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The degradation of cellulose and the production of cellulase, xylanase, mannanase and amylase by wood-attacking microfungi

Cellulosanedbrytning och produktion av cellulas, xylanas, mannanas och amylas hos vedangripande mikrosvampar.

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Abstract

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Thirty-six species of wood-inhabiting microfungi have been assayed for cellulase, xylanase, mannanase and amylase activity by the use of different test methods. The wood-degrading capabilities of the test organisms were previously known. Three of the species were unable to degrade birch wood. The other species represented three different degradation patterns, viz. 1) formation of soft rot cavities (Type 1 attack); 2) erosion of the wood cell walls (Type 2 attack); and 3) simultaneous Type 1 and Type 2 attack.

With one exception, all species were able to degrade starch. The production of the enzymes cellulase, xylanase and mannanase was demonstrated for twenty of the wood-degrading species. Five of the wood-degrading species, which all produced soft rot cavities in birch wood, failed to exhibit any of the enzyme activities. The remaining wood-degrading species could be shown to produce one or two of the enzymes. One of the species which was unable to degrade birch wood produced xylanase. Cellulase and mannanase were not produced by any of these species.

Twelve of the wood-degrading species appeared unable to degrade pure cellulose substrates when grown on agar media or in liquid cultures. Their only exhibition of cellulolytic activity was found in solid birch wood, where they formed soft rot cavities. These species have been referred to as "non-cellulolytic" soft rot fungi. Various explanations of the anomalous behaviour of these species are discussed.

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Contents

1 Introduction

In a previous paper (Nilsson 1973) the cellulolytic activity of 160 species of microfungi was compared with their ability to degrade birch wood *(Betula verrucosa* Ehrh.). The Rautela and Cowling (1966) method was used for assays of cellulolytic activity. With this method cellulolytic activity is measured as the depth of clearing of cellulose beneath fungal cultures growing on the top of agar columns in test tubes. The ability to degrade birch wood was determined by microscopic observations on sections from wood which had been attacked. Loss in weight of the birch wood was also determined for several of the species.

When using the decay method employed (Nilsson 1973), it was found that the microfungi could be classified into four groups with respect to their type of attack in birch wood: 1) fungi producing no attack, or producing no other attack than small bore holes through the cell walls; 2) fungi producing only soft rot cavities (Type 1 attack); 3) fungi producing only a type of erosion of the cell walls (Type 2 attack); and 4) fungi producing both soft rot cavities and erosion.

A comparison of the results from the assays of cellulolytic activity and the results from the wood decay tests showed that there was a good correlation between the actual clearing of cellulose and the ability to degrade birch wood. Of the 109 species which produced clearing of cellulose, 105 (96.3 %) were able to degrade birch wood. However, several of the species tested (9.4 %), which were able to produce soft rot cavities in the wood, failed to produce any clearing of cellulose. They would thus have been considered as non-cellulolytic if only the assay method for cellulolytic activity mentioned above had been used.

In order to investigate whether this anomalous behaviour is a consistent feature of this group of fungi or whether it is an effect due to the test method used for assaying cellulolytic activity, further studies have been carried out with other assay methods. The studies have also been extended to assays of xylanase, mannanase and amylase. It is likely that xylanase and mannanase are involved in the degradation of wood and it was intended to examine whether fungi which had failed to show cellulolytic activity also would fail to exhibit xylanase and mannanase activities. Amylase activity was assayed only for the purposes of comparison.

Thirty-six different species of microfungi were selected from our culture collections for these studies. Their type of wood attack was known and the species were selected so that representatives from all of the four groups of the fungi mentioned above were included.

The objects of the present investigation were two; 1) to demonstrate the production of cellulase, xylanase and mannanase, enzymes which *a priori* were assumed to be produced by the wood-degrading species, and 2) to obtain information about the conditions under which these enzymes are produced in order to achieve a better understanding of the mechanisms of wood degradation. No specific studies of the activities of the various enzymes were attempted. The purpose was merely to demonstrate the presence or absence of the various enzymes by the use of different test methods.

2 Material and methods

2.1 Organisms

Thirty-six different species of microfungi were studied. All species are listed in Table 1. Data on isolation, type of wood attack, weight losses of wood and cellulolytic activity can be obtained from a previous paper (Nilsson 1973) for all but four of the species, viz. *Ceratocystis albida* (Mathiesen-Käärik) Hunt, *Ceratocystis stenoceras* (Robak) C. Moreau, an unidentified *Cladosporium* species, tentatively called *Cladosporium* sp. A, and an unidentified imperfect fungus called Fungus D. Fungus D is very common in Sweden in chip piles and preservative treated poles. The strain used here was isolated 1968 from spruce pulpwood chips. *Ceratocystis albida* (strain B–23) was isolated 1952 by Dr. A. Käärik from galleries of *Pissodes pini* in a pine log. *Ceratocystis stenoceras* (strain B–104) was obtained from "Centraalbureau voor Schimmelcultures" in Baarn, and *Cladosporium* sp. A (strain SP78–4) was isolated 1971 from a pine foundation pile.

2.2 Substrates

Avicel. A microcrystalline cellulose preparation. Average particle size 38 μ (Kebo AB).

Walseth cellulose. Cellulose swollen in 85 percent o-phosphoric acid. Prepared from cellulose powder (Whatman CF 11) according to the description by Rautela and Cowling (1966).

Ball-milled cellulose. This cellulose was obtained from Dr. H.O.W. Eggins and Bernard King at the Biodeterioration Information Centre, University of Aston in Birmingham.

HCl-cellulose. Cellulose powder (Whatman CF 11) treated with concentrated hydrochloric acid according to a method described by Bose (1963).

Cotton wool. Chemically pure (Kebo AB).

Cellulose N (Nättraby cellstoff). This cellulose is a commercial product prepared from equal amounts of bleached pine sulphate pulp and bleached pine sulphite pulp. According to the manufacturer (Mölnlycke AB) this cellulose contains only small amounts of hemicellulose.

Jute fibres. From *Corchorus olitorius* (red jute). Received from Dr. N.J. Poole at the School of Agriculture, Aberdeen.

Birch wood meal. 80 mesh. Prepared from *Betula verrucosa.*

Larch xylan. (Koch-Light Laboratories Ltd.).

Glucomannan. Prepared from *Pinus silvestris* at Swedish Forest Products Research Laboratory. The preparation contained 78.2 percent mannose, 17.8 percent glucose, 1.4 percent galactose, 1.4 percent xylose and 1.2 percent arabinose.

Starch. Soluble starch. (E. Merck, Darmstadt).

2.3 Assay of cellulase, xylanase, mannanase and amylase with the Rautela-Cowling technique

The technique employed by Rautela and Cowling (1966) for testing the cellulolytic activity of fungi was adopted. Vertical agar columns (approx. height 40 mm), containing the various substrates were prepared in test tubes (18 mm diam.). The two media described in a previous study (Nilsson 1973) were

also used here. The media had the following compositons:

R–C medium (after Rautela and Cowling 1966)
$NH_4H_2PO_4$ 2.0 g, KH_2PO_4 0.6 g, K_2HPO_4 0.4 g, $MgSO_4 \cdot 7\ H_2O$ 0.89 g, thiamine HCl 100 µg, yeast extract 0.5 g, adenine 4.0 mg, adenosine 8.0 mg, agar 17 g and distilled water to make 1 liter.

B–VII medium (slight modification of Bravery's (1968) medium VII):
$(NH_4)_2SO_4$ 0.543 g, KH_2PO_4 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7\ H_2O$ 0.2 g, $CaCl_2$ 0.1 g, thiamine HCl 1 mg, agar 15 g and 1000 ml of deionized water.

In addition to these media, two further media were employed in test tubes with cellulose. These media had the following composition:

Medium A
$(NH_4)_2SO_4$ 2.5 g, KH_2PO_4 1.0 g, KCl 0.1 g, $MgSO_4 \cdot 7\ H_2O$ 0.5 g, $CaCl_2$ 0.1 g, $FeSO_4 \cdot 7\ H_2O$ 10 mg, $CuSO_4 \cdot 5\ H_2O$ 10 mg, yeast extract 0.5 g, agar 15 g and 1000 ml of deionized water.

Medium B
The same as medium A but 3.0 g KNO_3 instead of $(NH_4)_2SO_4$.

The media R–C, A and B contain yeast extract which might be used as a carbon source by certain fungi. Medium B–VII contains no yeast extract. The media A and B have a higher nitrogen content than media R–C and B–VII. If the amount of nitrogen is calculated from the nitrogen containing salts, medium A contains 530 mg, medium B 415 mg, medium R–C 243 mg and medium B–VII 115 mg N per liter.

The different carbohydrates to be tested were added to the four media in the following combinations:

Carbohydrate	*Concentration percent (W/v)*	*Medium*
Avicel	0.125	R–C
Avicel	0.125	B–VII
Walseth cellulose	0.25	R–C
Walseth cellulose	0.125	R–C
Walseth cellulose	0.25	B–VII
Walseth cellulose	0.125	B–VII
Ball-milled cellulose	0.2	B–VII
Ball-milled cellulose	0.2	A
Ball-milled cellulose	0.2	B
HCl-cellulose	0.125	B–VII
Xylan	0.125	B–VII
Glucomannan	0.1	B–VII
Starch	0.2	B–VII

The test tubes were fitted with cotton plugs and sterilized by autoclaving. The tubes were agitated by hand during the cooling of the agar in order to keep the carbohydrates uniformly suspended. The tubes were left in upright position when the agar solidified in order to obtain vertical columns. Opaque agar columns were obtained since the substrates used were insoluble, or only partly soluble, in water.

Most of the test tubes were inoculated with small pieces (approx. 2x2 mm) of mycelium and agar taken from actively growing cultures on malt extract (2.5 percent) agar. The test tubes with Walseth cellulose and ball-milled cellulose in B–VII medium were, however, inoculated with spores or aerial mycelium in order to avoid the addition of malt extract from the agar plates. Each fungus was inoculated in two replicate tubes.

All test tubes were incubated, standing perpendicular, at the ambient room temperature (23 – 25°C). Some of the fungi were also incubated at 15 and 30°C. The cotton plugs were covered with an aluminium foil to prevent the agar from drying out.

The depth of clearing was measured every week for up to twelve weeks. If clearing had occurred after three or six weeks, no further measurements were taken. The depth of clearing was measured in millimeters from the top of the agar to the front of the clear zone.

2.4 Cultivation on cellulose agar plates and assay of the cellulase produced.

Cellulose agar plates were prepared in 90

mm plastic Petri dishes with the following two media:

F6A
Avicel 10 g, asparagine 1.0 g, NH_4NO_3 1.0 g, KH_2PO_4 1.0 g, $MgSO_4 \cdot 7\ H_2O$ 0.5 g, $FeSO_4 \cdot 7\ H_2O$ 10 mg, $ZnSO_4 \cdot 7\ H_2O$ 10 mg, glucose 2.5 g, yeast extract 0.5 g, agar 15 g and 1000 ml of deionized water.

This is the same cellulose agar medium which was used in a previous study (Nilsson 1973) on the wood attack produced by microfungi.

B–VII cellulose agar
10 grams of Avicel was added to 1 liter of the medium B–VII described above.

The media were autoclaved and the plates were poured when the agar had cooled to about 45°C. The media were agitated when pouring in order to suspend the cellulose particles in the agar.

The agar plates with medium F6A were inoculated with small pieces of mycelium and agar taken from actively growing cultures on malt extract (2.5 percent) agar. The agar plates with B–VII medium were inoculated with spores or pieces of aerial mycelia. Three agar plates were inoculated with each fungus and each medium. For growth comparisons each fungus was also inoculated on three plates with malt extract (2.5 percent) agar.

