INDUCTION OF CELLULASE IN *TRICHODERMA VIRIDE* AS INFLUENCED BY CARBON SOURCES AND METALS

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Cellulase is an adaptive enzyme in most fungi (Reese and Levinson, 1952), although it is constitutive in cellulolytic bacteria (Hammerstrom *et al.*, 1955). Many polysaccharases are adaptive in fungi, including: pentosanase (Simpson, 1954), polygalacturonase (Phaff, 1947), chitinase (Reynolds, 1954), dextranase (Hultin and Nordström, 1949), and xylanase and mannanase (Sørensen, 1952). Since many of these substrates are insoluble, the question arises as to how an insoluble substrate can induce the formation of an extracellular enzyme.

Products of polysaccharide hydrolysis can often induce their respective polysaccharases: galacturonic acid for polygalacturonase in *Penicillium chrysogenum* (Phaff, 1947); xylose for pentosanase in several molds (Simpson, 1954); maltose for amylase in *Aspergillus niger* (Tanabe and Tonomura, 1953); *N*-acetylglucosamine for chitinase in *Aspergillus fumigatus* and *Myrothecium verrucaria* (Reese, unpublished data). The use of the product as an inducer often leads to lower enzyme yields than are obtained with the substrate. In most cellulolytic fungi tested, however, neither glucose nor cellobiose acted as inducers of cellulase (Reese and Levinson, 1952).

Further studies relating to this problem showed that, under certain conditions, some sugars can induce cellulase formation in *Trichoderma viride*. The present study is a reinvestigation of the induction of cellulase. For comparative purposes, some data on amylase production are also included.

METHODS

Trichoderma viride strain QM 6a was grown in liquid culture, 50 ml per 250-ml Erlenmeyer flask, and incubated at 29 C on a reciprocal shaker. Inoculum was 1 ml of a spore suspension (2 to 4×10^6 spores) prepared by suspending spores from potato dextrose agar slants in distilled water.

The culture medium in g/L was $(\text{NH}_4)_2\text{SO}_4$,

1.4 g; urea, 0.3 g; KH_2PO_4 , 2.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g; CaCl_2 , 0.3 g. The pH was adjusted to 5.3 with NaOH. Trace elements were added as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, ZnCl_2 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $20\text{MoO}_3 \cdot 2\text{H}_3\text{PO}_4 \cdot 48\text{H}_2\text{O}$. Unless otherwise indicated, the concentration of trace elements added for cellulase production was Fe, 1.0; Zn, 0.8; Mn, 0.5; and Co, 0.5 ppm. In some earlier experiments, yeast extract (Difco) was added at 0.1 g/L.

The carbon source was 0.5 per cent glucose unless otherwise noted. Sugars were usually autoclaved separately and added to the medium when cool. However, the results are no different when glucose is autoclaved with the medium. Other carbon sources included "solka floe" wood cellulose (SW40A, Brown Co.); methocel (degree of substitution 1.8, Dow Chemical Co.); carboxymethylcellulose (CMC 50T, degree of substitution 0.5, Hercules Powder Co.); sodium cellulose sulfate (degree of substitution 0.4, Eastman Kodak Co.); and dextran (synthesized by *Leuconostoc mesenteroides*, Northern Regional Research Laboratory). Other celluloses were prepared by us: the degraded cotton by treatment of cotton sliver with 85 per cent phosphoric acid after the method of Walseth (1952); the acetobacter cellulose from *Acetobacter acetigenum* strain QM B1562 (obtained from T. K. Walker); the Saprolegnia cellulose from a culture (QM 1881) supplied by Mr. Chris Martin of Harvard; the tunicate cellulose from *Phallusia mammilata*. Other chemicals were of reagent grade.

Cultures were harvested in duplicate at various times over a 2-week period. The mycelium was washed and dried at 80 C for weight measurements. Other determinations were made on the cell-free culture filtrate. Reducing sugar was determined as glucose by the dinitrosalicylic acid method of Sumner and Somers (1944). Total carbohydrate was determined as glucose by the orcinol method of Rimington (1941).

Protein was determined by the Folin method of Lowry *et al.* (1951) after precipitation with 5 per cent trichloroacetic acid. Bovine plasma albumin was used to prepare the standard curve from which the values were obtained.

Cellulase activity was determined by dilution as "cellulase units" by the method of Levinson and Reese (1950). The cellulase unit is that amount of enzyme which, in 10 ml of assay medium (0.5 per cent carboxymethyl cellulose 50T in 0.05 M citrate at pH 5.4), produces 4.0 mg of reducing sugar (as glucose) in 1 hr at 50 C.

