repressible by exogenous thiamine. This possibility was finally confirmed since a thiamine transport mutant of *S. cerevisiae,* PT-R2, previously isolated[7], showed no significant uptake of dimethialium.

From the findings obtained above, we conclude that dimethialium, which has the thiamine structure incapable of phosphorylation by thiamine pyrophosphokinase, is accumulated in yeast cells by an energy-dependent process mediated by the same carrier for thiamine. These results strongly suggest that thiamine can be transported and accumulated without obligatory phosphorylation in *S. cerevisiae.*

* We wish to thank the late Dr S. Yurugi, Takeda Research Laboratories, for a generous gift of dimethialium.

1 R.J. Kadner, in: Microbiology Series, vol. 4, p. 463. Ed. B.P. Rosen. New York 1978.
2 C. Chen, J. nutr. Sci. Vitam. *24,* 351 (1978).
3 I. Miyata, T. Kawasaki and Y. Nose, Biochem. biophys. Res. Commun. *27,* 601 (1967).
4 U. Ventura, G. Ferrari, R. Tagliabue and G. Rindi, Life Sci. *8,* 699 (1969).
5 A. Iwashima, H. Nishino and Y. Nose, Biochim. biophys. Acta *330,* 222 (1973).
6 Z. Suzuoki, K. Furuno, K. Murakami, T. Fujita, T. Matsuoka and K. Takeda, J. Nutrition, *94,* 427 (1968).
7 A. Iwashima, Y. Wakabayashi and Y. Nose, Biochim. biophys. Acta *413,* 243 (1975).
8 A. Fujita, in: Methods in Enzymology, vol. 2, p. 622. Ed. S.P. Colowick and N.O. Kaplan. New York 1955.
9 H. Okada and H.O. Halvorson, Biochim. biophys. Acta *82,* 538 (1964).
10 A. Iwashima and Y. Nose, J. Bact. *128,* 855 (1976).

Biochemical changes in mango after infection with *Rhizoctonia bataticola*

H.G. Vyas and H.S. Chhatpar

Department of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda (India), 21 May 1979

Summary. Rhizoctonia bataticola is responsible for the spoilage of mango fruits *(Mangifera india)* during post-harvest preservation and storage. Culture of *R. bataticola* exhibited significant pectinase and cellulase activity. In *Rhizoctonia*-infected fruits an increase of protease and cellulase activity, and a decrease in certain enzymes of carbohydrate metabolism, were observed in comparison to healthy fruits.

Post-harvest preservation, transit and marketing of mango fruits is a challenging problem. Earlier we reported the spoilage of mangoes by *Rhizoctonia bataticola*[1,2]. The present study focuses on biochemical changes after the infection process. An understanding of the physiology of the pathological process may help to develop methods for control.

The preparation of cell-free extracts and the determination of protein were performed as described earlier[3]. Ketoacids were estimated according to the method of Friedemann[4]. Assay methods for aconitase and isocitrate dehydrogenase were as described by Anfinsen[5] and Ochoa[6] respectively. Protease and cellulase activities were assayed by the methods of Ong and Gaucher[7] and Miller[8] respectively, whereas malate dehydrogenase and succinate dehydrogenase were measured according to Ochoa[9] and Slatter and Bonner[10] respectively. Pectinase was determined according to the method of McCready and McComb[11] and aldolase according to Sibley and Lehninger[12]. The mould *R. bataticola* was found to be responsible for the development of blackspot on *Alphonso mango.* An important problem in pathogenesis is the biochemical mechanism of penetration by degradation and solubilization of cell walls of plant tissues in different stages of disease. The encrusting and sheathing substances of plant cell walls such as cutin, suberin, lignin, pectin, protein and cellulose can all be degraded or modified by enzymes of a number of microbial pathogens. It has been shown in apple rot caused by *Penicillium expansum* and *Sclerotinia fructigena*[13] that polygalacturonase and macerating enzymes are important for pathogenesis. Furthermore, several soft rot pathogens like *Pythium* and *Rhizopus* are highly cellulolytic[14,15]. Cellulase activity has also been reported by Bateman[16,17] in bean hypocotyl tissue infected by *Rhizoctonia solani. R. bataticola* from mango tissues also showed considerable pectinase and cellulase activity when grown in the synthetic medium supplemented with carboxy methyl cellulose (table 1). When healthy and affected mango tissues were analysed for cellulase activity, about 4 times more acctivity was observed in black spotted tissues compared with healthy tissues (table 2).