The agar plates were placed in perforated plastic bags and incubated at the ambient room temperature for periods of three and six weeks. Some of the fungi growing on B–VII cellulose agar were incubated for fifteen weeks.

After incubation a visual estimation of the growth on the cellulose agar plates was made. The growth was compared with the growth on malt extract agar.

The cellulose agar plates were also examined for any visible clearing under or around the fungal colonies.

The agar-diffusion test described by Savory et al. (1967) was used to assay the cellulase produced in the cellulose agar media. This method employs the transfer of mycelium-agar plugs from the cellulose agar plates where the fungi have been growing. The agar plugs are cut with a cork-borer and transferred to a hole in a test agar plate which contains suspended cellulose. The cellulase present in the transferred plug will diffuse out into the surrounding agar and produce a clear zone. Savory et al. used an addition of sodium azide (0.005 mole/l) to the test agar in order to prevent growth of the fungi.

Two types of test agar plates were used in this study. One was prepared with Rautela-Cowling medium which was poured on plates instead of into the test tubes. The concentration of Walseth cellulose was 0.25 percent. The other plates contained the same medium and amount of Walseth cellulose, but sodium azide (0.3 g/l) was also added.

Mycelium-agar plugs were cut with a cork-borer (10 mm diam). from the plates with F6A and B–VII cellulose agar on which the fungi had grown for three, six or fifteen weeks. Two plugs were taken from each plate; one plug was taken close to the centre of the colony while the other was taken immediately behind the margin of the colony. The agar plugs were transferred to holes cut with the same cork-borer in the test agar plates. The plugs were inserted in the holes with the fungal growth uppermost. Approximately ten agar plugs were placed in each test plate.

The test plates without sodium azide were incubated for two days at 40°C. This temperature was employed in order to prevent fungal growth without the use of any poisonous additives. It is also known that cellulases generally have a higher activity at 40° than at 23–25°, which was the room temperature here. The test plates with sodium azide were incubated for two days at room temperature.

After incubation the test plates were examined for clearing around the agar plugs. No measurements of the width of the clear zones were made, only the presence or absence of clearing being noted.

2.5 Assay of cellulase and xylanase present in wood blocks attacked by the test organisms.

Decay tests were carried out with birch sap wood (*Betula verrucosa*) on cellulose and malt extract agar slopes in test tubes according to the methods described in a previous paper (Nilsson 1973). Each fungus was cultivated on the medium which had previously been found to support the heaviest attack.

After varying periods of time, usually after approximately 20, 40 or 60 days, the attacked wood blocks were removed from the test tubes and immediately sectioned with a razor blade. Both transverse and longitudinal sections were cut. The sections were 0.5 to 1 mm thick and the sides were 3 to 5 mm.

The sections were placed on two types of test plates immediately after cutting. The test plates were prepared according to Stranks and Bieniada (1971) in 90 mm plastic Petri dishes.

The test plates were prepared as a twin agar where the bottom layer contained agar (1 percent), sodium azide (0.005 percent) and 30 ml of 0.2 M sodium acetate buffer pH 5.5 per 100 ml. The top layer contained the same ingredients but the agar concentration was reduced to 0.5 percent. The substrate to be tested, in this case cellulose or xylan, was added to the top layer. When the agar had solidified, the substrate formed a thin monolayer between the two agar layers. The top layer in the test plates used here for assay of cellulase activity contained 0.25 percent Walseth cellulose instead of the recommended 2 percent. The top layer in the test plates for the assay of xylanase activity contained 2 percent larch xylan.

Stranks and Bieniada suggested the use of these plates for detection of cellulase and hemicellulases in culture filtrates. The formation of clear zones around small droplets of culture filtrate added to the test plates indicate cellulase or hemicellulase activity. In the present study, sections from attacked wood were used instead of culture filtrate. Tests showed that the fungi did not continue to grow in the wood sections since the test agar was poisoned with sodium azide. The enzymes present in the wood at the time of sectioning will diffuse out, if diffusible, in the agar and produce clear zones in the substrate (see Fig. 3). Henningsson et al. (1972) used this method to demonstrate the presence of cellulase and xylanase in birch wood chips attacked by a white rot fungus. This method is extremely sensitive, at least for the detection of cellulase, as is evident from dilution tests with a cellulase-containing solution (see section 2.6). The test agar plates were incubated for up to five days at 40°C. After incubation the test plates were examined for clearing under or around the wood sections.

2.6 Cultivation in liquid media. Weight losses of cellulosic substrates and assays of cellulase and xylanase.

The two following liquid media were used:

Medium EP
$(NH_4)_2\,SO_4$ 0.5 g, DL-asparagine 0.5 g, KH_2PO_4 1.0 g, KC1 0.5 g, $MgSO_4 \cdot 7\,H_2O$ 0.2 g, $CaC1_2$ 0.1 g, yeast extract 0.5 g and deionized water 1000 ml.

After autoclaving together with 100 mg cellulose N the pH was 5.4.

Medium EP contained the same ingredients as the medium described by Eggins and Pugh (1962), except for the agar and ball-milled cellulose present in the Eggins and Pugh medium.

Medium B–VII–L
As medium B–VII described previously, but since it was a liquid medium, no agar was added. In addition to thiamine, medium B–VII–L also contained 0.2 mg biotin and 0.2 mg pyridoxin per 1000 ml.

The pH was 4.6 after autoclaving together with 100 mg cellulose N.

The fungi were cultured in 100 ml Erlenmeyer flasks. In most experiments 20 ml medium was added to each flask together with 100 mg of the cellulose substrate

(weighed air-dry). The flasks were sterilized by autoclaving. Each flask was inoculated with 2 ml of a spore or mycelial suspension. Three or four replicate flasks were used in each test. Both shake and stationary cultures were used. The shake cultures were placed in a rotary shaker at 125 revolutions per minute. All cultures were incubated at ambient room temperature.

Growth on medium EP and B–VII–L with glucose as carbon source

All fungi which had failed to produce clearing of cellulose in the tests with the Rautela-Cowling technique and some of the fungi which had produced clearing only on B–VII medium were tested on the liquid media EP and B–VII–L in order to see if these media could support growth of the fungi. For this purpose glucose, 20 g/liter, was added to 1000 ml of each of the media. As a control, some of the fungi were grown on medium EP without glucose. Only stationary cultures were employed. The flasks were harvested after 14 or 15, 21 and 28 days, except for flasks containing medium B–VII–L which were harvested after 21 days. The mycelia were filtered off in glass crucibles and were dried and weighed to ascertain the dry weights of mycelium. pH was measured in the culture filtrates.

Determination of weight losses of cellulose N, Avicel and cotton produced by the test fungi. Assay of cellulase and xylanase in culture filtrates.

20 ml of medium was added to each flask together with 100 mg of the cellulose substrate. All of the fungi were tested on medium EP with cellulose N as substrate, some of them were also tested on Avicel and cotton. All of the species which failed to show activity on the EP medium were tested on B–VII–L medium with cellulose N as substrate. Some of these species were also tested on B–VII–L medium with Avicel or cotton as substrate. A number of the species which were active on EP medium were also cultivated on B–VII–L medium for comparison. Both shake and stationary cultures were employed. Non-inoculated flasks with EP medium and the cellulose substrates were used as controls.

After incubation the mycelium and remaining cellulose were filtered off in pre-weighed glass crucibles and washed several times with distilled water. The crucibles were dried overnight at 105°C and weighed. The weight loss of the cellulose substrates was calculated as the difference between the added amount of cellulose (100 mg) and the dry weight of the remaining cellulose plus mycelium. The weight losses reported were not corrected for the weight of mycelium, nor were they corrected by the values obtained from the non-inoculated control flasks.

The pH of the culture filtrates was measured and is reported as the final pH. The culture filtrates were also used for assays of cellulase and xylanase. The test agar plates recommended by Stranks and Bieniada (1971), but slightly modified as described in the preceeding section (2.5), were used for the assays. 80 µl of culture filtrate was added to a test plate with Walseth cellulose and 80 µl of the same filtrate was added to a test plate with larch xylan. Up to twenty droplets could be put on the same test plate if the enzyme activity was low. If enzyme activity was high, a maximum of six droplets could be added to each plate.

The test plates were incubated at 40°C for two days and they were then examined for clear zones. This incubation time was chosen since some of the filtrates showed such low activity that no clearing was perceptible after only one day. In the beginning of the experiments, some plates were kept for up to seven days but no case was found where clearing was perceptible first after three or more days. Some measurements were made of the width of the clear zones, but since exact measurements were difficult due to diffuse clearing and diffuse zone fronts, only the presence or absence of activity is reported in the results presented.

The sensitivity of the test method was studied here with a solution of cellulase from *Sporotrichum pulverulentum* Novobranova obtained from Dr. B. Pettersson at the Swedish Forest Products Research Laborato-

ry. This cellulase solution released 1.6 mg reducing sugar per ml of an Avicel suspension (1 %) after 4 hours at 30°C. Several dilutions were prepared of the cellulase solution with distilled water. 80 µl of each dilution was added to a test plate containing Walseth cellulose. Perceptible clearing was still obtained at a 400-fold dilution. This shows that the method is very sensitive for a detection of cellulase.

Growth in liquid cultures with birch wood meal. Assay of cellulase and xylanase.

A few of the fungi were tested in medium EP and medium B–VII–L with birch wood meal as the substrate. 100 mg wood meal (dried overnight at 105°C) was added to each flask plus 20 ml of the medium. Both shake and stationary cultures were employed. Weight loss determinations and assays of cellulase and xylanase were performed as described above.

Determination of weight losses of cotton and jute fibres

Five of the test fungi, *Bispora betulina, Ceratocystis albida, Phialophora* sp. A, *Wardomyces inflatus* and *Xylogone sphaerospora* were grown in medium EP on cotton and jute fibres in order to compare the degradation of non-lignified and lignified fibres.

500 mg of substrates (weighed air-dry) was added to each 100 ml Erlenmeyer flask together with 5 ml of medium EP. The flasks were inoculated and incubated as stationary cultures. Weight loss determinations, pH measurements and assays of cellulase and xylanase were carried out as described above. Non-inoculated flasks were used as controls.

Some microscopic studies of the exposed fibres were carried out after weighing. Small amounts of the fibres were spread on a glass slide, mounted in a glycerol-water mixture (1:1) and viewed under a light microscope using polarized light.

Effect of various amounts of glucose on the production of cellulase and xylanase

Bispora betulina, Ceratocystis albida, Cladorrhinum sp. A, *Phialocephala* sp. A, *Xylogone sphaerospora* and Fungus A were grown in stationary cultures in flasks containing 20 ml of medium EP to which varying amounts of glucose had been added. Approximately 100 mg of cellulose N added to each flask served as cellulose substrate. The flasks were harvested after the incubation times given in Table 9. The mycelium and cellulose were filtered off and discarded. pH was measured in the culture filtrates and assays of cellulase and xylanase in the filtrates were made on test plates as described above.

3 Results

3.1 Type of attack produced in birch wood

The type of attack produced by the fungi in birch wood is shown in Table 2. These data are taken from a previous paper (Nilsson 1973), except for the four species mentioned in Materials and methods. Their decay patterns were studied and it was found that *Ceratocystis albida* and *Ceratocystis stenoceras* only produced soft rot cavities and no erosion in birch wood. *Cladosporium* sp. A and Fungus D produced a weak erosion of the cell walls but no cavities.

3.2 Clearing produced in agar columns with cellulose, xylan, glucomannan and starch (Rautela-Cowling technique).

The average depth of clearing in two replicate tubes after 21 and 42 days on the various substrates is given in Table 2. Walseth cellulose was used in 0.25 percent concentration. The concentrations of the other substrates are given in Materials and methods. The depth of clearing of ball-milled cellulose on media A and B is not shown in the table since clearing was similar to that occurring on medium B–VII.

