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Glucose Inhibition of Cellulose Synthesis by Pyrenochaeta terrestris

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on cellulose and pectin and the presence of cellulase and pectinase in infected roots strongly suggests activity of these enzymes in pathogenesis.

Conclusion

Cellulase and pectinase enzyme systems are produced by *P. terrestris*. Mycelial growth on cellulose and cellulase synthesis did not vary greatly at temperatures from 15° to 30°C. Mycelial growth on pectin and pectinase production exhibited maximum response at 20°C. Growth on pectin equalled that on glucose and exceeded by many times that on cellulose. The data suggest pectinase may be of major importance in pathogenic behavior, while cellulase activity may play only a minor role. However, cellulase may be very important in saprophytic survival on plant debris in the soil. Attempts to correlate enzyme activity in infected roots with varietal response were not successful. A number of isolates of the fungus synthesized either enzyme equally well and other variations that were observed did not correspond to differences in pathogenicity.

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Glucose Inhibition of Cellulase Synthesis by *Pyrenochaeta terrestris*[1]

J. C. Horton and N. T. Keen[2]

Abstract. The rate of synthesis of *Pyrenochaeta terrestris* cellulase was determined on the substrates of glucose, cellulose, and cellulose + glucose. Enzyme production was rapid on cellulose, almost negligible on glucose, and intermediate on cellulose + glucose. On the latter substrate, cellulase appeared after the near exhaustion of glucose. The authors suggest that extremely low concentrations of soluble carbohydrate regulate cellulase synthesis.

Cellulase is an extracellular inducible enzyme found in many fungi (1, 2). Cellulase synthesis typically occurs when fungi are grown on cellulose. Cellobiose, a β-1, 4 dimer of glucose, is also claimed to function as an inducer (3). Glucose, the end product of cellulose hydrolysis, does not induce cellulase syn-

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thesis and even suppresses induction of cellulase (4, 5). Glucose inhibition of cellulase synthesis in *Pyrenochaeta terrestris* is the subject of this investigation.

Materials and Methods

The culture of *P. terrestris* used (Pt 8) was isolated from material collected at Clear Lake, Iowa. The organism was grown in standing culture in 250 ml erlenmeyer flasks with 50 ml of a modified Czapek's mineral solution per flask.[3] Carbon sources used were: cellulose[4] 1 g/1 (C), C.P. glucose 0.9 g/1 (G), and cellulose 1 g/1 + C.P. glucose 0.9 g/1 (C + G). Sterile flasks were seeded with 4 mm disks cut from the edge of fungus colonies grown on potato dextrose agar (Difco). They were incubated at room temperature. Sufficient flasks were prepared to make enzyme determinations in triplicate at two day intervals during the study. The contents of flasks were filtered through a coarse (C) porosity sintered glass funnel. Remnant cellulose and hyphae were discarded, and the culture filtrates were retained for cellulase analyses and glucose determinations. Estimates of cumulative cellulase synthesis were made by the carboxymethylcellulose/viscosity assay technique previously described (6). Glucose concentrations were determined enzymatically using the Glucostat method.[5] The lowest concentration of glucose detected by this method was approximately 0.07 mg/ml or 0.0004 molar.

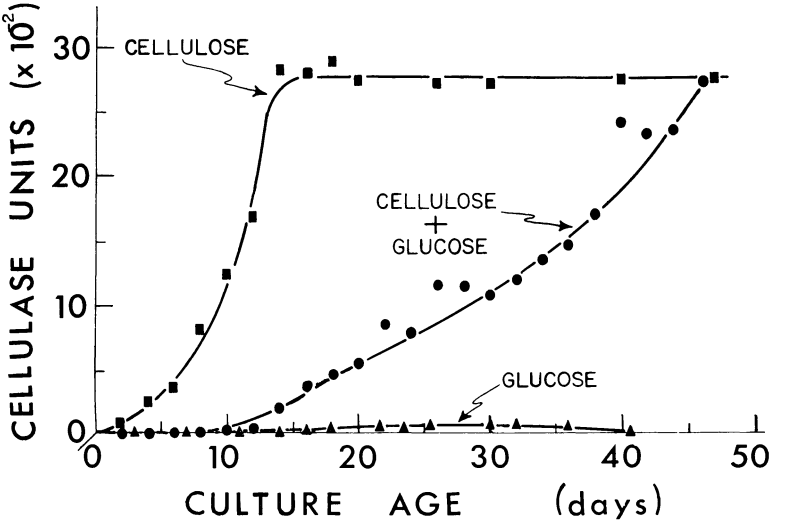

Figure 1. Cellulase synthesis by *P. terrestris* on various carbon sources.

[3] The following in 1.0 liter of solution: $NaNO_3$ 4.25 g, KCl 0.5 g, NaH_2PO_4 1.4 g, $MgSO_4$ 0.23 g, $FeSO_4$ 0.01 g.
[4] Alpha-Cel, sold by the Nutritional Biochemicals Corp., Cleveland, Ohio.
[5] Glucostat Enzymatic Glucose Reagent, sold by the Worthington Biochemical Corp., Freehold, New Jersey.

