malt medium. However, the pH of the culture filtrate is increased a little, when CMC is used as the carbon source.

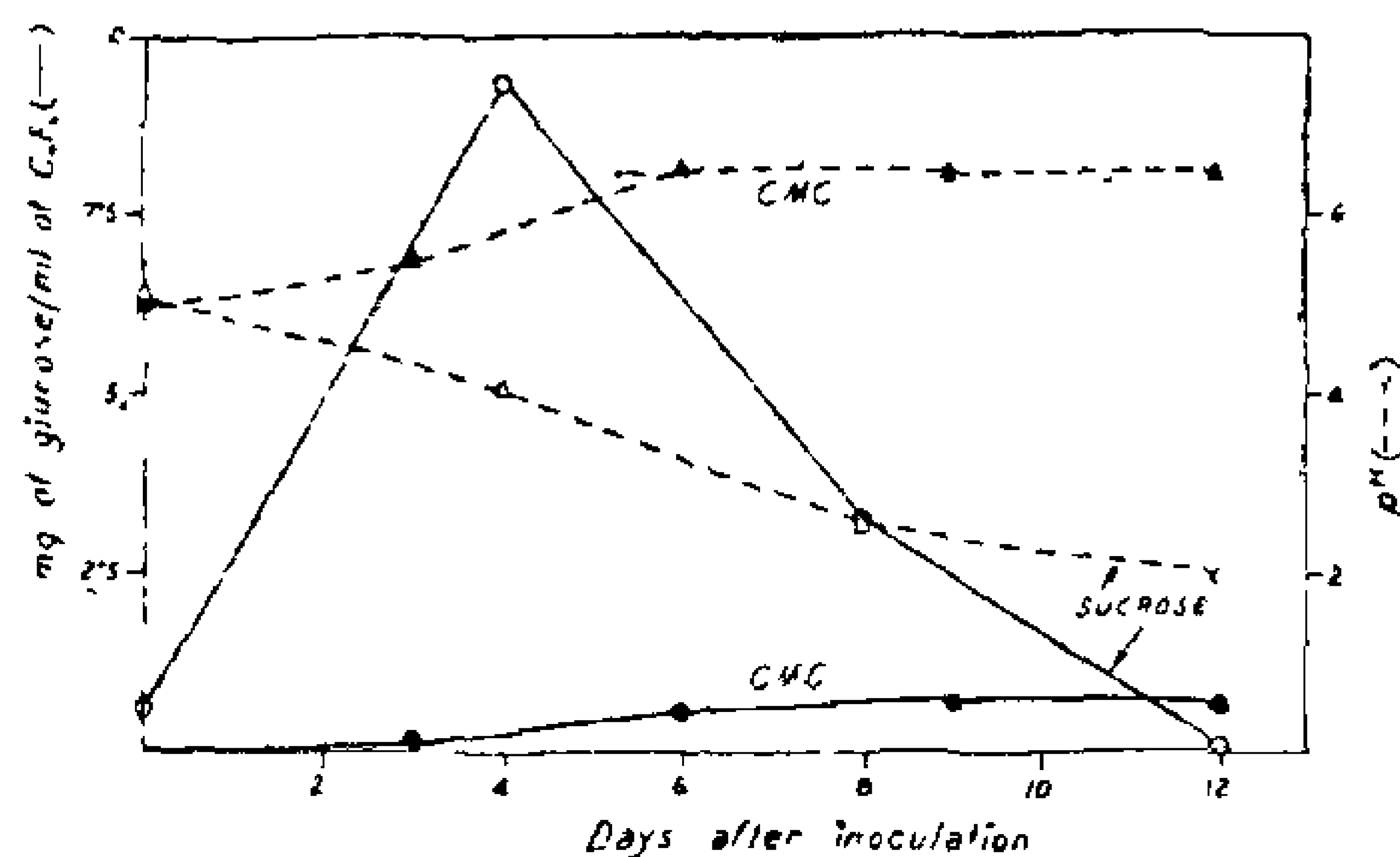

Fig. 1. Changes in the pH and concentration of reducing substances in the culture filtrate (C.F.) of *M. phaseolina* grown for different days in media containing different carbon sources.

The results given in Fig. 1 also indicate that the concentration of the reducing substances in the culture filtrate increases sharply when sucrose is used as the carbon source. It reaches maximum in 4 days after inoculation. On the other hand, when CMC is used as the carbon source the concentration of reducing substances in the culture filtrate increases gradually and it reaches plateau in 6–9 days after inoculation.

No extracellular CMCase (C_x-activity) could be detected when the fungus was grown in malt medium or medium containing sucrose as the carbon source. On the other hand extracellular CMCase (C_x-activity) in the culture filtrate could be detected by dinitro salicylic acid method (DNS method)[5], when the organism was grown in the medium containing CMC as the carbon source.