Earlier[1] we reported the accumulation of citrate in mango tissues infected by *R. bataticola.* This accumulation may be due to an increase in the activity of citrate synthase or a decrease in the activity of citrate catabolizing enzymes like aconitase and isocitrate dehydrogenase. It is known that the aconitase reaction is reversible and in equilibrium at pH 7.0, with a citrate:cis aconitate:isocitrate ratio of 90:4:6.

Table 1. Cellulase and pectinase activities from culture filtrates of *R. bataticola*

Enzymes	Enzyme activity (units/100 ml flask)
Cellulase (CMCase)	5.0
Pectinase	46.9

Table 2. Biochemical changes in healthy and infected tissues of ripe mango fruit

Biochemical changes	Mango tissue	
	Healthy	Infected
Aconitase	65.20	6.90
Isocitrate dehydrogenase	14.80	ND
Succinate dehydrogenase	1.90	1.20
Malate dehydrogenase	326.00	4.90
fructose-diphosphate aldolase	197.50	18.90
Protease	9.20	21.50
Carboxymethyl cellulase	6.80	27.80
Monocarboxylic keto acids	7.70	10.30
Dicarboxylic keto acids	8.70	13.50

ND: not detectable. Enzyme activities are expressed as units/mg protein; organic acids are expressed as mg/100 g pulp.

Thus for the active catabolism of citrate, very high levels of isocitrate dehydrogenase are required to remove the substrate isocitrate which may otherwise be converted to citrate by the reversible aconitase reaction. If the activity of isocitrate dehydrogenase decreases, citrate will accumulate in the tissues. In the present study the activities of aconitase and isocitrate dehydrogenase were found to be much lower in the infected tissues compared with the healthy ones (table 2). This indicates that the decrease of citrate catabolizing enzymes is one of the reasons for the accumulation of citrate in infected tissues.

The activities of fructose biphosphate aldolase, malate dehydrogenase and succinate dehydrogenase decreased in the infected tissues (table 2) indicating that the rate of respiration may be affected. A decrease in the activity of malate dehydrogenase has also been shown by Boser[18] in potato leaf roll. Since there was much lower succinate dehydrogenase activity, mitochondrial integrity might be affected after infection leading to a disturbance in the enzymes of the citric acid cycle. This consequently would cause an imbalance in the relative concentrations of metabolites of the tricarboxylic acid cycle. On the contrary the concentrations of monocarboxylic and dicarboxylic ketoacids were found to be higher in infected tissues compared with healthy tissues (table 2). This may be due to immobilisation of these metabolites in subsequent reactions or to low activities of specific transaminases. Furthermore, the overall protease activity was found to be higher in infected mango fruits than in healthy ones (table 2). This observation is in agreement with the postulation of Friedman[19], that greater virulence in the case of *Erwinia carotovora* can be attributed to an increase in proteolytic activity. The high overall protease activity may be responsible for the observed decrease in the activities of certain enzymes in the infected tissues.

1 M.B. Assnani, H.S. Chhatpar, M.H. Kadri, A.K. Mattoo and V.V. Modi, J. Food Sci. Tech. *8*, 208 (1971).
2 H.S. Chhatpar, V.V. Modi and V.B. Pandit, Curr. Sci. *44*, 674 (1975).
3 H.S. Chhatpar and V.V. Modi, Phytochemistry *13*, 165 (1974).
4 T.E. Friedemann, Meth. Enzym. *3*, 414 (1955).
5 C.B. Anfinsen, Meth. Enzym. *1*, 695 (1955).
6 S. Ochoa, Meth. Enzym. *1*, 699 (1955).
7 P.S. Ong and M.G. Gaucher, Can. J. Microbiol. *19*, 129 (1973).
8 G.L. Miller, Analyt. Chem. *31*, 426 (1958).
9 S. Ochoa, Meth. Enzym. *1*, 735 (1955).
10 E.C. Slatter and W.D. Bonner, Biochem. J. *52*, 185 (1952).
11 R.M. McCready and E.A. McComb, Analyt. Chem. *24*, 1986 (1952).
12 J.A. Sibley and A.L. Lehninger, J. biol. Chem. *177*, 859 (1949).
13 R.N. Goodman, Z. Kiraly and M. Zaitlin, in: The Biochemistry and Physiology of Infectious plant diseases, p.138. Ed. D. Van Nostrand. New York 1967.
14 D.N. Srivastava, E. Echandi and J.C. Walker, Phytopathology *49*, 145 (1959).
15 M.N. Winstead and G.L. McCombs, Phytopathology *44*, 153 (1961).
16 D.F. Bateman, Phytopathology *53*, 1178 (1963).
17 D.F. Bateman, Phytopathology *54*, 1732 (1964).
18 H. Boser, Phytopathology *33*, 197 (1958).
19 B.A. Friedman, Phytopathology *52*, 328 (1962).