The depth of clearing varied considerably among individual species. It was also dependent on time of incubation, type of medium and type of substrate. Fig. 1 shows the amount of clearing produced by *Xylogone sphaerospora, Cordana pauciseptata, Bispora betulina* and *Cladosporium resinae* in test tubes with B–VII medium containing 0.2 percent ball-milled cellulose.

The greatest clearing zones in the agar columns with cellulose as the substrate were obtained with Walseth cellulose. Smaller clearing zones were obtained on ball-milled cellulose and these zones tended to be more diffuse. Only a few of the fungi were able to produce any measurable clearing on Avicel and HCl-treated cellulose.

If clearing of Walseth cellulose and Avicel on the two media, R–C and B–VII, is compared, it is obvious that greater clearing zones were always obtained on medium B–VII. All of the species which produced clearing of

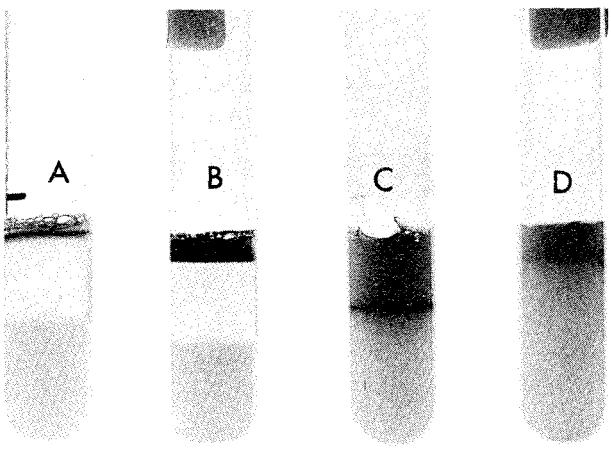

Figure 1.
Amount of clearing after 42 days in test tubes containing 0.2 percent ball-milled cellulose in B–VII medium. A. *Xylogone sphaerospora*, B. *Cordana pauciseptata*, C. *Bispora betulina*, and D. *Cladosporium resinae*. Distinct clearing is evident in tubes A and B. No clearing occurred in tubes C and D although a fair amount of growth was produced by *B. betulina* and *C. resinae*, which can be seen as a dark colouration in the agar columns.

Walseth cellulose on R–C medium also produced clearing of Walseth cellulose and ball-milled cellulose on B–VII medium. The difference in the amount of clearing on these media was, however, considerable for some of the fungi. *Acremonium atro-griseum, Cladorrhinum* sp. A, *Dictyosporium elegans, Phialophora fastigiata* and *Wardomyces inflatus,* which all produced less than a 1 mm clear zone of Walseth cellulose on R–C medium, produced clear zones of 9 to 13 mm on B–VII medium with the same substrate.

Chrysosporium pannorum was the only species which produced clearing of Avicel on R–C medium. When B–VII medium was used, ten species produced some clearing of Avicel, and twelve species produced clearing of the HC1 cellulose. The amount of clearing was, however, considerably less than that produced on Walseth cellulose.

Cladosporium sp. A, *Humicola alopallonella, Phialocephala dimorphospora, Scytalidium* sp. B and Fungus D all failed to produce any clearing of cellulose on R–C medium, even if the concentration of Walseth cellulose was reduced to 0.125 percent. Clearing was, however, obtained on B–VII medium with 0.25 percent of Walseth cellulose. *Phialocephala dimorphospora* failed to produce clearing of ball-milled cellulose on B–VII medium, while the remaining four species produced clearing. Of these five fungi, only *H. alopallonella* was able to produce a slight clearing of HC1 cellulose.

All of the species which produced clearing of Walseth cellulose on R–C medium also produced clearing of ball-milled cellulose on media A and B. The depth of clearing was very similar between medium A and medium B. Almost equally large zones were formed in ball-milled cellulose on medium B–VII as on media A and B. *Humicola alopallonella, Phialocephala dimorphospora, Scytalidium* sp. B and Fungus D failed, however, to produce any clearing on media A and B.

It is evident from Table 2 that all of the species which produced an erosion-type attack (Type 2) in the birch wood also produced at least some clearing of the cellulose. But *Acremonium atro-griseum, Humicola alopallonella, Petriellidium boydii* and *Phialocephala dimorphospora* which also produced clearing of the cellulose, have been found to be unable to cause any detectable erosion-type attack in birch wood. These species only form soft rot cavities in the birch wood (Type 1 attack). Similar results were already obtained in a previous study (Nilsson 1973).

The following sixteen species failed to produce clearing on any of the cellulose agars tested: *Bispora betulina, Catenularia heimii, Ceratocystis albida, Ceratocystis olivacea, Ceratocystis stenoceras, Cladosporium resinae, Gonatobotrys* sp. A, *Graphium* sp. A, *Mollisia* sp. A, *Phialocephala* sp. A, *Phialocephala* sp. C, *Phialophora verrucosa, Phialophora* sp. A, *Rhinocladiella* sp. A, Fungus A and Fungus B.

These fungi were incubated for up to 12 weeks on Walseth cellulose with R–C and B–VII medium, but even after this time no clearing had occurred. Tests on cellulose agar with a lower concentration of Walseth cellulose, 0.125 percent instead of 0.25 percent, also gave negative results. The sixteen species were also tested on B–VII medium with 0.125 percent Walseth cellulose at 15 and 30°C for up to six weeks, but still no clearing was obtained.

All of these fungi produced at least some growth on the cellulose agars. As can be seen in Fig. 1, some of them produced a fair amount of growth without being able to form any clearing zones. To ensure that clearing was not obscured by the mycelia which penetrated into the agar, microscopic studies of the top layers of the agar columns were carried out as described in a previous paper (Nilsson 1973). However, no evidence of degradation of the cellulose was noted for any of the species mentioned above.

Only three of the sixteen species, viz. *Ceratocystis olivacea, Cladosporium resinae* and *Phialophora verrucosa,* are unable to produce other attack in birch wood than small bore holes through the cell walls. The remaining thirteen species all produce soft rot cavities in birch wood (see Table 2).

All species which produced clearing of cellulose, except *Humicola alopallonella* and *Phialocephala dimorphospora,* also produced clearing of xylan and glucomannan. *H. alopallonella* produced no clearing of glucomannan and *P. dimorphospora* produced no clearing of xylan.

Of the sixteen species which failed to produce clearing of any of the cellulose substrates, the following formed clear zones in both xylan and glucomannan agar: *Phialophora* sp. A, Fungus A and Fungus B. Five of the species, *Bispora betulina, Cladosporium resinae, Graphium* sp. A, *Mollisia* sp. A and *Phialocephala* sp. A produced clearing of xylan, but not of glucomannan. The remaining eight species: *Catenularia heimii, Ceratocystis albida, Ceratocystis olivacea, Ceratocystis stenoceras, Gonatobotrys* sp. A, *Phialocephala* sp. C, *Phialophora verrucosa* and *Rhinocladiella* sp. A failed to produce clearing of both xylan and glucomannan.

Phialophora verrucosa was the only species which failed to produce clearing of starch. All of the other species already produced appreciable clearing after 21 days.

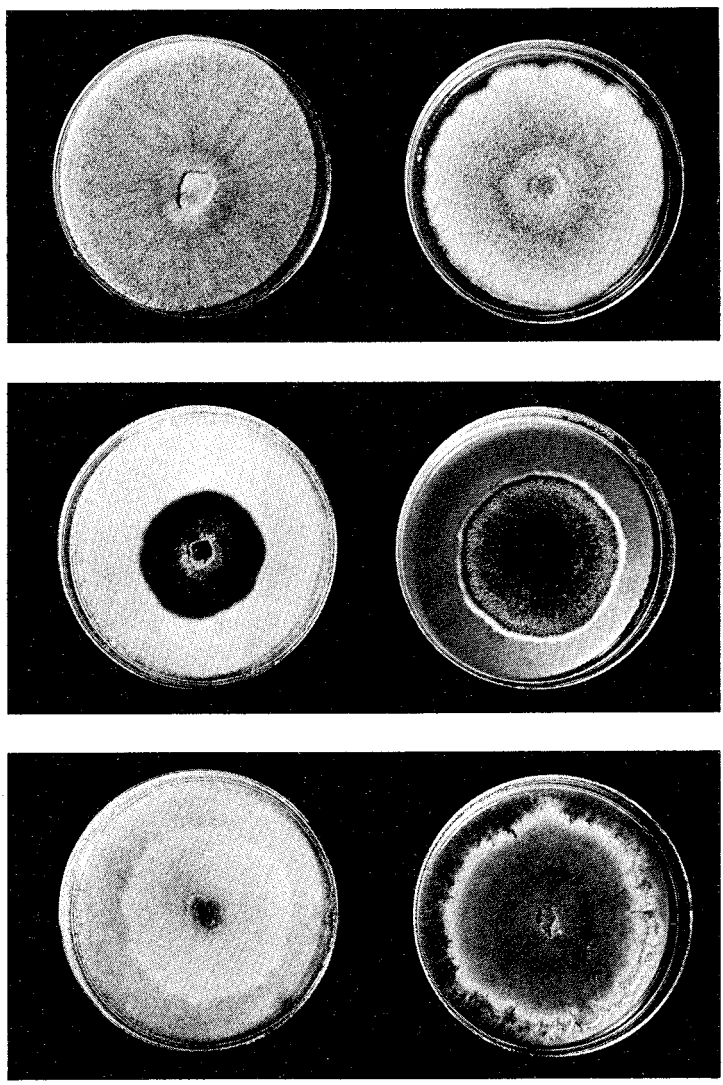

Figure 2.
Comparison of growth of *Petriellidium boydii* (A), *Phialophora* sp. A (B) and *Rhinocladiella* sp. A (C) on malt extract (2.5 %) agar (right) and F6A cellulose agar (left).

3.3 Growth and cellulase production on cellulose agar plates

All results from these experiments are shown in Table 3.

There were great variations in the amount of growth produced on the three types of agar media employed. Growth was generally best on malt extract agar, followed by F6A cellulose agar and less on B–VII cellulose agar. Fungi such as *Cladorrhinum* sp. A, *Coniothyrium fuckelii* var. *sporulosum, Humicola grisea, Petriellidum boydii, Phialophora fastigiata, Phialophora hoffmannii, Scytalidium lignicola, Wardomyces inflatus* and *Xylogone sphaerospora* produced equal or better growth on F6A cellulose agar when compared with malt extract agar. All of these species also produced clearing of cellulose with the Rautela-Cowling technique (see Table 2). Although *Cordana pauciseptata, Rhinocladiella anceps* and *Scytalidium* sp. B also produced clearing of cellulose with the same technique their growth was sparse on F6A cellulose agar. The remainder of the tested species showed sparse or moderate growth on F6A cellulose agar. Fig. 2 shows the growth of three species on F6A cellulose agar as compared with growth on malt extract agar.

All species, except *Scytalidium* sp. B, produced much less growth on B–VII cellulose agar as compared with malt extract agar and in most cases also when compared with F6A cellulose agar. *Humicola alopallonella, Scytalidium* sp. B and Fungus D were the only species which produced equal or increased growth on B–VII than on F6A cellulose agar. *Scytalidium* sp. B grew almost equally well on B–VII cellulose agar as on malt extract agar.

The truly non-celluloytic fungi *Ceratocystis olivacea, Cladosporium resinae* and *Phialophora verrucosa* produced equal or better growth on both of the two cellulose agar media than several of the species which were able to produce soft rot in birch wood but which had failed to produce clearing in cellulose agar columns. Such species were *Catenularia heimii, Ceratocystis albida, C. stenoceras, Gonatobotrys* sp. A, *Phialocephala* sp. C and *Rhinocladiella* sp. A.