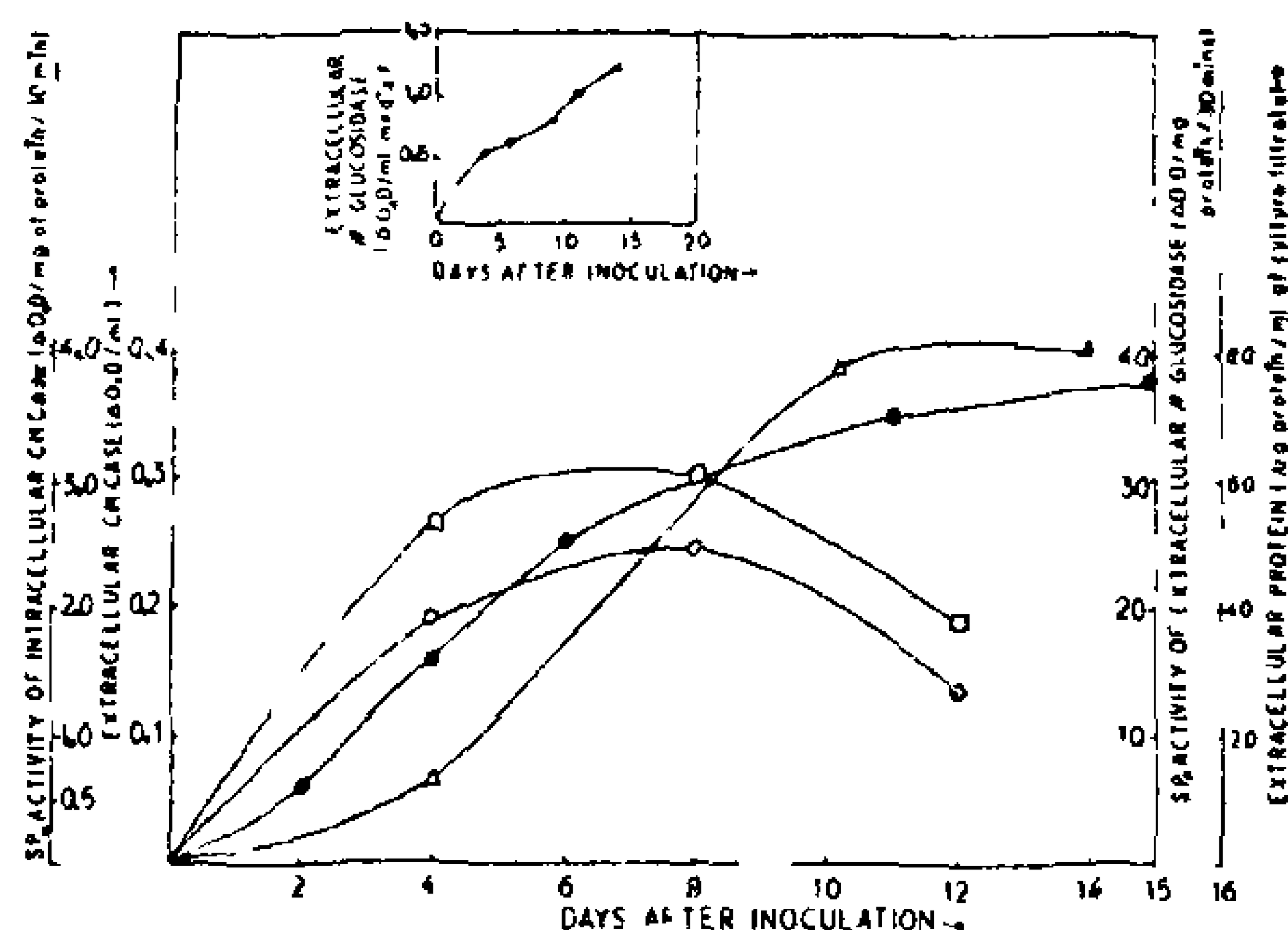

Fig. 2. Activities of different extra cellular and intra-cellular enzymes produced by *M. phaseolina* after different days of inoculation in CMC medium. The inset of the figure represents the changes of the extra-cellular glucosidase activity in the culture filurate.

△—△ : Extra-cellular protein (μg)/ml of C.F.;
●—● : Extra cellular CMCase/ml of C.F.;
□—□ : Specific activity of extra cellular β-glucosidase;
○—○ : Specific activity of intra cellular CMCase.

The results given in Fig. 2 indicate that the extracellular CMCase activity per ml of culture filtrate increases with the increase in number of days after inoculation. The activity reaches maximum in 12 to 15 days of the growth. The production of extracellular protein estimated by the method of Lowry *et al.*[6] has also been found to follow the same pattern as that of CMCase. As a result of this the specific activity of extra cellular CMCase is maximum in between 4–6 days of growth of the pathogen. No filter-paper activity could be detected in the crude culture filtrate. FPase activity was measured using filter-paper as substrate for the measurement of total cellulase which is, at present, thought to be composed of endo-β-1, 4-glucanase (C_x), exo-β 1, 4-, glucanase (C_1) and β-glucosidase[7]. However, the concentrated ammonium sulfate fraction (0–70%) of the culture filtrate showed some filter-paper activity (data not given). Our inability to detect filter-paper activity in crude culture filtrate may be due to two major

factors : (i) the presence of certain specific inhibitor for filter paper activity or (ii) the very low concentration of the enzyme present in the crude culture filtrate. The specific activities of the other two enzymes, *viz.*, β-glucosidase measured using *o*-nitrophenyl-β-D-glucoside[3] and intracellular CMCase estimated by the DNS method[5] were found to reach maximum in between 6–9 days of the growth of the fungus