Amylase activity was determined similarly by the release of reducing sugar from 0.15 per cent soluble starch. The amylase unit is that amount of enzyme which, in 10 ml of assay medium, releases 4.0 mg of reducing sugar (as glucose) in 1 hr at 50 C, pH 5.4.

RESULTS

Mineral requirements for cellulase production. The mineral composition of the medium had a marked effect on the production of cellulase when 0.5 per cent glucose was used as the carbon source. Varying the levels of magnesium and

calcium affected both cellulase production and sugar consumption (table 1). In the absence of magnesium, growth is delayed (as shown by slow utilization of sugar) and cellulase production is prevented. In the presence of 0.003 per cent $MgSO_4$, good growth with slight cellulase production takes place. $MgSO_4$ at 0.03 per cent also results in good growth, but cellulase production is even poorer than on 0.003 per cent $MgSO_4$. Calcium cannot be substituted for magnesium in the growth function. Supplementation of magnesium with 0.003 per cent $CaCl_2$, however, greatly increases cellulase production, and supplementation with 0.03 per cent $CaCl_2$ improves it still further. The data suggest that calcium may act in part to counteract some inhibitory effect of magnesium. Strontium can partially replace calcium (table 1). Barium has no effect.

The effect of varying calcium chloride concentration was tested in further detail in the presence of 0.03 per cent magnesium sulfate (figure 1). Cellulase activity was slight when no $CaCl_2$ was added. The addition of 0.001 per cent $CaCl_2$ (3.6 ppm Ca) caused a marked increase in cellulase activity. The activity continued to increase to a maximum value at 0.02 per cent (72 ppm Ca) and then decreased. Growth was uniform over this range of calcium chloride concentration. Sugar was consumed in all cultures at 2 days, indicating little effect of calcium concentration on the growth rate.

Trichoderma viride grows well when no yeast extract, calcium, or trace metals are added to our medium. Trace elements required for growth of this fungus are apparently supplied in adequate amounts in the inoculum and as impurities in the

TABLE 1
Effect of Ca, Mg, Sr on sugar consumption and cellulase production on 0.5% glucose

Additions			Sugar Remaining		Max Cellulase Production
$MgSO_4$	$CaCl_2$	$SrCl_2$	2 days	4 days	
%	%	%	mg/ml	mg/ml	units/ml
0.0	0.0	0.0	3.7	2.1	0.0
0.0	0.003	0.0	3.9	1.9	0.0
0.0	0.03	0.0	3.5	1.3	0.0
0.0	0.0	0.03	4.0	2.1	0.0
0.003	0.0	0.0	0.3	0.0	0.7
0.003	0.003	0.0	0.0	0.0	2.0
0.003	0.03	0.0	0.0	0.0	3.6
0.003	0.0	0.03	0.0	0.0	1.2
0.03	0.0	0.0	0.0	0.0	0.2
0.03	0.003	0.0	0.0	0.0	1.0
0.03	0.03	0.0	0.0	0.0	3.2
0.03	0.0	0.03	0.0	0.0	0.9

Yeast extract, 0.01%.
Trace elements: Fe, 1.0; Zn, 0.8; Mn, 0.5; Cu, 0.1; Co, 0.025; Mo, 0.025 ppm.
 $CaCl_2$ and $MgSO_4$ omitted from basal medium.
Sugar was completely consumed in all cultures at 11 days.

Figure 1. Effect of Ca concentration on cellulase (Cx) production on 0.5% glucose. Trace elements added were Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm.

sugar and nutrient salts used. However, for cellulase production not only calcium but certain trace metals must be added to the medium (table 2). The presence or absence of calcium or trace elements had little effect on the growth of the organism as measured by maximal weight. In separate experiments, it was found that the addition of Fe, Mn, or Zn at twenty times the above concentrations did not cause decreased growth, thus showing that the elements were acting at levels above those required for maximum growth, but well below toxic levels. The trace elements had a marked effect, however, on cellulase yields. Best yields were obtained in the presence of Fe, Mn, Zn, and Co. Omission of Zn reduced the yield; omission of any one of the other three had little effect. Further experiments showed that any combination of Fe or Mn with Zn or Co gave a good cellulase yield. Cobalt was the only trace element active alone. Study of the effect of cobalt concentration on cellulase production and growth (figure 2) showed that the cellulase yield increased as the cobalt was increased up to 10 ppm and then declined as cobalt was further increased to 100 ppm. Cellulase did not appear in these cultures until the third day (fourth day for 50 and 100 ppm). Growth was not affected by cobalt concentration up to 0.5 ppm. From 1.0 to 10 ppm, maximum weight was slightly reduced and growth rate was decreased, i. e., the sugar was consumed more slowly and peak weight was reached at 3 days instead of at 2. At 50 and 100 ppm, peak weight was not reached until 4 days after inoculation. In a control with full trace elements (Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm), cellulase appeared at 2 days and maximum yield was 4.7 units per ml.