It was difficult, due to obscuring mycelia, to observe any clearing of the cellulose agar on which the fungi grew. However, the following species produced visible clearing in F6A cellulose agar: *Cladorrhinum* sp. A, *Coniothyrium fuckelii* var. *sporulosum, Humicola grisea, Petriellidium boydii, Phialophora hoffmannii, Scytalidium lignicola* and *Xylogone sphaerospora. Cladosporium* sp. A, *Scytalidium* sp. B and Fungus D produced visible clearing on B–VII but not on F6A medium.

No differences in cellulolytic activity were shown if the test plates were incubated at 40°C or if the test agar was poisoned with sodium azide. Thus, separate results with the two types of test plates are not shown in Table 3. *Cladorrhinum* sp. A and *Petriellidium boydii* produced some growth on the plates incubated at 40°C but this did not obscure the clearing.

All of the species which had produced clearing of cellulose with the Rautela-Cowling technique on R–C medium, except for *Rhinocladiella anceps,* were found to produce cellulase when growing on F6A cellulose agar. But *Humicola alopallonella, Scytalidium* sp. B and Fungus D only produced cellulase when they grew on B–VII cellulose agar. Irrespective of media, no celluloytic activity was found for *Phialocephala dimorphospora* or *Rhinocladiella anceps,* nor for any of the sixteen species which had failed to produce clearing of cellulose with the Rautela-Cowling technique (see section 3.2), even if the incubation time was extended to fifteen weeks.

3.4 Presence of cellulase and xylanase in birch wood attacked by the test organisms

Thirty of the test fungi were used in experiments to demonstrate the presence of cellulase and xylanase in attacked birch wood. No cellulolytic activity had been found for fourteen of these species when using the two methods described previously, and six of the species had failed to produce clearing of xylan with the Rautela-Cowling technique.

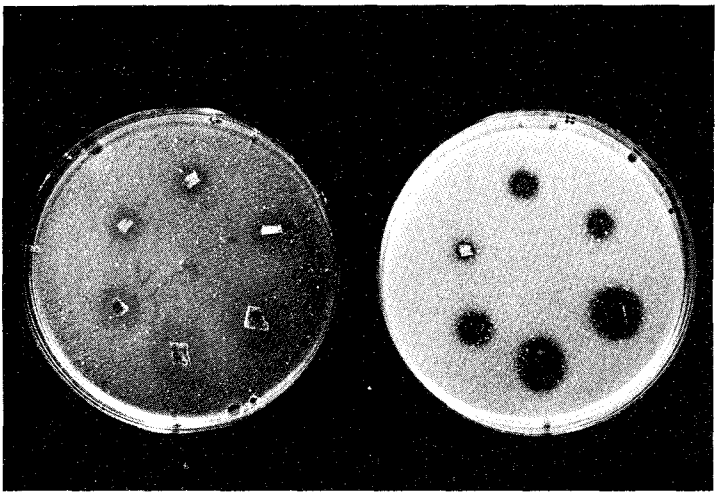

Figure 3.
Clearing of Walseth cellulose (left) and xylan (right) around sections cut from birch wood blocks attacked by *Xylogone sphaerospora* for 27 days. Sodium azide is incorporated in the test agar plates to prevent growth of the fungus.

The results are illustrated in Table 4. Clearing of the Walseth cellulose in the test plates was obtained around sections cut from birch wood blocks attacked by sixteen of the thirty fungi. Among these sixteen species were four species, *Bispora betulina. Phialocephala* sp. A, *Phialophora* sp. A and Fungus A, which had not shown any cellulolytic activity in the two previous tests. Fig. 3 shows clearing of Walseth cellulose and xylan around sections cut from birch wood attacked by *Xylogone sphaerospora.*

No clearing of cellulose was obtained around sections cut from wood blocks attacked by *Catenularia heimii, Ceratocystis albida, Ceratocystis stenoceras, Cladosporium resinae, Gonatobotrys* sp. A, *Graphium* sp. A, *Mollisia* sp. A, *Phialophora verrucosa, Rhinocladiella* sp. A and Fungus B. All of these species had also failed to show cellulolytic activity in the two previous tests. Microscopic examination of the attacked wood blocks from which the sections had been cut showed that all of these species, except *Cladosporium resinae, Mollisia* sp. A and *Phialophora verrucosa,* had caused considerable degradation of the wood in the form of soft rot cavities. *Cladosporium resinae* and *Phialophora verrucosa* had only produced bore holes through the wood cell walls while *Mollisia* sp. A had produced a few cavities.

Cellulase was demonstrated in the birch wood for twelve of the species, which had also shown cellulolytic activities in the previous tests. However, no clearing of the cellulose was found around sections cut from wood attacked by *Dictyosporium elegans, Humicola alopallonella, Petriellidium boydii* and *Wardomyces inflatus,* although microscopic examination of the wood blocks from which the sections had been cut, showed that substantial degradation had occurred.

The presence of xylanase in wood was closely correlated with the presence of cellulase. Except for three of the fungi. viz. *Bispora betulina, Ceratocystis stenoceras* and *Pseudeurotium zonatum,* xylanase was always found in wood blocks together with cellulase. Cellulase, though not xylanase, activity was detected in wood blocks attacked by *Bispora betulina* and *Pseudeurotium zonatum* whereas xylanase but not cellulase was detected in wood blocks attacked by *Ceratocystis stenoceras.*

Xylanase activity could not be detected around sections cut from wood attacked by nine of the twenty-four species, which previously had been found to produce clearing of xylan with the Rautela-Cowling technique. Of all of the species which had failed to produce clearing of xylan with the same technique, *Ceratocystis stenoceras* was the only species for which xylanase could be demonstrated in attacked wood.

3.5 Growth on medium EP and B–VII–L in liquid cultures with glucose as the source of carbon

The results illustrated in Table 5 show that all of the tested fungi could grow on the two media, EP and B–VII–L, with glucose as the source of carbon. No growth or very slight growth occurred on medium EP without glucose.

Phialocephala sp. C and *Rhinocladiella* sp. A showed slightly better growth on B–VII–L than on EP medium. All of the other species produced equal or, in some cases, considerably more growth on EP medium.

All of the species lowered the pH of both of the media during growth on glucose. A rise in pH was noted in the flasks with medium EP without glucose.

Differences in growth rates between the test species grown on EP medium can be noted in the table. Some species had evidently reached their maximal mycelial weights already after 14 days, while others still showed an increase in mycelial weight up to 28 days.

3.6 Degradation of cellulose N, Avicel and cotton and the production of cellulase and xylanase on these substrates in liquid cultures

The results with medium EP are shown in Table 6 and the results with medium B–VII –L are illustrated in Table 7. The weight losses reported represent the average of three or four flasks.

The non-inoculated controls with EP as the medium showed weight losses of the cellulose substrates which ranged from 0.8 to 1.0 percent in shake cultures and from 2.1 percent to 5.1 percent in stationary cultures. Since great errors are involved in the procedure of determining weight losses of the cellulose substrates, only weight losses above 10 percent are regarded as significant.

The results show a pattern similar to that of the results of the clearing tests using the Rautela-Cowling technique. Most of the species which had produced clearing of cellulose were also able to cause weight losses of cellulose and cellulase and xylanase could be found in the culture filtrates.

The weight losses were generally higher on medium EP than on medium B–VII–L. Exceptions were found for *Acremonium atro-griseum* and *Cordana pauciseptata* which produced higher weight losses of cotton on medium B–VII–L than on EP medium. Their weight losses of Avicel and cellulose N were, however, higher on EP than on B–VII–L medium. *Rhinocladiella anceps* and *Scytalidium* sp. B were also exceptional in causing higher weight losses of cellulose N on B–VII–L medium than on EP medium. The last two fungi produced no weight losses of cellulose N on EP medium after 42 days whereas the weight losses on B–VII–L medium were 26.8 and 46.0 percent respectively after 21 days.

On medium B–VII–L only very few of the fungi produced cellulase and xylanase which could be detected in the culture filtrates. This also applies to species which caused significant weight losses on B–VII–L medium.

Differences were noted between shake and stationary cultures. *Coniothyrium fuckelii* var. *sporulosum, Pseudeurotium zonatum, Scytalidium lignicola* and Fungus D caused higher weight losses on medium EP in shake cultures than on the same medium in stationary cultures, while the reverse was found for *Dictyosporium elegans, Humicola alopallonella, Phialophora fastigiata* and Fungus A (see Table 6). Especially great differences were noted for *Phialophora fastigiata* growing on cotton in EP medium. After 42 days the weight loss of cotton in stationary cultures was 38 percent while only 1 percent was lost in shake cultures. Fungus A behaved similarily on cellulose N on EP medium. The weight loss was nearly 46 percent after 42 days in stationary cultures while no weight losses occurred in shake cultures.

If the weight losses obtained in medium EP for the different celluloses are compared, it appears that cellulose N in most cases was

more rapidly degraded than Avicel, while cotton was the most resistant substrate. But individual variations were found, *Chrysosporium pannorum,* for instance, caused a higher weight loss of Avicel than of cellulose N. Some of the fungi showed considerable variations in their ability to degrade the different cellulose substrates. *Acremonium atro-griseum* caused weight losses of 35.0 and 48.4 percent of Avicel and cellulose N respectively, while the weight loss of cotton was only 4.1 percent. When medium B–VII –L was used, the fungus produced during the same time a weight loss of 33.2 percent of cotton while Avicel and cellulose N lost only 15.2 and 13.5 percent respectively.

Dictyosporium elegans and Fungus A caused weight losses only in cellulose N. Avicel and cotton were not attacked. *Phialophora fastigiata* did not degrade Avicel but caused high weight losses of both cotton and cellulose N.

Among the species which were tested on medium EP, the following produced cellulase and xylanase that could be detected in the culture filtrates, both in shake and stationary cultures: *Coniothyrium fuckelii* var. *sporulosum, Phialophora fastigiata, Scytalidium lignicola* and Fungus D. *Dictyosporium elegans* and *Pseudeurotium zonatum* produced both cellulase and xylanase in stationary cultures but in culture filtrates from shake cultures no xylanase could be detected. The reverse was found for *Petriellidium boydii. Humicola alopallonella* and Fungus A did not produce any of the two enzymes in shake cultures, both produced cellulase in stationary cultures and Fungus A also produced xylanase. *Mollisia* sp. A produced xylanase but not cellulase in shake cultures. None of the enzymes were found in stationary cultures.

On medium B–VII–L comparisons can only be made for *Phialophora* sp. A and Fungus D. Cellulase and xylanase were detected in stationary cultures of Fungus D, while no xylanase was found in shake cultures. *Phialophora* sp. A showed xylanase activity on Avicel after 42 days. No cellulase activity was found on any of the substrates in stationary cultures. None of the enzymes were found in shake cultures.

If weight loss is used as a criterion for cellulolytic activity, the following fungi, which caused more than 10 percent weight loss, may be considered cellulolytic: *Acremonium atro-griseum, Chrysosporium pannorum, Cladorrhinum* sp. A, *Coniothyrium fuckelii* var. *sporulosum, Cordana pauciseptata, Dictyosporium elegans, Humicola alopallonella, Humicola grisea, Petriellidium boydii, Phialophora fastigiata, Phialophora hoffmannii, Pseudeurotium zonatum, Rhinocladiella anceps* (only on medium B–VII –L), *Scytalidium lignicola, Scytalidium* sp. B (only on medium B–VII–L), *Wardomyces inflatus, Xylogone sphaerospora,* Fungus A and Fungus D.