Extracellular CMCase activity was also detected when solid medium was used for the growth of the fungus. The enzyme activity was detected by 1% cetryltrimethyl ammonium bromide[9]. The clear zone was formed due to the production of C_x-cellulase or CMCase. The production of other exrtacellular enzymes was detected by growing the pathogen in solid media supplemented with different carbon sources. The results thus obtained suggest that extracellular amylase as tested by iodine solution[10] and lipolytic activity detected by the method described by Sierra[11] were found to be positive (The data not given). When *M. phaseolina* was grown in solid medium containing 0·4% gelatin, extracellular protease activity was also detected. The protease activity was detected by both TCA and saturated solution of ammonium sulfate as described by Hankin and Anagnostakis[10].

At this point it is worth mentioning that *M. phaseolina* has already been reported to produce extracellular cellulase (C_x-activity) when grown in a medium containing carboxymethyl cellulose[12]. However, Dube and Gour failed to detect the extracellular CMCase activity by using DNS method. On the other hand our results strongly suggest that both CMCase and " Filter paper activity ", if there be any, produced in the culture media of *M. phaseolina* could be detected by the DNS method. Moreover, we report here the nature of increase in both extra and intra-cellular β-glucosidase activities estimated by using *o*-nitrophenyl β-D-glucoside[7]. The results given in the inset of Fig. 1 indicate that extracellular β-glucosidase activity increased gradually after inoculation of the fungus *M. phaseolina* and follows the same pattern like that of extracellular CMCase. No extracellular CMCase activity could be detected when sucrose was used as the carbon source instead of carboxymethyl cellulose. From the results presented in this communication it can be concluded that the jute pathogenic fungus *M. phaseolina* can produce several extracellular enzymes including C_X-activity in the culture filtrate.

The authors are grateful to the Director, JARI, Barrackpore, W.B., for generous supply of the strain of *M. phaseolina* (JARI 25). The work has been financed partly by the grant of C.S.I.R., New Delhi, to Dr. S. Dube, Pool-Officer, Department of Biochemistry, Calcutta University.

Department of Biochemistry,
University College of Science,
35, Ballygunge Circular Road,
Calcutta 700 019,
and
Indian Jute Industries Research Assn.
17, Taratola Road,
Calcutta 700 053, *January* 8, 1979.

SAGAR C. SAHA.
SYAMALIMA DUBE.
ARUNIK SANYAL.
DIPAK K. DUBE.
SANDIP N. SINHA.

1. Bateman, D. F., *Phytopathology*, 1963, **53**, 197.
2. —, *Ibid.*, 1964, **54**, 1372.
3. Mahadevan, A. and Chandramohan, D., *Phytopath. Medit.*, 1967, **6**, 86.
4. Capellini, R. A., and Peterson, J. L., *Methods in Microbiology*, 1965, **4**, 64.
5. Mandels, M., Andreotti, R. and Roche, C., *Biotech. Bioeng. Symp.*, 1976, **6**, 21.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L, and Randal, R., *J. Biol. Chem.*, 1951, **193**, 265.
7. Wood, T. M., *Biotechnol. and Bioeng. Symp.*, 1975, No. 5, 111.
8. Michell, K. H., Karonovsky, N. J. and Kornovsky, M. L., *Biochem. J.*, 1970, **116**, 207.
9. Lester Hankin and Anagnostakis, S. L., *J. Gen. Microbiol.*, 1977, **98**, 109.
10. Hankin, L. and Anagnostakis, S. L., *Mycologia*, 1975, **67**, 597.
11. Sierra, G., *Antonie Von Leenwenhock Ned Tijdschr. Hyg.*, 1967, **23**, 15.
12. Dube, H. C. and Gour, H. N., *Curr. Sci.*, 1975, **44**, 818.

TWO NEW HOSTS FOR *XANTHOMONAS VESICATORIA* (DOIDGE) DOWSON

A BACTERIAL leaf spot disease of *Argemone mexicana* L and *Tinospora cordifolia* (Willd.) Miers belonging to the families Papaveraceae and Menispermaceae, respectively was observed during routine surveys at Indian Institute of Horticultural Research, Hessaraghatta farm, Bangalore, for the first time in August, 1978. Some of these infected plants were growing as weeds in tomato crcp. The symptoms produced en these hosts were as follows :

(*a*) *Argemone mexicana*

The infection starts as water-soaked spots on the leaves which soon become dark brown to black in colour, circular to irregular in shape and 5 to 10 mm in size (Fig. 1). The spots may appear on any part of the leaf lamina but they generally originate from margin and tips. The infection is also observed on the stem as minute dark brown to black spots.

(*b*) *Tinospora cordifolia*

The disease is characterised as minute water-soakde spots on the leaves. Later, these spots become pale