removes ionized calcium from the cytosol, thus inhibiting the contractile processes[3]. Increased levels of c-AMP also inhibit platelet reactions[4]. The fact that the 1st aggregation wave was normal in our patients argues against the possibility of increased c-AMP[1]. However, as suggested by Holmsen[5], the degree of response depends on the intensity of the stimulus. A weak one will only produce a 1st aggregation phase. Similarly, it might also be possible that the inhibition produced by c-AMP was not strong enough to alter the 1st phase. Recent work by Johnson et al.[6] in dogs demonstrated that c-AMP plays an important role in regulating the response of platelets to the aggregating products of prostaglandin metabolism. For this reason we have studied the activity of adenylate-cyclase and the steady-state concentration of c-AMP in platelets of patients with Huntington's disease.

Venous blood from 7 Huntington's disease patients and 11 normal controls was collected in a fasting state, between 08.00 h and 09.00 h. The control group was matched for age and sex. None of the patients were under medication. Informed consent was obtained in all cases.

Adenylate-cyclase activity was determined in a platelet rich fraction by homogenizing the final pellet with 2 ml of 2 mM Trismaleate buffer pH 7.4, containing 2 mM EGTA[7]. The standard assay system (final volume 0.5 ml) was as described[8]. Cyclic-AMP was determined by radio-immunoassay (New England Nuclear). Statistical analysis was by Student's t-test.

As shown in the table, the adenylate-cyclase activity and cyclic-AMP content in platelets of Huntington's disease patients and controls were similar. It seems, therefore, that the platelet defect observed in Huntington's disease is not due to an increase in the levels of cyclic-AMP.

Adenylate-cyclase activity and cyclic-AMP content in platelets of Huntington's disease patients

Groups	Adenylate-cyclase activity (pmole/mg protein/2.5 min)	Cyclic-AMP (pmole/mg protein)
Huntington's disease	67.2± 10.1*	2.8±0.8
Controls	69.1±4.2	2.8±0.6
p	NS	NS

* Values expressed as mean± SE.

1 M. Diez-Ewald, E. Bonilla and J.V. González, Prog. Neuro-Psychopharmac. *4*, 277 (1980).
2 H.J. Weiss, New Engl. J. Med. *293*, 531 (1975).
3 J.G. White, G.H.R. Rao and J.M. Gerrard, Am. J. Path. *77*, 135 (1974).
4 E.W. Salzman, New Engl. J. Med. *286*, 358 (1972).
5 H. Holmsen, in: Platelets: production, function, transfusion and storage, p.207. Eds M.G. Baldini and S. Ebbe. Stratton Inc., New York 1974.
6 G.J. Johnson, G.H.R. Rao, L.A. Leis and J.G. White, Blood *55*, 722 (1980).
7 J.P. Blass, S.D. Cederbaum and R.A. Kark, Clin. chim. Acta *75*, 21 (1977).
8 Y.C. Clement-Cormier, J.W. Kebabian, G.L. Petzold and P. Greengard, Proc. natl. Acad. Sci. USA *71*, 1113 (1974).

Characterization of cellulase from *Humicola lanuginosa*

P.O. Olutiola

Microbiology Department, University of Ife, Ile-Ife (Nigeria), 8 January 1982

Summary. In a carboxymethylcellulose-yeast extract medium, *Humicola lanuginosa* synthesized cellulase. The purified enzyme possessed a molecular weight of 71,000 daltons and exhibited optimum enzymatic activity at pH 5.0 and 45 °C.

Some species of *Humicola,* like a number of other thermophilic fungi, are able to hydrolyze cellulose substrates[1,2]. However, some workers have shown that *H. lanuginosa* is unable to synthesize cellulases[3-5]. Recently a group of workers reported an isolate of *H. lanuginosa* which was cellulolytic[6]. During our work on decomposition of organic matter by thermophilic fungi, we isolated a strain of *H. lanuginosa* which produced cellulase. The present work examines the production and purification of the enzyme.