Both cellulase and xylanase were detected in culture filtrates from all the species mentioned above, except for the culture filtrates from *Humicola alopallonella* and *Scytalidium* sp. B in which no xylanase was detected. Among the remainder of the tested species *Cladosporium* sp. A was found to produce cellulase and *Mollisia* sp. A and *Phialophora* sp. A were found to produce xylanase. None of these enzymes was detected in culture filtrates from the rest of the fungi.

The pH after cultivation was higher than the pH of the non-inoculated controls on medium EP. The highest pH values were found in culture filtrates from flasks where no cellulose degradation had occurred. On medium B–VII–L, the pH was the same or lower than the pH of the medium after autoclaving. Especially low pHs were measured in culture filtrates from flasks with medium B–VII–L where significant degradation of the cellulose had occurred.

3.7 Degradation of birch wood meal and production of cellulase and xylanase on this substrate in liquid cultures

Chrysosporium pannorum, Bispora betulina, Phialophora sp. A and *Phialocephala* sp. A were grown in liquid cultures on birch wood meal as described in Materials and methods (section 2.6). The incubation was extended to 42 days but even after this time no significant weight losses were obtained, nor

was cellulase and xylanase detected in any of the culture filtrates.

3.8 Degradation of cotton and jute fibres and production of cellulase and xylanase on these substrates in liquid cultures

Table 8 shows the results obtained from liquid cultures with cotton and jute fibres as substrates.

Bispora betulina and *Phialophora* sp. A did not cause any significant weight losses of cotton and jute fibres, nor could cellulase and xylanase be detected in the culture filtrates. No degradation of any of the two fibres was observed in the microscope.

Judging from the small and insignificant weight losses, no attack was caused by *Ceratocystis albida* on cotton fibres. Neither could any degradation of the cotton fibres be seen in the microscope. The pH of the culture filtrates was considerably higher than that of the non-inoculated controls. Significant weight loss was, however, obtained in the jute fibres, 30.8 percent after 42 days. Microscopic examination of the jute fibres revealed numerous soft rot cavities. Cellulase and xylanase could not, however be detected in the culture filtrates. The pH of the filtrates was lower than that of the controls.

Wardomyces inflatus was capable of attacking both cotton and jute. After 42 days the weight losses were 12.9 and 19.8 percent respectively. Microscopical examination revealed erosion in the cotton fibres and erosion and soft rot cavities in the jute fibres. Both cellulase and xylanase were detected in the culture filtrates from the flasks with cotton after 42 days, though neither of these enzymes appeared in the jute cultures. The pH of the culture filtrates from both of the substrates was higher than the pH in the control flasks.

Xylogone sphaerospora was the only species which produced a higher weight loss of cotton than of jute, 59.5 and 27.2 percent respectively, after 42 days. Cellulase and xylanase were detected in filtrates from jute fibre cultures, whereas only cellulase was detected in the cultures with cotton. Only an erosion type of attack was seen under the microscope in both types of fibres. The pH of the culture filtrates was lower, especially the pH of filtrates from cultures with cotton, than the pH of the controls.

3.9 Effect of various amounts of glucose added to medium EP on the production of cellulase and xylanase.

Table 9 shows the influence of the addition of various amounts of glucose to the EP medium on the production of cellulase and xylanase.

The addition of up to 0.1 percent glucose as "start-glucose" did not stimulate *Bispora betulina, Ceratocystis albida* or *Phialocephala* sp. A to produce cellulase or xylanase. Table 9 shows that no cellulase or xylanase could be detected in any of the culture filtrates. The pH was higher than that of the autoclaved EP medium before inoculation.

It can be noted in Table 9 that increasing amounts of glucose retarded the production of cellulase and xylanase by *Cladorrhinum* sp. A, *Xylogone sphaerospora* and Fungus A. The addition of glucose had a greater influence on the production of xylanase than on that of cellulase. 0.25 percent glucose prevented the formation of detectable amounts of xylanase by *Cladorrhinum* sp. A and *Xylogone sphaerospora.* The latter species produced detectable cellulase at 0.5 percent glucose, whereas cellulase could not be found for *Cladorrhinum* sp. A at this glucose concentration.

Fungus A produced cellulase after seven days in cultures with 0.005 and 0.01 percent glucose, but none in cultures with 0.025 percent. However, after 14 days cellulase was found in cultures with 0.1 percent glucose, which was the highest concentration tested with this fungus. Xylanase was also found after 14 days in cultures with 0.1 percent glucose.

The pH of the culture filtrates from *Cladorrhinum* sp. A, *Xylogone sphaerospora* and Fungus A were in most cases lower than the start pH, especially if increasing amounts of glucose had been added.

3.10 Comparison of the results obtained by the different methods

Table 10 shows which of the enzymes cellulase, xylanase, mannanase and amylase could be demonstrated for each of the species by using the different methods. The results should be compared with the wood-degrading ability of the different species as given in Table 2. The results of tests of the growth on cellulose agar plates and the clearing of cellulose in these plates are not included in Table 10.

Cellulase could be demonstrated for twenty-four of the thirty-six species. Of these twenty-four species, twenty were found to be active when the Rautela-Cowling technique was employed. Twenty species produced detectable amounts of cellulase in liquid cultures. The weight loss test with a cellulose substrate (cellulose N) resulted in nineteen positive results and the agar-plug method yielded eighteen positive results. All species were not tested by the wood block method but when this method was used, sixteen out of thirty species were found to be cellulolytic. Thirteen of the wood-degrading species failed to show cellulolytic activity on pure cellulose substrates. All of them were able to produce soft rot cavities within the birch wood. They will subsequently be referred to as "non-cellulolytic" soft rot fungi.

The highest number of positive results for xylanase activity was obtained with the Rautela-Cowling technique. When this technique was used, twenty-seven of the thirty-six species were found to produce xylanase. Eighteen species produced detectable amounts of xylanase in liquid cultures and when the wood block method was used, xylanase activity could be demonstrated for fourteen of the thirty species tested by this method. Tests for mannanase and amylase were carried out only with the Rautela-Cowling technique so no comparisons can be made with the other methods.

All species except *Phialophora verrucosa* produced amylase. The following twenty species were found to also produce all of the three other enzymes: *Acremonium atrogriseum, Chrysosporium pannorum, Cladorrhinum* sp. A, *Cladosporium* sp. A, *Coniothyrium fuckelii* var. *sporulosum, Cordana pauciseptata, Dictyosporium elegans, Humicola grisea, Petriellidium boydii, Phialophora fastigiata, Phialophora hoffmannii, Phialophora* sp. A, *Pseudeurotium zonatum, Rhinocladiella anceps, Scytalidium lignicola, Scytalidium* sp. B, *Wardomyces inflatus, Xylogone sphaerospora,* Fungus A and Fungus D.

Ten of these species exhibited both cellulase and xylanase activity in all the tests used. One of these ten species, Fungus D, was not tested by the wood block method. It produced, however, positive results in all the other tests.

Nine of the twenty species mentioned above failed to show cellulase or xylanase activity in one or more of the tests. *Cladosporium* sp. A did not cause any weight losses of cellulose N and xylanase could not be detected in culture filtrates. This species and *Scytalidium* sp. B were not tested by the wood block method. *Scytalidium* sp. B did not produce detectable amounts of xylanase in liquid cultures. *Dictysporium elegans, Petriellidium boydii* and *Wardomyces inflatus* did not show cellulase and xylanase activity with the wood block method. No xylanase activity was found when employing the same method for *Pseudeurotium zonatum*. Cellulolytic activity could be demonstrated only by the wood block method for *Phialophora* sp. A, whereas xylanase activity was found with all the other three test methods used. Fungus A did not produce clearing of cellulose with the Rautela-Cowling technique nor did it produce cellulase when grown on cellulose agar plates. But this species caused high weight losses of cellulose N in liquid cultures with EP medium and cellulase was detected in the culture filtrates.

Two of the cell wall degrading enzymes could be demonstrated for the following species: *Bispora betulina* (cellulase and xylanase), *Humicola alopallonella* (cellulase and xylanase), *Phialocephala dimorphospora* (cellulase and mannanase), *Phialocephala* sp. A (cellulase and xylanase) and Fungus B (xylanase and mannanase). *Bispora betulina* and *Phialocephala* sp. A did not show cellulolytic activity in any other test than the wood block test. *Bispora betulina* failed to show xylanase activity in this test. Both the species produced clearing of xylan with the Rautela-Cowling technique but none of them was able to produce detectable amounts of xylanase in liquid cultures. *Humicola alopallonella* failed to show cellulase and xylanase activity in the wood block test, nor was any xylanase produced in liquid cultures.

Phialocephala dimorphospora produced clearing of cellulose with the Rautela-Cowling technique but did not produce cellulase when growing on cellulose agar plates. Nor did this species produce any weight losses of cellulose N in liquid cultures and cellulase could not be detected in the culture filtrates. This species was not tested with the wood block method. Fungus B exhibited xylanase activity only in test with the Rautela-Cowling technique.

Only one cell wall degrading enzyme, viz. xylanase, could be demonstrated for *Ceratocystis stenoceras, Cladosporium resinae, Graphium* sp. A and *Mollisia* sp. A.

If the results given in Table 10 are compared with the wood-degrading ability of the different species shown in Table 2, it is obvious that most of the wood-degrading fungi produced at least one of the three cell wall degrading enzymes studied. All the sixteen species which produced an erosion-type attack (Type 2 attack) in birch wood could be shown to produce all three enzymes.

With the test methods used, five of the wood-degrading species failed to show cellulase, xylanase and mannanase activity. These fungi were: *Catenulari heimii, Ceratocystis albida, Gonatobotrys* sp. A, *Phialocephala* sp. C and *Rhinocladiella* sp. A. Table 2 shows that these species are able to produce soft rot cavities (Type 1 attack) but no erosion-type attack (Type 2 attack) in birch wood.

Of the three species *Ceratocystis olivacea, Cladosporium resinae* and *Phialophora verrucosa*, all of which were incapable of degrading birch wood, only *C. resinae* showed xylanase activity. Cellulase and mannanase could not be demonstrated for any of them.

4 Discussion

4.1 Wood degradation and enzyme production

Wood is degraded by a great number of different species of fungi belonging to the Basidiomycetes, Ascomycetes and Fungi imperfecti. The Basidiomycetes cause white or brown rot while the wood-attacking Ascomycetes and Fungi imperfecti cause soft rot. The term "soft rot", coined by Savory (1954) for the attack by microfungi and characterised by cavity formation in the secondary walls of the wood cells, has in later years been used for all types of wood degradation caused by microfungi, irrespective of whether cavities are formed or not.

The main constituents of wood are cellulose, hemicelluloses and lignin. The white rot fungi decompose all three components, whereas the brown rot fungi leave most of the lignin as a residue. Few studies have been made on the changes in chemical composition of wood attacked by pure cultures of microfungi. Only one species, *Chaetomium globosum*, has been studied in detail. These studies were carried out by Savory and Pinion (1958), Levi and Preston (1965) and Seifert (1966). Their results and scattered data from other investigations (Merrill et al. 1965, Bergman & Nilsson 1967 and Lundström 1973) indicate that the main targets for the wood-degrading microfungi are cellulose and hemicelluloses, although small reductions in the lignin content have been reported.

A prerequisite for fungi capable of degrading wood is the production of extra-cellular enzymes that catalyze the dissolution of the polymeric wood components. So far as the author is aware, no fungus has proved capable of removing only one of the three main components of wood. The removal of only one component is probably physically blocked by the other components. Removal of two of the components is, however, possible as shown by the brown rot and soft rot fungi. These fungi which degrade the cellulose and hemicelluloses of the wood must produce extra-cellular cellulases and hemicellulases. The main constituents of hemicelluloses are xylan in hardwoods and mannan in softwoods. The brown rot and soft rot fungi would consequently be expected to produce cellulase, xylanase and mannanase. A simultaneous production of xylanase and mannanase is most probable since production of only one of these enzymes would restrict the degradation ability to either hardwoods or softwoods.