Essentially no cellulase is produced by *T. viride* on cellobiose in the absence of calcium and trace elements. On lactose, some cellulase is produced in the absence of calcium and trace elements, but the cellulase yield is much increased in their presence. On cellulose sulfate, neither growth nor cellulase production occurs in the absence of either calcium or trace elements, while, in their presence, growth occurs and cellulase is found in the culture filtrate. On CMC and on celluloses such as solka floc, growth and cellulase production occur when calcium and minor elements are omitted from the medium.

These data suggested that the cellulose might

TABLE 2

Effect of trace elements and calcium on growth and cellulase production on 0.5% glucose

Calcium	Additions				Max Wt	Max Cellulase Production
	Trace elements				mg/ml	units/ml
—	—	—	—	—	1.7	0
—	Fe	Mn	Co	Zn	1.9	0.3
+	—	—	—	—	2.4	0
+	Fe	Mn	Co	Zn	1.9	4.5
+	Mn	Co	Zn	(—Fe)	1.9	4.0
+	Fe	Mn	Co	(—Zn)	2.2	1.5
+	Fe	Co	Zn	(—Mn)	2.0	4.5
+	Fe	Mn	Zn	(—Co)	2.0	3.8
+	Fe	—	—	—	2.3	0.1
+	Zn	—	—	—	2.1	0.1
+	Mn	—	—	—	2.3	0
+	Co	—	—	—	2.3	2.0

Calcium, CaCl_2 0.03%. Trace elements, Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm.

Figure 2. Effect of Co on cellulase (Cx) production on 0.5% glucose in the absence of other trace elements. CaCl_2 was present at 0.03%.

contain in itself the required minerals. A sample of solka floc was therefore ashed (600 C) and the ash taken up in HCl and added to various modifications of the glucose medium in amount equivalent to 0.4 per cent cellulose (table 3). The addition of this ash to the glucose medium lacking in calcium and trace elements resulted in a cellulase yield of 2.0 units per ml. Ash alone or with Ca or trace elements, however, did not equal the combination of Ca plus trace elements, which gave a cellulase yield of 4.5 units/ml.

The data suggest that the mineral requirements for cellulase production are the same on glucose,

TABLE 3

Effect of cellulose (solka floc) ash on growth and cellulase production from 0.5% glucose

Additions			Max Wt	Max Cellulase Production
Ca	Trace elements	Ash		
—	—	—	mg/ml	units/ml
—	—	—	1.7	0
—	—	+	2.1	2.5
+	—	—	2.6	0.3
+	—	+	2.2	2.4
—	+	—	1.9	1.1
—	+	+	2.1	2.5
+	+	—	2.2	4.8
+	+	+	2.0	4.8

Calcium, CaCl_2 0.03%.

Trace elements: Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm.

Ash equivalent to 0.4% solka floc.

cellobiose, lactose, or cellulose, but that the impurities in cellulose can to a large degree supply these needs.

Carbon sources for cellulase production. 1. Cellulase is an adaptive enzyme in *T. viride*: *T. viride* was grown on 62 compounds at 0.5 per cent as sole carbon source. Cellulase was produced only on glucose, cellobiose, lactose and cellulose (table 4). Cellobiose, an important product of cellulose breakdown, is a poor inducer. Glucose, which can also result directly from the action of *T. viride* cellulase (Reese, 1956), gives somewhat better yields. Lactose is an excellent inducer, equal to cellulose itself. Lactose resembles cellobiose very closely, differing only in the configuration around the number 4 carbon of the glycosidic unit. Oxidation of the reducing group of lactose (or of glucose or cellobiose) gives a product having no inducing ability.