Materials and methods. The isolate (UNIFE 147) of *Humicola lanuginosa* (Griff. and Maubl) Bunce employed was taken from decomposing compost and was identified by Professor M.H. Zoberi. The growth medium and the inoculation techniques were as previously described[7]. Experimental flasks were incubated at 45 °C for 6 days. Ability of the organism to degrade soluble and insoluble celluloses, measurement of cellulolytic activity and other experimental procedures were as previously described[7]. Purification procedures involving a combination of ammonium sulphate precipitation, batch adsorption on DEAE-Sephadex A-25, ultrafiltration (UM-10), gel filtration and ion-exchange chromatography were performed as described elsewhere[7-10]. One unit of cellulase activity was defined as the amount of enzyme in 1 ml of the reaction mixture that released reducing sugars equivalent to 1 µmole glucose. Specific activity was defined as units per mg protein.

Results and discussion. Humicola lanuginosa failed to grow in defined liquid media containing soluble (carboxymethyl-

Table 1. Growth and synthesis of cellulase by *H. lanuginosa* grown in liquid synthetic medium containing 1% carboxymethycellulose and different concentrations of yeast extract

Concentration (%, w/v) of yeast extract added to 1% (w/v) of carboxy-methylcellulose	Mycelial dry wt (mg)	Cellulase activity (units/mg protein)
0.00	0.0±0.0*	0.0 ± 0.0
0.05	75.7±2.8	0.21±0.02
0.10	99.5±1.5	0.36±0.01
0.15	152.8±2.9	0.65±0.03
0.20	186.8±1.1	0.91±0.05
0.25	219.6±1.8	1.10±0.02
0.30	221.5±2.1	1.13±0.01
0.35	229.1±3.7	1.13±0.03
0.40	230.5±2.8	1.12±0.05

* Each value represents the mean of 6 replicates with SE.

Table 2. Purification of cellulase from *H. lanuginosa* grown in liquid synthetic medium containing 1% carboxymethylcellulose and 0.3% yeast extract

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (fold)
Crude culture filtrate	5200	4610	1.13	100	1.0
Batch adsorption (DEAE-Sephadex A-25)	4595	1514	3.03	88.4	2.7
UM-10 concentration	4251	196.8	21.6	81.8	19.1
1st $(NH_4)_2SO_4$ precipitate	3905	57.8	67.6	75.1	59.8
Sephadex G-100 chromatography	3764	27.5	136.9	72.4	121.2
CM-Sephadex C-50 chromatography	3625	13.0	278.8	69.7	246.7
2nd $(NH_4)_2SO_4$ precipitate	3568	8.9	400.9	68.6	354.8

cellulose or cellobiose) or insoluble (cellulose film, cellulose powder, viscose cellulose or Whatman No. 1 filter paper) celluloses, but lacking yeast extract. When yeast extract was present, growth occurred, but only when the carbon source was carboxymethylcellulose or cellobiose. However cellulase activity was detectable only when carboxymethylcellulose was present, indicating non-constitutive synthesis of the enzyme. Growth and cellulase synthesis by *H. lanuginosa* increased as the initial concentration of yeast extract increased (table 1). However, yeast extract concentrations above 0.3% (w/v) produced no further increase in cellulase synthesis. It is suggested that yeast extract contains some 'constituents' or 'factors' able to induce cellulase synthesis in *H. lanuginosa*. This isolate of *H. lanuginosa* differs from the non-cellulolytic one of Fergus[4] and Chang[3], but resembles the cellulolytic isolate of Mishra et al.[6].

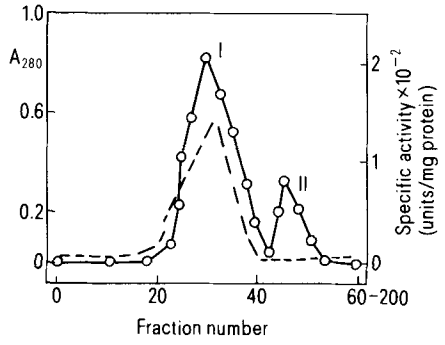

Figure 1. Separation of proteins in culture filtrates of *H. lanuginosa* by gel filtration, and enzymic activity of the fractions towards carboxymethylcellulose. O——O, protein; ---, cellulase activity. Each fraction tube contained 5 ml.

