compared with that of the synthetic substances. Cellulose-trisulphuric acid and chitin disulphuric acid show a particularly high inhibition. In view of the chemical structure of hyaluronic acid, the inhibitory action of the substances considered is interesting. As suggested by McClean[3], they appear to compete with hyaluronic acid for the enzyme, thus blocking its action on hyaluronic acid. The reaction is another example of the competitive inhibition in biological systems such as is observed in the sulphonamide–*p*-aminobenzoic acid antagonism.

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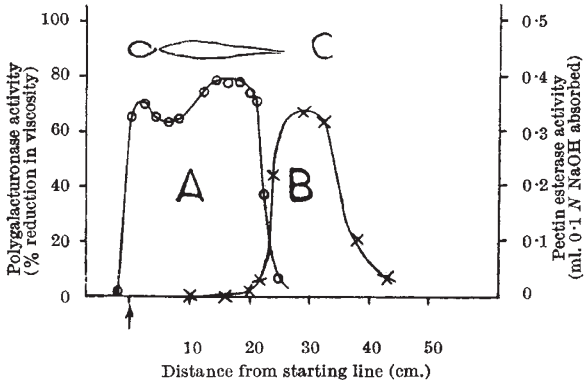
Estimation and Separation of the Pectin-Esterase and Polygalacturonase of Micro-fungi

VARIOUS methods for the estimation of pectin-esterase and polygalacturonase have been used[1,2]; but for rapid examination of cultures of micro-fungi, a modification of the 'cup-plate' diffusion method is useful. The polygalacturonase assay is carried out on a medium containing 1·5 per cent agar and 1 per cent sodium pectate at *p*H 5·0; and after incubation at 37° C. for 18 hr., the plates are sprayed with acid, when the enzyme activity is revealed as clear circular zones on an opalescent background. For the pectin-esterase assay, buffer-free agar containing 1 per cent purified pectin and methyl red adjusted to *p*H 6·0 is used, and the activity is indicated as red zones on a yellow background, due to the lowering of the *p*H by demethylation of the pectin. With each enzyme there is a linear relationship between the diameter of the zone and the logarithm of the enzyme concentration. Other enzymes (amylase, cellulase, xylanase, arabanase) may be determined similarly[3], and the activities calculated by the usual method[4,5].

Pectin-esterase and polygalacturonase have been separated by chromatography on sheets of Whatman No. 1 and No. 3 papers, using various concentrations of buffered salt solutions as developing agents. After development, the chromatograms are laid on slabs of the gel media described above to indicate the presence of pectin-esterase and polygalacturonase. Under suitable conditions the pectin-esterase travels more rapidly, and may be separated completely from the slower-moving polygalacturonase.

The area occupied by the enzyme on the chromatogram, and also the zone produced by diffusion into an agar substrate gel from the paper strip, is linearly related to the amount of enzyme applied to the paper. The activity may also be determined by dissecting duplicate strips and placing the pieces directly into tubes of the respective substrates, in which polygalacturonase is estimated by a viscometric method[4], and the pectin-esterase activity by titration[7]. This method is less sensitive than the agar-gel technique,



Paper chromatogram of a pectic enzyme preparation from a micro-fungus: (*A*) ○—○, zone of polygalacturonase activity; (*B*) ×—×—, zone of pectin-esterase activity; (*C*) tracing of polygalacturonase activity on pectic-acid – agar gel

owing to the possible overlap of zones when dissecting the paper (see graph, where the polygalacturonase activity shows two peaks), whereas on an agar-gel a duplicate strip gave clear evidence of two separate zones of activity.

With some preparations the amylase, pectin-esterase and the polygalacturonase may be resolved into two components. The pectin-esterases from different fungi differ in the total amount of de-esterification of pectin they can accomplish, and in their reaction velocities, which may be correlated with the fact that some preparations appear to have two components, and others only one.

With ammonium sulphate a gradient exists (determined by estimation in the dissected paper strip, or application of a strip to an agar-gel followed by treatment with barium chloride to indicate the concentration of sulphate ion), which *decreases* from the top of the paper down to the solvent front. Other factors, including *p*H and absorption on the cellulose, also operate.

The technique has been found useful for comparative studies on amylases and related enzymes. Recently[6], partial chromatographic separation of proteins and some enzymes has been reported, using a colorimetric technique for indicating all protein components. The two methods in combination should be useful for indicating inactive components, and overlapping enzyme activities.

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Note added in proof. With acetone–water mixtures as solvents, polygalacturonase and some amylases (β, bacterial, salivary, malt) may be resolved into two, and fungal amylase into four, components; duplicate chromatograms by the technique of Quastel and Franklin do not indicate this resolution.

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