PECTIN AND POLYGALACTURONATE *TRANS*-ELIMINASES IN *FUSARIUM MONILIFORME* AND *CEPHALOSPORIUM SACCHARI*

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FROM cane varieties CO 449 and CO 658, exhibiting wilt symptoms, *Fusarium moniliforme* Sheldon and *Cephalosporium sacchari* Butler were isolated. Extensive occurrence of macerated pith tissues with brown discolouration has been reported to be the typical internal symptom of the disease. Tissue maceration has been attributed to the activity of cell-wall dissolving enzymes[1,2] and both the pathogens are known to produce such enzymes.[3,4] Recently Sherwood[5] and Mahadevan and Chandramohan[6] showed that *trans*-eliminase types of pectic enzymes caused tissue maceration. The production of *trans*-eliminases by *F. moniliforme* and *C. sacchari* is reported in this note.

In 500 ml. Erlenmeyer flasks, 25 g. of wheat bran and 15 ml. water were mixed, sterilized, separately inoculated with the isolates and incubated at room temperature (28 ± 2° C.) for 6 days. Two drops of toluene and 200 ml. distilled water were added to the growth, allowed to autolyse for 12 hr., filtered through a cheese cloth, centrifuged at 3,000 rpm. for 15 min. and the clear supernatant was used as the enzyme.[7] The enzyme activity was determined by the loss in viscosity of sodium polypectate or pectin at pH 8·6 in an Ostwald-Fenske viscometer size 300 at 30° C. in a water-bath. The reaction mixture consisted of 4 ml. of 1·2% pectin or sodium polypectate at pH 8·6 in boric acid-borax buffer, 1 ml. of the buffer and 2 ml. of the filtrate at pH 8·6.[6] Maceration of potato medullary discs was determined by placing 5 potato discs of 1 mm. thickness, 9 mm. diameter in a sterile petri-dish containing 15 ml. of the filtrate at pH 8·6, 1 ml. of 4% sodium fluoride (against contaminants) and testing the coherence of discs by touching them with a glass rod at 4 hr. interval.[8]

The autolysed filtrates of *F. moniliforme* and *C. sacchari* reduced the viscosities of pectin and sodium polypectate and macerated potato discs within 24 hr. *Fusarium* produced more of the enzyme than *Cephalosporium*; the viscosity losses of sodium polypectate and pectin were respectively 35 and 27% with the former while in the latter, they were 29 and 22% at the end of 2 hr. When the enzyme substrate mixture was analysed for the presence of thiobarbituric acid (TBA) reacting substances (to 3 ml. of the clarified mixture, 10 ml. of 0·01 M thiobarbituric acid and 5 ml. of 0·5 N HCl were added, boiled in a boiling water-bath for 1 hr., cooled, the volume was made up and the percent transmittance was determined in a Beckman DU Spectrophotometer at a wavelength of 480–580 mμ), it was found that the maximum absorption was at 547 mμ, indicating that *trans*-eliminative split of pectic substances occurred. TBA reacting substances were also released by the enzyme preparation from potato cells. The *trans*-eliminative split of pectin and sodium polypectate by the culture filtrates was conclusively demonstrated on the basis of increased absorption maximum of the clarified reaction mixture at 232 mμ determined in a Beckman DU Spectrophotometer at a wavelength of 210–310 mμ[9] (the reaction mixture was clarified by precipitating the enzyme proteins with 3 ml. of 5% trichloro-acetic acid). Hence based upon subtsrate specificity, the release of TBA-reacting substances and increased absorption maximum of reaction mixture at 232 mμ, it may be concluded that both the fungi produced in the wheat bran medium pectin and polygalacturonate *trans*-eliminases.[10] Singh[3] showed that enzyme preparations of *F. moniliforme* and *C. sacchari* macerated potato discs especially in a pH range of 7·8–8·6. It is likely that *trans*-eliminases might have been involved in the various enzyme sources used by Singh.

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