However it differs from the latter workers' isolate in being unable to hydrolyze insoluble cellulose, thus indicating absence of C_1 cellulase[11,12].

When subjected to Sephadex G-100 chromatography, 2 peaks of absorption, with molecular weights of approximately 71,000 (peak I) and 19,000 (peak II) daltons, were obtained (fig. 1). Cellulase activity was present only in peak I. Ion-exchange chromatography of peak I resolved only 1 absorption peak, with a cellulase activity of about 279 units per mg protein (table 2). The final purification step yielded a cellulase enzyme with a purification of approximately 355-fold, and a specific activity of about 400 units/mg. The purified enzyme exhibited maximum activity at pH 5.0 and 45 °C (fig. 2). The enzyme was stimulated by Ca^{++}, Mg^{++} and cysteine, but inhibited by metal chelating agents (EDTA and NaCN), thiol enzyme inhibitors (IAA and pCMB) and 2,4-dinitrophenol (uncoupler of oxidative phosphorylation) suggesting that the enzyme may require metal ion activator(s), free sulphydryl group and metabolic energy for activity[13].

Decomposing organic matter usually consists of numerous fungi some of which degrade insoluble celluloses to soluble forms[4,6]. Thus the physiological significance of *H. lanuginosa* cellulase will be the hydrolysis of such soluble celluloses to simpler sugars which can be more readily metabolized by the parent organism.

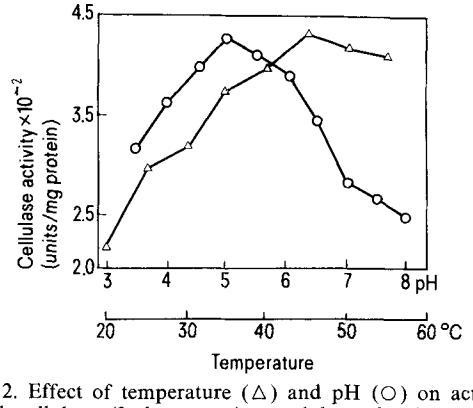

Figure 2. Effect of temperature (△) and pH (○) on activity of purified cellulase (2nd ammonium sulphate fraction) obtained from culture filtrates of *H. lanuginosa*.

1 D.E. Cooney and R. Emerron, in: Thermophylic fungi. An account of their biology, activities, and classification, p. 188. W.H. Freeman Publ. Co., San Francisco 1964.
2 M.K. Jain, K.K. Kapoor and M.M. Mishra, Trans. Br. mycol. Soc. *73*, 85 (1979).
3 Y. Chang and H.J. Hudson, Trans. Br. mycol. Soc. *50*, 649 (1967).
4 C.L. Fergus, Mycologia *61*, 120 (1978).
5 S.L. Rosenberg, Mycologia *70*, 1 (1978).
6 M.M. Mishra, K.K. Kapoor, M.K. Jain and C.P. Singh, Trans. Br. mycol. Soc. *76*, 159 (1981).
7 P.O. Olutiola, J. gen. Microbiol. *102*, 27 (1976).
8 P.O. Olutiola and O.O. Cole, Physiol. Plant. *50*, 26 (1980).
9 P.S. Ong and G.M. Gaucher, Can. J. Microbiol. *22*, 165 (1976).
10 C. Fanelli, M.G. Cacane and F. Cervone, J. gen. Microbiol. *104*, 305 (1978).
11 K. Selby and C.C. Maitland, Biochem. J. *104*, 716 (1967).
12 P. Whithney, J.M. Chapman and J.B. Heale, J. gen. Microbiol. *56*, 215 (1969).
13 M. Dixon and E.C. Webb, in: Enzymes, p. 950. William Clowes and Sons Ltd. London, Beccles and Colchester 1971.