It is interesting to note that many fungi which have proved to be cellulolytic have also been found to produce xylanase and mannanase. This also applies to fungi whose wood-degrading abilities are unknown (cf. Lyr & Novak 1961).

Lyr (1963) showed that the wood-rotting Basidiomycetes, *Schizophyllum commune, Trametes versicolor, Phellinus igniarius, Collybia velutipes, Fomes marginatus, Coniophora cerebella, Piptoporus betulinus* and *Gloeophyllum saepiarium* produced both xylanase and mannanase. He also found that both of these enzymes were produced by the cellulolytic mould *Trichoderma viride* and the well-known soft rot fungus *Chaetomium globosum*. Xylanase production by *C. globosum* has also been found by Sørensen (1952 and 1957) and Fuller (1970).

Ahlgren and Eriksson (1967) showed that three wood-rotting fungi, *Chrysosporium lignorum*[1)], *Fomes annosus* and *Stereum sanguinolentum* produced cellulase, xylanase

[1)]The fungus called *Chrysosporium lignorum* is, according to "Centraalbureau voor Schimmelcultures" in Baarn, identical with *Sporotrichum pulverulentum* Novobranova.

and mannanase. Lyr and Novak (1961), who studied the production of these enzymes among ten species of Fungi imperfecti, found that the three enzymes were produced by all of the species. Gascoigne and Gascoigne (1960) found xylanase activity among several species of fungi which are also known to be cellulolytic. In a study of a soil fungus population, Domsch and Gams (1969) found that *Cephalosporium (= Acremonium) furcatum, Chrysosporium pannorum, Coniothyrium fuckelii* var. *sporulosum, Cylindrocarpon didymum, Cylindrocarpon magnusianum, Doratomyces microsporus, Epicoccum nigrum, Gliomastix (= Acremonium) murorum, Margarinomyces (= Phialophora) luteoviridis, Oidiodendron echinulatum,* species in the *Phialophora fastigiata* group, *Phoma eupyrena, Pseudogymnoasus roseus, Pseudeurotium zonatum. Trichocladium opacum* and *Verticillium nigrescens* could decompose xylan. Nilsson (1973) found that all of the mentioned species were cellulolytic and all of them, except *Oidiodendron echinulatum,* were able to degrade birch wood.

Thus, it is obvious that there is a connection between the ability to produce cellulase and the ability to produce xylanase and mannanase. This is also shown by the fact that xylanase and mannanase are often produced even if the fungus is cultivated on pure cellulose. Eriksson and Goodell (in press), who prepared a number of cellulase-less mutants of the wood-rotting fungus *Polyporus adustus*, found that most of the mutants lacked xylanase and mannanase as well. One of the cellulase-less mutants was mutagenized and cellulose-degrading revertants were selected. These revertants were not only found to be capable of degrading cellulose, but they also degraded xylan and mannan. On the basis of their results, Eriksson and Goodell suggested that the induction of cellulase, xylanase and mannanase is controlled by a single regulator gene.

Table 10, which summarizes the results of all experiments in the present investigation, shows that all three enzymes could be demonstrated for twenty of the thirty-three wood-degrading species. Five species were shown to produce two of the enzymes and for three of the species only one enzyme was demonstrated. Five species, all producing soft rot cavities in birch wood, seemed to lack cellulase as well as xylanase and mannanase. The apparent lack of one, two or all three of the enzymes by the wood-degrading species is most likely due to imperfect test methods and not to a real incapability to produce these enzymes. This will be discussed later in more detail.

Of the three species which were unable to degrade wood, viz. *Ceratocystis olivacea, Cladosporium resinae* and *Phialophora verrucosa*, only *C. resinae* produced xylanase. Cellulase and mannanase were not produced by any of the species.

It was observed in a previous study (Nilsson 1973) that all species which were able to produce an erosion-type attack (Type 2 attack) on the cell walls of birch wood were also able to produce clearing of Walseth cellulose. The same was found in the present investigation and, furthermore, it was ascertained that such species could also be shown to produce the enzymes xylanase and mannanase. It is obvious that hyphae which are growing in the cell lumina and which act upon the surface of the cell wall must produce more or less diffusible enzymes which accomplish the degradation of the components of the cell wall by means of erosion.

In the investigation mentioned above, it was also observed that a few of the species which were able to produce clearing of Walseth cellulose did not produce erosion-type attack in birch wood. It was suggested that this failure could be due to a lack of wall-degrading enzymes like xylanase and mannanase. Another explanation was that the S_3 layer of the wood fibres represented a resistant barrier which could not be degraded by these species and through which their enzymes could not diffuse. *Acremonium atro-griseum* and *Petriellidium boydii* are such species which failed to produce erosion of the cell walls of birch wood although they were able to produce clearing of Walseth cellulose. The birch wood was, however, degraded by these species through cavity formation. As may be seen in Tables 2 and 10, these species are able to produce

diffusible xylanase and mannanase as well as diffusible cellulase. Thus, it is evident that their failure to produce an erosion type of attack is not due to a lack of these enzymes but is more probably due to their inability to degrade the S_3 layer.

A literature survey covering the degradation of cell wall components by those species in the present study which are fully identified has been made. For several of the species no information about cellulolytic activity or degradation of xylan and mannan could be found. Some of the data collected is found below. The data presented by the author in a previous paper (Nilsson 1973) are not included.

Ceratocystis albida (= Ophiostoma albidum). Käärik (1960) found that cellulose was a very poor source of carbon for this species, as well as for other *Ceratocystis* species. However, she stated that all species were "able to make a starvation type of growth on it".

Chrysosporium pannorum. Degradation of filter paper by this species was found by Schaefer (1957). Domsch and Gams (1969) found that the fungus degraded xylan and CMC.

Cladosporium resinae. Some variation seems to exist among different strains. Parbery (1969) found that one out of five isolates was able to degrade cellulose.

Coniothyrium fuckelii var. *sporulosum.* Domsch and Gams (1969) found that this fungus degraded xylan and CMC.

Humicola alopallonella. Meyers and Reynolds (1959) found that this species was cellulolytic.

Humicola grisea. Traaen (1914) already showed that this fungus decomposed cellulose. Later, several investigations have proved the cellulolytic activity of this species (cf. Domsch & Gams 1970). Poole and Taylor (1973) found production of cellulase and xylanase.

Phialophora fastigiata. Reese et al. (1950) showed that *P. fastigiata* reduced tensile strength of cotton duck. Domsch and Gams (1969) found that this species degraded xylan and CMC. Fuller (1970) found that production of cellulase in liquid cultures but no xylanase was found. King and Eggins (1973) showed that the fungus reduced the strength of filter paper.

Phialophora verrucosa. Reese et al. (1950) found that this species was unable to reduce the strength of cotton textile.

Pseudeurotium zonatum. Domsch and Gams (1969) showed that this species degraded xylan and CMC.

Scytalidium lignicola. King and Eggins (1973) showed that this fungus could degrade cellulose.

If the above data are compared with the results obtained in the present study, it is evident that there is good agreement. Unfortunately, no studies seem to have been done earlier with the most interesting species in this study, i.e. those which degrade wood but fail to show production of the necessary enzymes. The only exception is the study of *Ceratocystis albida* by Käärik (1960).

Production of amylase is not necessary for the degradation of wood but might be beneficial for fungi colonizing wood substrates since starch is an important reserve nutrient in many trees. The present investigation shows that the ability to degrade starch is common among wood-inhabiting microfungi. This was also found by King and Eggins (1973) who found amylolytic activity in all of the thirty-three different mould and staining fungi which were tested.

4.2 Test methods and results

In the following the results obtained with the different test methods will be discussed.

Numerous methods have been used for assays of cellulolytic activity among microorganisms. Eriksson (1969) gives a list of various assay methods used for determining cellulolytic activity. His list contains the following methods:

1) loss in weight of insoluble substrates
2) decrease in mechanical properties of fibres or films
3) change in turbidity of cellulose suspension
4) increase in reducing ends groups
5) decrease of viscosity of cellulose derivatives
6) colorimetric determination of dissolved decomposed products of cellulose
7) measurements of clearance zones in cellulose agar

To this list may be added:
8) microscopic observations of morphological changes in a cellulose substrate (like fibres or cellophane)
9) growth on cellulose agar

Except for method 9, these methods can be used both for determining the cellulolytic activity of growing microorganisms and the activity of isolated cellulases.

Methods 3 and 7 are very much of the same type. These assay methods, which aim at a clearing of cellulose substrates, have been extensively used in this study. Methods 1, 8 and 9 have also been used. All of the methods referred to may be considered to be indicative of a C_1 enzyme *sensu,* Reese, Siu and Levinson (1950).

Measurements of the decrease of viscosity of cellulose derivatives (method 5) have been widely used for determining the cellulolytic activity of microorganisms. Most of the studies have been carried out with carboxymethyl cellulose (CMC) as substrate. Yet Reese and Levinson (1952) already found several non-cellulolytic organisms that produced CMC-degrading enzymes. These enzymes are usually referred to as C_X enzymes. The production of C_X enzymes by non-cellulolytic fungi has later been demonstrated in other investigations. Wood (1969) referred to these fungi as "pseudo-cellulolytic" species. The aforementioned method has applications in the studies of the components of the cellulase system, but it should not be used for assays of the cellulolytic activity of microorganisms.

Reese, Siu and Levinson (1950) suggested that truly cellulolytic fungi possess an additional enzyme, C_1, which enables them to degrade native cellulose. King and Vessal (1969) also defined the C_1 enzyme as an enzyme which is required for the hydrolysis of highly oriented solid cellulose, like cotton and Avicel, by $\beta - 1 \rightarrow 4$ glucanases ($=C_X$). Since wood cellulose also is a highly oriented solid cellulose, it follows from the definition that all fungi which are able to degrade wood must produce C_1 enzymes. Thus, the inability of certain fungi to degrade cotton or Avicel does not necessarily mean that they lack C_1 enzymes. It may possibly be that the culture conditions are unsuitable for the production of this enzyme.

Whether the degradation of a modified cellulose such as Walseth cellulose indicates C_1 activity or merely C_X activity has already been discussed in a previous paper (Nilsson 1973). The findings, that most of the fungi which degraded Walseth cellulose were also able to degrade wood cellulose, and the fact that CMC-degrading fungi failed to degrade Walseth cellulose, support the former theory.

As can be seen in Table 10, a high number of positive results were obtained by means of the Rautela-Cowling technique. This technique has several advantages. It is simple to carry out and, as pointed out by Rautela and Cowling (1966), cellulolytic activity is "determined directly on a continuous, cumulative basis". Other substrates than cellulose, e.g. hemicelluloses and starch, can also be tested with this technique, as has been done in the present study. This method also seems to be quite sensitive and appears to give positive results even for species with weak cellulolytic activity.