Trypsinogen-kinase from *Aspergillus fumigatus*

M. Panneerselvam and S.C. Dhar[1]

Biochemistry Laboratory, Central Leather Research Institute, Madras-600 020 (India), 20 June 1979

Summary. The activation of bovine trypsinogen by an extracellular acid proteinase from *A. fumigatus* is described. The enzyme activates trypsinogen optimally at pH 3.5 and 32 °C. The effect of substrate and enzyme concentrations on the activation has been studied and the K_m-value has been determined.

It has been reported that several fungal acid proteinases have the unique ability to activate pancreatic trypsinogen to trypsin. This property was first discovered by Kunitz[2] for an unidentified strain of *Penicillium*. The activity was later demonstrated in *Aspergillus oryzae*[3], in *Aspergillus saitoi*[4,5] and in *Rhizopus chinensis*[6]. Hofmann[7] described the presence of this activity in *Aspergillus carbonarius* and in a number of *Penicillia* including 2 strains of *Penicillium janthinellum*. In an earlier communication[8], the production of extracellular acid proteinase by *Aspergillus fumigatus* has been reported. The object of the present study is to investigate the trypsinogen-activating property of the acid proteinase.

Materials and methods. Bovine trypsinogen (pure) and bovine trypsin (twice crystalline) were obtained from Sigma Chemical Co., USA, and vitamin-free casein was purchased from ICN Pharmaceuticals, Inc., USA. All other chemicals and reagents were of analytical grade.

Partial purification of the acid proteinase from *A. fumigatus:* Culture filtrate (3 l) obtained by culturing *A. fumigatus* in a liquid medium at room temperature (30–32 °C) for 3 days and by filtering off the mat, as described earlier[8], was centrifuged at 5000 × g. The centrifugate was collected and the pH of the centrifugate was adjusted to 4.8. 45 g of DEAE cellulose which had been equilibrated to pH 4.8 by repeated washing with 0.1 M citrate of pH 4.8 were added to the centrifugate with stirring for 1 h at 4 °C. After this period the slurry was packed in a column, and the enzyme was collected in a 500 ml fraction by passing through the column 0.2 M citrate of pH 4.0 containing 0.2 M NaCl.

To the eluate, solid ammonium sulphate was added with gentle stirring till 60% saturation was obtained. The precipitate thus formed was collected by centrifugation, and was dialyzed against bi-distilled water at 4 °C for 40 h. The dialyzed enzyme solution was freeze dried in a Virtis-lyophilizer. The dried acid proteinase thus obtained was used for the present investigation.

Trypsinogen activation assay. The assay for the acid proteinase, based on its ability to activate trypsinogen at pH 3.0–4.0 was determined according to the method described by Kunitz[9] using casein as substrate with slight modifications as suggested by Hofmann and Shaw[10]. The trypsin standard curve was also prepared according to the same method.

Activation of trypsinogen. 1.5 ml of 10^{-5} M trypsinogen solution which was prepared by dissolving 5.0 mg of trypsinogen in 10 ml to 0.0025 M HCl and mixing with 20 ml of 0.1 M citrate of pH 3.4 was warmed at 36 °C and mixed with 0.5 ml of enzyme solution (5 mg/100 ml solution of 0.1 M KH_2PO_4) to start the activation reaction.

Trypsin assay. After exactly 10 min of reaction, 2 ml of casein solution (prepared by mixing equal volumes of 2%