Celluloses of various origins (plant and animal) were all consumed, but the yields of cellulase varied considerably (table 4). Degrading cotton by ball milling increased the amount of enzyme produced, but degradation with 85 per cent phosphoric acid (Walseth) led to decreased yields. *T. viride* grew on substituted celluloses of low degrees of substitution and produced cellulase, but the mycelial weights were rather low. Other experiments show that methocel (1.8 degrees of substitution) is not hydrolyzed by cellulase, does not support growth, nor does its presence

TABLE 4

Growth and cellulase production on various substrates at 0.5%

Substrate (insoluble)*	Max Cellulase Production	Substrate (soluble)	Max Cellulase Production	Max Wt
	units/ml		units/ml	mg/ml
Cotton (ball milled)	30	α -Lactose	22	2.2
Cotton (40 mesh)	21	β -Lactose	21	2.3
Partially de-graded cotton (Walseth)	8	Glucose	4	2.4
Wood cellulose (solka floc)	29	Cellobiose	1	2.2
Fungus cellulose (Saprolegnia)	4	Cellulose- SO_4	3	0.8
Bacterial cellulose (Acetobacter)	5	Carboxy methyl cellulose 0.5 degrees of substitution	4	0.4
Animal cellulose (tunicate)	7	Pectic acid	0.3	0.1

* All these insoluble substrates were degraded by *Trichoderma viride*. Residue weights ranged from 0.5 mg/ml (Walseth cotton) to 3.5 mg/ml (40 mesh cotton).

Calcium, CaCl_2 0.03%.

Trace elements, Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm.

in glycerol medium lead to cellulase production. A comparison of the relative inducing efficiencies of the various celluloses is difficult because of differences in the surface areas, degrees of swelling (hydration) and other factors which influence not only the availability of the substrate to the organism, but adsorption of the cellulase from the culture filtrate.

No cellulase was induced on the following compounds. Maximum mycelial weight in mg/ml is shown in parenthesis after the compound. These maximum weights were reached in from 2 to 21 days after inoculation. Pentoses: ribose (2.1), L-arabinose (2.0), D-arabinose (1.9), xylose (1.9), lyxose (1.6). Hexoses: fructose (2.3), galactose (2.1), mannose (2.1), fucose (1.4), rhamnose (1.0), D-sorbose (0.2), L-sorbose (0.2). Disaccharides: trehalose (2.2), maltose (2.2), melibiose (2.2), turanose (1.4), sucrose (0.2). Trisaccharides

raffinose (0.9), melezitose (0.2). Alcohols: mannitol (2.5), glycerol (2.1), inositol (1.4), sorbitol (1.2). Sugar acids: Ca cellobionate (2.6), Ca arabonate (2.2), Ca gluconate (1.4), gluconolactone (δ) (1.1), galacturonic acid (0.9), Ca lactobionate (0.9), glucuronolactone (0.2), Ca saccharate (0). Glucosides: β -methyl glucoside (1.6), salicin (1.1), α -methyl glucoside (0.3). Substituted sugars: *N*-acetyl glucosamine (1.9), glucosamine-HCl (1.2), glucose-1-PO₄ (0.7), cellobiose octa acetate (0). Polymeric carbohydrates: starch (1.9), glycogen (1.8), dextran (0.2), xylan (0), chitin (0). Proteins: Na caseinate (1.0), wool (slight). Misc.: ascorbic acid (0.4), Na stearate (slight).

Xylan can be used under certain conditions by *T. viride*, but cellulase is not produced with it as substrate.

Amylase was produced on almost every compound that supported growth. Yields, however, varied widely. Best yields in units per ml were on: turanose (7); glycogen and glucose-1-PO₄ (6); starch and melibiose (5); maltose (4); *Saprolegnia* cellulose, and cellulose-SO₄ (3); cellobiose, CMC 50T, ball-milled cotton, β -methyl glucoside, salicin, and cotton sliver (2); mannose, galactose, lactose, dextrose, fructose, trehalose, raffinose, glycerin, mannitol, solka floc, and *Acetobacter* cellulose (1).

2. Growth and enzyme production on glycerol, dextrose, and cellulose (figures 3 and 4): The course of growth, pH, development of enzymes, protein in the filtrate and carbohydrate level were followed on 0.5 per cent glucose, 0.4 per cent solka floc + 0.1 per cent glycerol, and 0.5 per cent glycerol. The three substrates were used with and without calcium and trace elements. The results of experiments with trace elements are given in figure 3.