It was already shown in a previous paper, Nilsson (1973), that cultivation on medium VII suggested by Bravery (1968) yielded a higher number of positive results for cellulolytic activity than cultivation on the medium formulated by Rautela and Cowling (1966). Several species which failed to produce clearing on R–C medium did clear the cellulose on B–VII medium. This was also confirmed in the present study in the case of two additional species, viz. *Cladosporium* sp. A and Fungus D. This phenomenon might be

explained by the presence of an alternative carbon source (yeast extract) in the R–C medium, since it was demonstrated by Bravery (1968) that small amounts of alternative carbon sources, like asparagine and yeast extract, inhibited clearing of cellulose agar by certain soft rot fungi. The decreased amount of clearing of cellulose produced by all active species on medium R–C compared with the clearing that occurred on B–VII medium (see Table 2) might also be due to inhibition by the presence of yeast extract in the former medium. Alternative carbon sources like glucose, asparagine and yeast extract appear, however, to exert a varying influence on the cellulolytic activity depending on the species of fungi and also on the culture conditions. As can be seen from the results of the tests with cellulose in liquid cultures (Tables 6 and 7), the presence of asparagine and yeast extract in the EP medium appeared to have no adverse effects on the cellulolytic activity of the tested fungi. Park (1973), who made a study similar to that of Bravery (1968), tested several modifications of the Eggins and Pugh (1962) medium. He reached the conclusion that the presence of asparagine might have disadvantages, whereas yeast extract seems to have no disadvantages. Dennis (1972), who studied the clearing of cellulose agar by yeasts *(Trichosporon* spp.) found that small amounts of yeast extract and asparagine stimulated the activity of some of the isolates, but reduced the activity in others. Norkrans and Aschan (1953), who cultivated different strains of *Collybia velutipes* on a weak yeast-glucose-cellulose medium, found that the cellulolytic activity did not change much when cultivated on a richer yeast extract medium. If the strains were cultivated on a glucose-free medium, the activity was the same or decreased.

It is obvious from the results presented in Table 2 that the amount of clearing of cellulose with the Rautela-Cowling technique depends very much on the type of cellulose substrate. The greatest amount of clearing was obtained on Walseth cellulose and ball-milled cellulose. The preparation of these two types of celluloses involves treatments that increase the proportion of amorphus cellulose and also the intrinsic porosity. Both are factors which will increase the susceptibility to hydrolysis (cf. Norkrans 1950, Walseth 1952 and Stone et al. 1969).

Table 3 shows the results of three different assay methods of cellulolytic activity; 1) growth on cellulose agar, 2) clearing of cellulose underneath or around fungal colonies growing on cellulose agar plates and 3) clearing of cellulose in test plates around transferred mycelium-agar plugs. The only difference between method 2 and the Rautela-Cowling technique is that the former is carried out in petri dishes while the latter employs test tubes.

It is evident from the results that growth on cellulose agar is a very uncertain criterion for cellulolytic activity. Visual estimation of the amount of growth of fungi on agar is difficult since the fungi might produce different types of colonies. Some fungi produce a dense mycelium on a restricted area on a certain medium, while on other media the same species will produce a widely spreading but thin mycelium. The estimation of growth and non-growth on cellulose agar media of the types used in the present investigation seems impossible since the truly non-cellulolytic fungi also produced some growth on the B–VII medium which contains no source of carbon other than cellulose. It is possible that these fungi are able to make some growth on impurities containing carbon, which may be present in the Avicel that was used as cellulose substrate. The other cellulose agar medium used, F6A, contains both glucose and yeast extract. This explains why all of the tested species were able to grow on this medium. In fact, two of the non-cellulolytic species grew better on F6A celluloses agar than some of the cellulolytic species. The species which had failed to produce clearing of cellulose with the Rautela-Cowling technique (see Table 2) made sparse or moderate growth even on the F6A cellulose agar. They did not appear to be able to utilize the cellulose in the medium, since no clearing of the cellulose could be detected in the agar nor was any cellulase detected with the mycelium-agar plug technique of Savory et al. (1967).

A rather paradoxical conclusion which can

be drawn from the results obtained in the present study is that cellulose agar substrates are not suitable for the isolation of certain cellulolytic fungi.

The two agar media used for these studies contained Avicel, a cellulose which is more resistant to hydrolysis than Walseth cellulose and ball-milled cellulose. This might explain the failure of most of the species to produce visible clearing in the cellulose agar on which they grew. It was already shown in Table 2 that clearing of Avicel was very weak, as compared with the clearing of the two other celluloses. But the obscuring by dense mycelia, which made it difficult to detect any clearing probably also contributed to the low number of positive results.

The agar-diffusion method employing mycelium-agar plugs adopted after Savory et al. (1967) had no advantages over the Rautela-Cowling technique. The agar-diffusion method is more time consuming and the assays of cellulolytic activity are made after certain arbitrarily chosen times. The number of positive results was lower with this method than with the Rautela-Cowling technique (see Table 10). Cellulolytic activity could not be detected with the mycelium-agar plug technique for two of the fungi, *Phialocephala dimorphospora* and *Rhinocladiella anceps,* which had produced clearing of cellulose with the Rautela-Cowling technique.

If the production of cellulase on the cellulose agars F6A and B–VII is compared for the fungi which produced clearing of cellulose only on medium B–VII when the Rautela-Cowling technique was used (see Table 2), it is evident that the glucose, and possibly also the yeast extract, present in the cellulose agar F6A did inhibit the cellulolytic activity of these fungi. Of these fungi only *Cladosporium* sp. A was able to produce detectable amounts of cellulase on F6A agar.

The test method described in Materials and methods (section 2.5) which employs the detection of cellulase and xylanase present in wood blocks attacked by the test organisms, is based on different premises than the other test methods used in the present investigation. With the other test methods nothing is known about the formation of cellulase on the substrate used. But when solid wood is used as substrate, the degradation of the wood cell walls in the form of erosion or cavity formation indicates that cellulase is present. The degradation of the cellulose in the cell walls is easily detected by microscopic studies using polarized light and is in itself a demonstration of cellulolytic activity. By means of the wood block method it could be shown that the cellulase present in the wood could degrade a pure cellulose such as Walseth cellulose (see Table 4).

The wood block method was the only means by which cellulase could be demonstrated for *Bispora betulina, Phialocephala* sp. A and *Phialophora* sp. A. The xylanase activity of *Ceratocystis stenoceras* was demonstrated only by this method. Fungus A, which had failed to demonstrate cellulolytic activity in the previous tests discussed, was found to be cellulolytic when the wood block method was used and also when cultured on cellulose in liquid media.

The species *Catenularia heimii, Ceratocystis albida, Ceratocystis stenoceras, Gonatobotrys* sp. A, *Graphium* sp. A, *Mollisia* sp. A, *Rhinocladiella* sp. A and Fungus B, all of which failed to show cellulolytic activity with the other test methods used, also failed with the wood block method. However, microscopic studies showed that numerous cavities were present in all the wood blocks attacked by these species, with the exception of wood blocks attacked by *Mollisia* sp. A in which only a few cavities were found. It was further ascertained that several species which showed cellulolytic activity in other tests failed in the wood block method. Two explanations can be given for the failures in the wood block method: 1) the enzymes are produced in only minute amounts in the wood, adequate for wood degradation but not sufficient for diffusion out into the test agar; 2) the enzymes are firmly bound either to the wood substrate and/or the mycelium and are thus not able to diffuse freely out into the test agar.

The method in which the fungi were cul-

tured on cellulosic substrates in liquid media provided two criteria for cellulolytic activity. The first was weight loss of the substrate and the other detection of cellulase in the culture filtrate. A weight loss of the cellulosic substrate is not necessarily correlated with detectable amounts of cellulase in the culture filtrate. This can be seen in Table 7 where, for example, *Acremonium atrogriseum, Cordana pauciseptata* and *Scytalidium lignicola* is shown to have caused high weight losses although no cellulase could be detected in the culture filtrates. This might be explained by binding of the enzyme to the mycelium and/or to the substrate. The same fungi, however, produced detectable cellulase on medium EP. Medium B–VII–L is a rather poor medium compared with medium EP. Medium EP contains yeast extract and 212 mg N/liter while medium B–VII–L lacks yeast extract and contains only 115 mg N/liter. When growing on medium B–VII–L the fungi must use their enzymes more economically than when growing on the richer medium. Free enzymes in the solution can be regarded as a wastage of available resources, especially nitrogen.

The fact that cellulolytic enzymes are detected in the culture filtrates is not always correlated with significant weight losses of the substrate. Table 6 shows that cellulase was detected in culture filtrates from *Cladosporium* sp. A on cellulose N, *Dictyosporium elegans* on Avicel and cotton and *Phialophora fastigiata* on Avicel while no significant weight losses occurred. Thus the use of two methods for determining cellulolytic activity increased the number of positive results.

The differences in nitrogen content between the two media might explain why the weight losses were generally lower on the B–VII–L medium. However, two species, *Acremonium atro-griseum* and *Cordana pauciseptata,* caused higher weight losses of one of the substrates, viz. cotton, on medium B–VII–L than on medium EP. It is difficult to explain why the weight losses of the other two substrates, Avicel and cellulose N, were lower on the B–VII–L medium than on medium EP. The results show that the susceptibility of a certain cellulose substrate not only depends on the structure of the substrate but also on the medium in which the tests are carried out. The order of increasing susceptibility of different types of cellulose under one condition might therefore change drastically under other conditions.

Differences in the distribution of the cellulose substrates within the nutrient solution are also likely to influence the results. Cotton, for example, is partly floating and partly immersed in the solution, while the Avicel particles are completely immersed. These particles will sink to the bottom of the flasks if stationary cultures are used. Thus, apparent differences in the susceptibility of the cellulose substrates as found here when growing fungi in liquid cultures must not be considered to reflect an absolute indication of the susceptibility of these substrates to fungal degradation.

It was expected that the species which produced clearing on B–VII but failed to produce clearing of cellulose on the R–C medium (see Table 2) would give the best results on B–VII–L medium since this medium lacks alternative carbon sources. But of these species both *Cladosporium* sp. A and *Humicola alopallonella* produced better results on the EP medium. Only *Scytalidium* sp. B showed a positive response to medium B–VII–L. The same positive response was found for *Rhinocladiella anceps,* although this species was also able to produce clearing of cellulose on the R–C medium.

If the results from the experiments with liquid cultures are compared with the results obtained with the Rautela-Cowling technique, it is found that essentially the same fungi have shown positive results by the two test methods. There are only two exceptions: one is *Phialocephala dimorphospora* which failed to give any indications of cellulolytic activity when cultured in liquid media; the other was Fungus A which had failed to produce clearing of cellulose with the Rautela-Cowling technique. This fungus produced significant weight losses in liquid cultures and cellulase was found in the

culture filtrates. No explanation has been discovered for the behaviour of Fungus A, but the physiological conditions are probably not suitable for the production of cellulase when the fungus grows on agar.

With the exception of Fungus A, all soft rot fungi which failed to produce clearing of cellulose with the Rautela-Cowling technique also failed when cultured in liquid media on cellulose substrates. The conclusion which can be drawn is that these species did not produce cellulase under the prevailing conditions. It might be argued that cellulases were not detected in the culture filtrates because these species may have only cell-bound enzymes. But even if the enzymes are cell-bound, attack on the cellulose would still be possible and this would lead to a weight loss of the substrate. However, no weight losses were recorded for the species mentioned.

The high numbers of positive results for cellulolytic activity obtained from the tests on cellulose in liquid cultures, shown in Table 10, were achieved only by the use of two nutrient media of different composition. If only medium EP had been used the number of positive results from the weight loss determinations would have been 17 instead of 19 and cellulase would have been detected in the culture filtrates for 18 instead of 20 species. The number of positive results would have been far less if only medium B–VII–L had been used. When the Rautela-Cowling technique was used, all positive results could be obtained on a single medium, viz. medium B–VII. It is obvious that this technique is preferable to tests on cellulose in liquid media if the objective is a survey of the cellulolytic activity of microfungi.