Results and Discussion

Production of cellulase was compared with culture age (Figure 1). Consistently low levels of enzyme activity were detected in flasks containing only glucose. When cellulose was the sole carbon source, cumulative cellulase activity rapidly reached a maximum in 15 days. Cellulase activity on cellulose + glucose reached the same maximum, but only after 46 days.

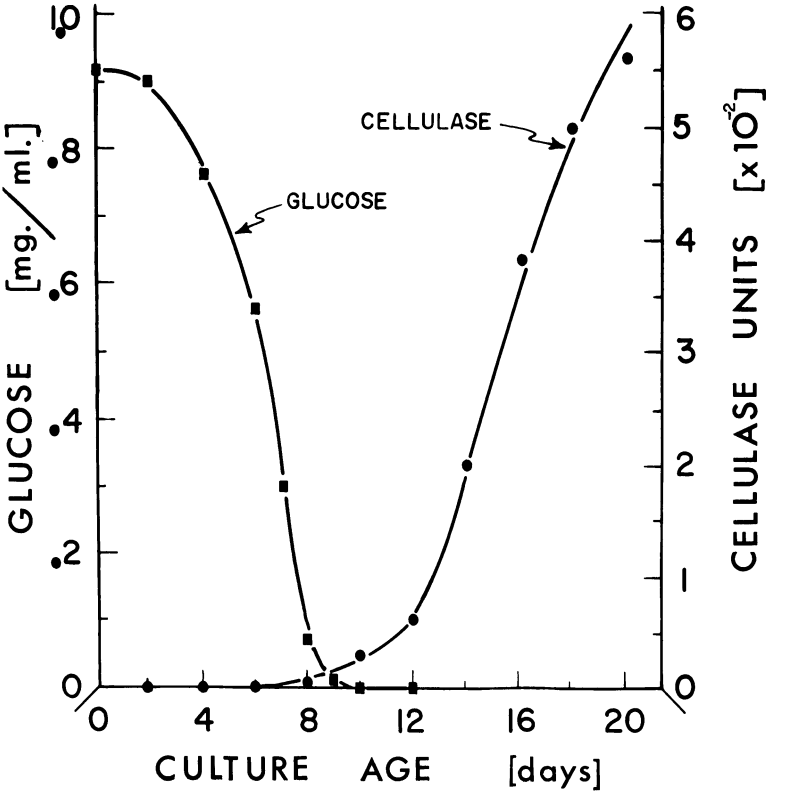

Figure 2. Glucose utilization and cellulase synthesis by *P. terrestris*.

Cellulase concentrations were related to detectable levels of glucose in cultures where the substrate was C + G (Figure 2). Enzyme synthesis on this substrate was detected only when glucose neared depletion. The curve for cellulase production on C + G was expected to be parallel but displaced in time with respect to the curve for synthesis on cellulose. The skewed C + G curve (Figure 1) suggests an inhibitory effect continuing after supplemental glucose was no longer detected. Were glucose completely exhausted, the C + G curve should parallel the C curve. The skewing indicates that glucose concentration was not

reduced to the level presumably present in the cellulose cultures until after 46 days. The prolonged effect of glucose at levels below those detectable suggests that glucose inhibits enzyme synthesis at high concentrations and regulates it at low concentrations. We postulate that the level of soluble carbon compounds such as glucose may regulate microbial cellulase synthesis on cellulose. As soluble carbon sources are depleted, cellulase is synthesized until an equilibrium becomes established between synthesis and inhibition of synthesis by the products of hydrolysis. The establishment of a static, maximum enzyme level in the cellulose cultures exemplifies this situation. The cellulose source presumably contains only low levels of soluble carbohydrates. Thus, little inhibition of synthesis is exercised and cellulase synthesis proceeds rapidly. As enzyme concentration increases, more soluble hydrolysis products become available to the fungus and inhibitory effects eventually create an equilibrium between synthesis and enzyme degradation.

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Androhermaphrodites of *Lychnis Alba*

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Abstract. *Lychnis alba* was found to produce four categories of flowers; i.e. pistillate, staminate, gynohermaphrodite and androhermaphrodite. Hermaphrodites are formed when a rudimentary pistil (pistillodium) or stamens (staminodea) develop into mature organs on the ovaries of otherwise pistillate or staminate flowers. Androhermaphrodites, in particular, were studied and their structure and behavior found to be essentially similar to corresponding parts of regular staminate and pistillate flowers. Ovaries of androhermaphrodite flowers exhibited variations in style number ranging from one to five. Ovaries with two to five styles were self- or cross-fertile with any good *Lychnis alba* pollen. One-styled ovaries of androhermaphrodite flowers were of unusual interest because they occurred so commonly, showed a range of development from a pistillodium to a mature ovary, and because they were characteristically self- and cross-sterile.

Lychnis alba Mill. (Caryophyllaceae) is dioecious with definite pistillate flowers on one plant and definite staminate flowers on a separate one. However, it appears that the dioecious habit is

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