genase activity between fibres were not as distinct as the differences in adenosine triphosphatase activity. The proportion of aerobic and anaerobic fibres varied between species, but was not influenced by body size. Phosphorylase activity tended to show an opposite trend to that of adenosine triphosphatase. The horse had nearly 100% of its fibres high in phosphorylase activity, but in the shrew diaphragm no fibres with phosphorylase activity were seen.

Fast-contracting muscle fibres were also high in phosphorylase activity, showing that faster-contracting fibres used mainly an anaerobic metabolism. In smaller animals the proportion of fibres with high adenosine triphosphatase activity exceeded that of fibres with high phosphorylase activity, so aerobic metabolism may be used by fast-contracting fibres.

Some Properties of a Protease from *Bacillus polymyxa*

By P. J. GRIFFIN and W. M. FOGARTY. (*Department of Industrial Microbiology, University College, Dublin* 4, *Irish Republic*)

Preliminary investigations dealing with the elaboration of proteolytic and starch-degrading enzyme systems by *Bacillus polymyxa* N.C.I.B. 8158 and the influence of an acid-catalysed extract of peat on these processes have been described (Griffin & Fogarty, 1971*a*). The protease alone was secreted in high yield when arabinose was used as carbon source. The effects of different factors influencing the proteolytic and milk-clotting activity of the enzyme have been recorded (Griffin & Fogarty, 1971*b*).

The protein in the cell-free supernatant from a peat extract–arabinose medium was fractionated by precipitation with ammonium sulphate, dialysed and concentrated with acetone. Further purification was effected by gel filtration on Sephadex G-100. An overall 70-fold increase in specific activity was thus achieved.

Dialysis of the protease against 20mM-phosphate buffer, pH7.0, resulted in irreversible loss in activity. Addition of 1mM-calcium chloride to the phosphate buffer reversed this effect. Dialysis against 20mM-tris–HCl buffer, pH7.0, resulted in only a slight loss in activity. The ionic composition of the buffer used had a significant effect on enzyme activity, e.g. Britton and Robbinson's buffer, phosphate buffer maleate buffer, and citrate–phosphate buffer gave activities of 83, 85, 67.7 and 53% respectively, of that observed with tris–HCl buffer, pH7.0.

The enzyme showed highest activity at pH7.0 for casein and hide powder, 83% and 82% of the respective substrates being rendered soluble in 5% (w/v) trichloroacetic acid in 2h. The protease caused considerable breakdown of a variety of proteins.

There was complete inhibition of activity by 1mM-EDTA and by 1mM-*o*-phenanthroline, 66% inhibition by 2mM-$\alpha\alpha'$-bipyridyl, 7% inhibition by 5mM-sodium oxalate and 34% inhibition by 5mM-sodium citrate. The effects of a variety of other inhibitors on activity of the enzyme were also examined.

Dialysis of the enzyme preparation against 1mM-EDTA, pH7.0, for 3h and then against 20mM-tris–HCl buffer, pH7.0, for 12h, followed by addition of Mn^{2+} (final concentration 1mM), gave an increase of 56% in activity over the original non-dialysed material. Dialysis against 1mM-EDTA for 12h resulted in no activity on the addition of Mn^{2+}.

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Griffin, P. J. & Fogarty, W. M. (1971*a*). *J. gen. Microbiol.* (in the Press).

Griffin, P. J. & Fogarty, W. M. (1971*b*). *Proc. 1st Res. Conf. Food Science and Technology, Cork* (in the Press).

Extracellular Enzymes of a *Bacillus* sp. Associated with Increased Permeability in Sitka Spruce (*Picea sitchensis*)

By O. P. WARD and W. M. FOGARTY. (*Department of Industrial Microbiology, University College, Dublin* 4, *Irish Republic*)

Destruction of tori and bordered pit membranes in the sapwood of Sitka spruce (*Picea sitchensis*) during water storage has been shown to result in increase in permeability of the material to treatment with preservative (Dunleavy & Fogarty, 1971). Evidence indicates that destruction of the pit membranes may be caused by the action of bacterial extracellular enzymes. The importance of pectinolytic activity in the degradation of the pits has been suggested (Ward & Fogarty, 1971). Certain enzymes, e.g. cellulases, are known to have highly detrimental effects on the structural properties of wood. We now report the results of a study of the nutritional factors influencing the elaboration of extracellular enzymes by an organism associated with permeability increase in water-stored spruce.

The bacterium, a *Bacillus* sp., isolated from water-stored Sitka spruce (W. M. Fogarty, unpublished work), was grown at 27°C in liquid media