T. viride grows rapidly on 0.5 per cent glucose, consuming all of the sugar and reaching a maximum weight in 2 days. The weight decreases rather rapidly thereafter. Because of this decline, comparison of maximum weights in cultures harvested daily must be interpreted with caution. The pH falls as the sugar is consumed and then rises as the weight decreases. Growth on glycerol follows a similar pattern, although peak weight is reached a day later than in glucose cultures.

On cellulose + glycerol, the glycerol is quickly consumed and the cellulose is the principal growth substrate. In the absence of glycerol, there is a

lag of several days before growth begins on solka floc. Since mycelial weights could not be obtained, growth is indicated by the decrease in weight representing degradation and consumption of the cellulose. The pH also falls during growth on cellulose, but the subsequent rise in pH is much slower than on glucose or glycerol.

Cellulase appears in glucose cultures at about the time that weight is at the peak and pH is at its lowest point. Although not apparent in figure 3, cellulase never appears until the glucose is all consumed. We have not been able to detect cellulase in the mold mycelium prior to its appearance in the medium. The enzyme appears all at once and does not increase or decrease on longer incubation. Cellulase was produced only in traces unless both calcium and trace elements were present.

No cellulase was produced in glycerol cultures, regardless of addition of calcium or trace elements.

On cellulose, cellulase increases rapidly during the initial stages of growth and then more slowly until the fourteenth day. Cellulase yield is much higher than on dextrose.

Amylase was produced in all cultures, regardless of additions of calcium or of trace elements. The presence of calcium reduces amylase production in glucose and in glycerol cultures. On cellulose, amylase appears later than cellulase and may be more closely connected with autolysis. The addition of trace elements has little effect on amylase production.

The release of protein into the medium during growth is closely correlated with the appearance of cellulase, the activity being ca. 50 cellulase units per mg of protein. Similar data were obtained by Miller and Blum (1956) for *Myrothecium verrucaria*. Appreciable protein was found only in filtrates containing cellulase (figure 4). No such relation is found for amylase. The points in figure 4 represent cultures growing on glucose and on cellulose plus glycerol, 2 to 14 days after inoculation. Several points at or near the origin are omitted.

The carbohydrate released into the medium is chiefly nonreducing in nature and seems to have no relationship to the appearance or yields of cellulase or of amylase.

3. The effect of gradual addition of glucose: The thought that glucose may be the true inducer in cellulose-grown cultures, and that the high

Figure 3. Development of *Trichoderma viride* on glucose (0.5%), on glycerol (0.5%), and on cellulose (0.4% solka floc + 0.1% glycerol). Trace elements added were Fe 1.0, Zn 0.8, Co 0.025 ppm. Ca = 0.03% CaCl_2 . +Ca. x—x, -Ca o---o.

yields on cellulose could be due to slow release of sugar over a considerable time, suggested the addition of glucose in small daily amounts. In all cases, the daily increment method gave

much lower cellulase yields than the method of adding the entire amount at the beginning of the incubation period. For example, four daily increments of 0.1 per cent glucose gave a maxi-

Figure 4. Relationships between amylase, cellulase (Cx) and protein in the culture filtrate during growth on cellulose (0.4% solka floc + 0.1% glycerol) and on glucose (0.5%). x, +Ca (0.03% CaCl_2) + trace elements (Fe 1.0, Zn 0.8, Co 0.025 ppm.); ●, -Ca + trace elements; △ +Ca - trace elements; □ -Ca - trace elements.

Figure 5. Effect of glucose (D) and lactose (L) on growth and production of cellulase (Cx) after 7 days. Trace elements added were Fe 1.0, Zn 0.8, Mn 0.5, Co 0.5 ppm.

mum cellulase value of 0.3 units, while 0.4 per cent glucose at zero time gave a maximum cellulase value of 2.5 units.

4. Comparison of glucose and lactose as inducers: Growth on glucose and on lactose up to 1.0 per cent concentration is essentially equal (figure 5), but lactose is a much better inducer of cellulase. On lactose, cellulase yield increases as sugar concentration rises to 0.75 per cent and then levels off. Other tests show that cellulase appears in lactose (and cellulose) cultures, while the substrate is still present and continues to increase for some time. Glucose and galactose are not detectable in lactose cultures by chromatograms, nor does reducing sugar accumulate in cellulose cultures.

