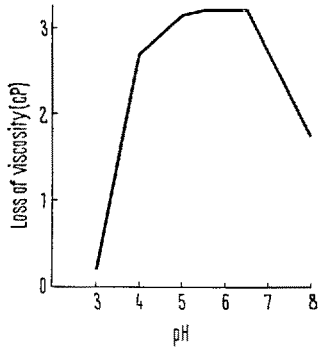
## Production of Pectolytic and Cellulolytic Enzymes by *Cercosporella herpotrichoides* Fron

The disease symptoms of eye spot disease of wheat caused by *Cercosporella herpotrichoides* are marked in advanced stages by a rotting of the stem basis of the plants. It may therefore be presumed that the fungus possesses cell wall degrading, especially pectolytic and cellulolytic enzymes. The results of recent histochemical investigations[1] apparently confirm this presumption. Our own experiments were, at first, carried out in order to examine whether the pathogen is capable of producing in vitro pectolytic and cellulolytic enzymes.

*Methods.* The fungus was cultured on a nutrient solution of the following composition: 5000 mg/l Citrus pectin, 5000 mg/l Maltzin, 3000 mg/l $NaNO_3$, 500 mg/l $MgSO_4 \cdot 7\,H_2O$, 500 mg/l KCl, 10 mg/l $FeSO_4 \cdot 7\,H_2O$, 1000 mg/l Yeast extract (DIFCO), dissolved in $0.2M$ phosphate buffer, pH 5.4. In some experiments also carboxymethylcellulose (CMC) and/or glucose were used as carbohydrate sources. The medium was distributed in 300 ml Erlenmeyer flasks in portions of 100 ml each, sterilized at 120 °C and inoculated with agar discs (9 mm diam.) carrying the fungus. Incubation was performed at 19–22 °C as still culture.

Subsequently, the culture filtrates were tested for the presence of enzyme activity using the following methods: Polygalacturonase (PG): Viscosimetrically at pH 4.5 with pectin or sodium polypectate as a substrate. Pectin methylesterase (PME): Titration with NaOH of carboxyl groups produced from pectin. Pectin lyase (PL): Determination of changes in absorption at 230 nm or thiobarbituric acid method with subsequent measurement of absorption at 550 nm. Cellulase (Cx): Viscosimetrically at pH 6.0 with carboxymethylcellulose as a substrate. Heat-inactivated culture filtrates served as controls. As to further details, an earlier publication[2] should be consulted.

| Weeks | Polygalacturonase activity | | |
|---|---|---|---|
| | Loss of viscosity of a pectin solution (cP) | (pH) of culture filtrate | Dry weight of mycelium (mg) |
| 0 | – | 5.40 | – |
| 2 | 2.50 | 5.30 | 175 |
| 4 | 3.45 | 5.65 | 408 |
| 6 | 3.30 | 5.70 | 449 |
| 8 | 3.45 | 5.75 | 443 |
| 10 | 3.40 | 5.85 | 465 |
| 12 | 3.45 | 5.90 | 477 |




Degradation of a CMC solution as a function of pH (Loss of viscosity of a 0.8% solution of CMC during 30 min).

For determination of heat stability, the culture filtrates were kept in test tubes at different temperatures in a water bath for 30 min, then rapidly cooled to room temperature and tested for enzyme activity.

*Results.* Already after incubation for 2 weeks, a notable polygalacturonase activity could be detected. The fact that it was greatest in nutrient solutions containing pectin as a component, permits the conclusion that this pectin-splitting enzyme is largely formed adaptively. The same is true for pectin methylesterase and pectin lyase as was demonstrated in later experiments. During 12 weeks of incubation the activity of polygalacturonase, pH value and dry weight of mycelium developed as described in the Table. The data in the Table reveal that the PG activity under these conditions increases only until the 4th week and does not change significantly thereafter. The same applies to the dry weight of mycelium. The pH of the buffered medium shows only minor changes; later, however, it increases slightly. The pH optimum for activity of PG is in the range of pH 4.0 to 5.0. This enzyme is very sensitive to high temperatures. Keeping the culture filtrate at 50 °C for 30 min results in an almost complete loss of pectolytic activity.

In the culture filtrates a pectin methylesterase can also be demonstrated, the pH optimum of which lies around pH 7.5. Additions of NaCl increase the activity with an optimal concentration at about $0.085M$. Keeping the culture filtrate at 50 °C for 30 min reduces considerably the PME activity.

A further pectin-splitting enzyme, pectin lyase, is present in the culture filtrates. Its pH optimum was determined at pH 8.0. The activity of this enzyme is stimulated by addition of Ca; $10^{-3}M$ $CaCl_2$ proved to be most favourable. $MgCl_2$ and $SrCl_2$ also had a slight stimulating effect on the activity. On exposure to higher temperatures the PL reacted as sensitively as the enzymes mentioned before.

Furthermore, a Cx activity of the culture filtrates can be proved. This activity is not specifically dependent on the added carbohydrate source, but develops more or less parallel to the growth of mycelium. Optimum activity lies at pH 6 and thus corresponds to the optimum of other Cx cellulases of fungal origin (Figure).

The heat sensitivity of this cellulase is lower than that of the pectic enzymes. Even a temperature of 90 °C does not completely destroy the cellulolytic activity during 30 min.[3]

*Zusammenfassung.* In Kulturfiltraten von *Cercosporella herpotrichoides*, dem Erreger der Halmbruchkrankheit des Weizens, wurden folgende Enzyme nachgewiesen: Polygalacturonase, Pektinmethylesterase, Pektin-Lyase und Cellulase. Es wird vermutet, dass diese Enzyme, die in einigen Eigenschaften kurz charakterisiert werden, für die Pathogenese von Bedeutung sind.

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[1] L. DEFOSSE, Phytopath. Z. *70*, 1 (1971).
[2] F. GROSSMANN, Phytopath. Z. *62*, 371 (1968).
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