No cellulase is induced on 0.1 per cent glucose; maximum cellulase production increases as concentration rises to 0.5 per cent and then declines. Cellulase does not appear in glucose cultures until the sugar is all consumed. The lag between the disappearance of the sugar and the appearance of cellulase increases from a few hours at 0.5 per cent to several days at 1 per cent glucose. If glucose cultures are continued for a second week, cellulase sometimes increases in the 1 per cent medium to as much as 12 units. By the third week, traces of cellulase appear in 2 per cent glucose cultures.

DISCUSSION

Our data show that cellulase is formed when *Trichoderma viride* is grown on cellulose, lactose, glucose, or cellobiose. Cellulose and lactose can be assumed to be inducers of cellulase in the usual connotation of the term.

Glucose does not appear to be an inducer of cellulase for the following reasons. A rather high initial glucose concentration is required for production of cellulase, but cellulase does not appear until the glucose has been removed from the medium. Several compounds which are probably metabolized through glucose, such as starch, maltose, trehalose, and β -methyl glucoside, support excellent growth but do not induce cellulase. It is possible that glucose is metabolized to an inducer, possibly a β -glycoside.

The importance of the metals to cellulase induction may account for our previous inability to obtain cellulase when growing *T. viride* on glucose. We have been unable to satisfactorily explain the role of metals in cellulase production. In the succeeding paragraphs, we shall attempt

to relate our observations to those described for similar systems.

The lag in cellulase appearance after depletion of glucose increases as the initial glucose concentration increases. At concentrations of glucose above 1 per cent, the yield of cellulase is decreased. A similar inhibitory effect of glucose on amylase production is found in *Aspergillus niger* (Tanabe *et al.*, 1954). It is possible that products of glucose metabolism may combine with metals, making them unavailable to the fungus. Upon consumption of these products the metals would be released.

We have no evidence that the minerals required for cellulase production activate cellulase itself. It has been claimed that manganese stimulates cellulase activity (Fukumoto and Kishi, 1952; Toyama, 1956), but the data we have seen are not convincing. Otherwise we know of no claim that cellulase requires any metal ions for its activity. No prosthetic group or co-enzyme has been demonstrated as yet.

The balance between different ions may be more important than their individual concentrations. Magnesium is required for cellulase production, but as its concentration is increased it appears to inhibit. This inhibition is counteracted by calcium.

We find calcium and trace metals required by *T. viride* for cellulase production but not for growth except on pure cellulose (Cellulose-SO₄). Fahraeus (1947) found that cytophaga would not grow on pure cellulose (cotton wool) unless calcium and manganese were added to the medium. Likewise, calcium is required for the production of extracellular proteases by various bacteria, but not for their growth, except on pure protein (Merrill and Clark, 1928; Haines, 1933; Gorini and Audrain, 1951). Calcium as a firmly bound prosthetic group is required to stabilize the protease, which, in the absence of calcium, is inactivated as rapidly as it is produced (Gorini, 1951). It is possible that calcium acts similarly in cellulase.

Under certain conditions, proteases are released as inactive zymogens and activated either autocatalytically or by added proteolytic enzymes (Gorini and Lanzavecchia, 1954). While it would be attractive to consider that cellulase is released as an inactive zymogen and is activated by a protease requiring calcium, our data do not support this idea.

Metallic ions may be affecting the release of the

enzyme. Fukumoto *et al.* (1954) found that traces of zinc promoted the secretion of protease, but inhibited amylase secretion for *Bacterium liquefaciens*. It is unlikely that all of the protein appearing with cellulase is cellulase. There may be a general release of cell proteins into the medium. However, the fact that we cannot find cellulase in the mycelium prior to its appearance in the filtrate suggests that the picture is not a simple matter of release.

The metals may, on the other hand, prevent some component necessary for induction from leaking out of the cells. Morton and Broadbent (1955) found that the release of amino acids from the cells of several fungi was inhibited by the addition of trace metals.

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SUMMARY

Cellulase is an adaptive enzyme in *Trichoderma viride*. It is produced on cellulose, lactose, glucose, and cellobiose, but not on a wide variety of other substrates. The question of whether glucose is the true inducer has been discussed.

Calcium and certain trace elements are required for cellulase production, although the growth of the mold (except on pure cellulose) is not affected by their addition to the medium. The trace element requirement can be supplied by cobalt alone, but best cellulase production occurs if iron or manganese, and zinc or cobalt are also added.

The level of cellulase activity is closely correlated with the amount of protein in the culture filtrate.

Amylase is produced on most substrates supporting growth. Calcium in the medium decreases amylase production. Amylase activity is not correlated with the amount of protein in the culture filtrate.

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