Birch wood meal in liquid cultures appears to be an unsuitable substrate for tests of cellulolytic activity since no activity was obtained, even with the highly cellulolytic species *Chrysosporium pannorum*. Degradation of the substrate did not occur, neither could cellulase nor xylanase be detected in the culture filtrates. As can be seen in Table 6, the same fungus caused an extensive degradation of Avicel and cellulose N in liquid cultures and both cellulase and xylanase were found in the culture filtrates. The purpose of using birch wood meal as a substrate was that it contains all the constituents of solid birch wood, in which several of the test organisms made their only manifestation of cellulolytic activity in the form of cavities. But even the cavity-forming species tested were unable to degrade the birch wood meal and to produce cellulase and xylanase.

It is a common observation that lignified substrates are less susceptible to degradation than non-lignified substrates. This seems especially to be the case when isolated cellulase systems are used. However, Table 8 clearly shows that some species of microfungi are able to degrade a lignified fibre more readily than a non-lignified fibre. *Ceratocystis albida* is an exemple of such a species. This fungus failed to degrade the cotton fibres while the jute fibres were degraded to a significant degree although jute fibres contain about 11 percent lignin (Roelofsen 1959). *Wardomyces inflatus* was able to degrade cotton but the weight losses of jute were higher. Only *Xylogone sphaerospora* caused higher weight losses of cotton than of jute. Of the five species used in this experiment, all except *X. sphaerospora* were able to produce soft rot cavities in birch wood. Under the conditions employed here, *Ceratocystis albida* and *Wardomyces inflatus* were also able to produce cavities in the lignified jute fibres but not in the non-lignified cotton fibres.

C. albida, which evidently is unable to produce cellulases in liquid cultures on pure cellulose substrates (cf. Tables 6 and 7), could thus not degrade the cotton fibres. *Wardomyces inflatus* which is able to produce cellulases under the same conditions (cf. Tables 6 and 7) degraded the cotton fibres in this experiment but the degradation of the jute fibres was more extensive due to prominent cavity formation in these fibres.

Bispora betulina and *Phialophora* sp. A are able, as will be shown in a later paper, to produce cavities in jute fibres. However, cavities are not formed by these species in fibres which are immersed in a nutrient

solution as they were in the present investigation. Both of the species are also unable to produce cavities in cotton fibres in liquid cultures. Cellulase was not produced when they were cultured in liquid cultures on pure cellulose substrates or on jute fibres (cf. Tables 6, 7 and 8). These facts explain why the two fungi failed to degrade the cotton and the jute fibres.

Both the cotton and jute fibres were degraded by *Xylogone sphaerospora* by a type of erosion attack. No soft rot cavities were formed in any of the fibres. The erosion is caused by enzymes which are released into the liquid medium by the fungus. The breakdown of the jute fibres under these circumstances appears to be limited by the presence of lignin. The fungus consequently caused the highest weight losses on the non-lignified cotton fibres.

The observations made in the experiments discussed above might also explain the results obtained by Chakravarty et al. (1962). They compared the degradation of jute fibres with degradation of filter paper. All species tested except one, *Sordaria hypocoproides,* caused the highest weight losses when cultured on filter paper. *S. hypocoproides,* however, caused higher weight loss of jute fibres than of filter paper. It might be possible that this species is also able to produce cavities within the jute fibres and that the amount of cellulase released into the nutrient medium is small. But so far as the present author is aware, no studies of the cavity-forming ability of this fungus have been carried out.

Simpson and Marsh (1964) discovered that *Aspergillus niger* and certain other *Aspergillus* species caused a strength loss of cotton fabric only when a small amount of glucose was added to the test medium. Similarly, Basu and Ghose (1960) found that additions of sugar, especially xylose, stimulated the production of cellulase by certain species of fungi which had been found to be unable to produce cellulase when growing on pure cellulose. The addition of small amounts of glucose as "start-glucose" to medium EP had, however, no positive effect on the production of cellulase by *Bispora betulina, Ceratocystis albida* and *Phialocephala* sp. A (see Table 9). Thus, the initial lack of an easily available carbon source does not explain why these species failed to show cellulolytic activity.

The addition of 0.1 percent glucose to medium EP delayed cellulase production of Fungus A only slightly. Since the EP medium also contains small amounts of asparagine and yeast extract it is evident that this species is not prevented from producing cellulase by small amounts of alternative carbon sources.

The cellulase production of *Cladorrhinum* sp. A and *Xylogone sphaerospora* was delayed by glucose additions and *Cladorrhinum* sp. A produced no cellulase at a glucose concentration of 0.5 percent. It has been shown by Bemiller et al. (1969) and others that the cellulase production does not start until the glucose in a nutrient solution has been consumed. The more sugar added, the longer time it will take for the consumption of glucose and the initiation of cellulase production. This fact will also explain the delay in cellulase production observed in the experiments carried out here.

The conclusion drawn from the experiments presented here and from the preceeding discussion is that there is no single simple method for the assay of the cellulolytic activity fo microfungi. This does not apply solely to fungi isolated from wood, since fungi with an apparent lack of cellulolytic activity but which are able to produce cavities in various cellulosic substrates, might also be isolated from other sources, for example soil and compost heaps. It has been shown by Baker (1939) and by Nilsson (1974) that in nature cavity formation of the soft rot type is not restricted to wood but might also occur in other materials containing cellulose fibres.

If a total survey of the cellulolytic activity is objective, the following procedure is recommended:

1) All the isolated species should be tested by means of the Rautela-Cowling technique, employing Walseth cellulose as the

substrate. 0.25 percent appears to be a suitable concentration of the cellulose. Ball-milled cellulose might also be used but the number of positive results is likely to decrease. Avicel and similar types of celluloses should be avoided. The use of medium B–VII is recommended since it yielded a greater number of positive results than the medium used by Rautela and Cowling (1966). All of the species which produce clearing of the cellulose can safely be regarded as cellulolytic but obviously nothing is known about the cellulolytic activity of the remaining species since certain soft rot fungi of the type described in the present study will not produce any clearing.

2) All of the species which have failed to produce clearing of cellulose are tested on birch wood (*Betula* species) for the ability to form soft rot cavities. Some other hardwood species might equally well be used, such as aspen (*Populus* species) or beech (*Fagus silvatica*), but it must be ascertained that the wood used is not very resistant to attack by soft rot fungi. The decay method used by Nilsson (1973) and which employs agar slopes in test tubes is recommended. The time required for cavity formation varies among the species. Most of the species tested here have formed numerous cavities in birch wood already within 2–4 weeks. The species which produce soft rot cavites must also be regarded as cellulolytic and be included in the total number of cellulolytic species. It is highly probable that most of the remaining species are non-cellulolytic, though it cannot be totally excluded that some of these species are able to degrade cellulose under certain special conditions. The procedure described above will, nevertheless, increase the number of cellulolytic species, even if not all of the cellulolytic species are detected.

The recommended procedures are restricted to assays of the cellulolytic activity of microfungi. Problems concerning the assays of the cellulolytic activity of the basidiomycete fungi will be discussed in a later paper.

The test method described above may appear tedious and time-consuming but until new methods for assaying cellulolytic activity are found, it is a procedure which will give reliable information of the cellulolytic activity of species of microfungi.

4.3 The anomalous behaviour of the "non-cellulolytic" soft rot fungi

It was previously suggested that the wood-degrading species would produce all the three cell wall degrading enzymes cellulase, xylanase and mannanase. This assumption was also confirmed for about half of the number of the species tested. But some wood-degrading species failed to exhibit one or more of these enzymes when assayed with the test methods employed. Various explanations of this behaviour are possible. The lack of activity against xylan and mannan could be due to alterations of these substrates during their preparation from wood so that their structure differs too greatly from the natural. It is also possible, in fact, that some fungi lack these enzymes. It is more difficult to explain the failure of certain species to degrade the pure cellulose substrates. This phenomenon and others concerning the production of cellulase will now be discussed in more detail.

Cellulase is the only enzyme which is with certainty known to be produced by the wood-degrading species since degradation of cellulose occurred in the birch wood fibres. This degradation was easily detected in the microscope using polarized light. Since no chemical analysis of birch decayed by the test organisms was made, nothing is really known about the degradation of xylan. It may, however, be assumed to be very probable that the xylan was also decomposed.

The discussion will be focused on the following organisms, all of which produced soft rot cavities in the birch wood but failed to exhibit cellulolytic activity on pure cellulose substrates: *Bispora betulina, Catenularia heimii, Ceratocystis albida, Ceratocystis stenoceras, Gonatobotrys* sp. A, *Graphium* sp. A, *Mollisia* sp. A, *Phialocephala* sp. A, *Phialocephala* sp. C, *Phialophora* sp. A, *Rhinocladiel-*

la sp. A and Fungus B. These fungi have previously been referred to as "non-cellulolytic" soft rot fungi. The presence of cellulase in attacked birch wood blocks could be demonstrated for some of these species. The experiments carried out using the Rautela-Cowling technique and the cultivation of the fungi on pure cellulose substrates in liquid media showed that no cellulase was produced under the prevailing conditions. These findings suggest that cellulase of the mentioned species is an inducible enzyme and that the induction is regulated by factors which are so far unknown.

Various explanations may be advanced for the failure of the "non-cellulolytic" soft rot fungi to degrade pure cellulose substrates under the conditions prevailing in the present investigation.

1) the "non-cellulolytic" soft rot fungi have a very weak cellulolytic activity.

There have been some false opinions regarding the cellulase activity of the soft rot fungi (cf. Lyr 1960, Levi 1964 and Wilhelmsen 1965). It has been claimed that these fungi produce less of these enzymes than the white and brown rot fungi. Such generalisations should not be drawn unless they are based on a large number of observations. Seifert (1966) could, in fact, show that in the early stages, cellulose degradation of beech wood by the soft rot fungus *Chaetomium globosum* was greater than the degradation caused by one white and one brown rot fungus.

In fact, the soft rot species tested here can be regarded to have a rather high cellulase activity when growing on birch wood. If a typical soft rot cavity is observed, only a single hypha is present in each cavity. The cavity may be very large in relation to the size of the hypha. This indicates a high enzyme activity per mycelium unit.

The opinion that the soft rot fungi have a low cellulolytic activity probably is a result of the fact that several of these fungi cause comparatively small weight losses of the wood blocks used in decay tests. It has been shown by Lundström (1973) that wood blocks in laboratory tests are most heavily degraded in the outer layers. Weight losses reported from laboratory tests refer to the weight loss in the whole blocks. Thus, a thin outer layer of the wood blocks might be very highly degraded even if the total loss in weight is small. Microscopic observations of the soft rot attack on birch wood blocks by the "non-cellulolytic" soft rot fungus *Ceratocystis albida* (Nilsson, not published) have shown that the wood fibres in the outer layers of the birch wood blocks may already be degraded to 50 – 90 percent after three weeks. Thus the low weight losses caused by some soft rot fungi seem to a higher degree to be due to a restriction of the attack to the outer layers of the wood than to a low cellulolytic activity.

2) The cellulase production is temperature-dependent.

It has been demonstrated that the cellulolytic activity of some fungi is quite sensitive to temperature. Marsh et al. (1949) and Reese and Levinson (1952) have shown that *Cladosporium herbarum* has cellulolytic activity at 25°C but not at 30°C. Hirsch (1954) found that *Neurospora crassa* had a very weak cellulolytic activity at 25°C compared with the activity at 35°C. The wood-degrading species in the present investigation, which had failed to produce clearing of cellulose at the ambient room temperature (23–25°) were also incubated at 15°C and 30°C. Since no clearing of the cellulose occurred even after incubation for six weeks at these temperatures, it must be regarded as unlikely that the failure to produce clearing zones in the cellulose was due to a temperature factor.

3) Some growth factors are lacking in the media used.

The "non-cellulolytic" soft rot fungi produced at least some growth on the cellulose agar media used. All species also grew in the liquid media EP and B–VII–L when glucose was added. Thus, it is not likely that their failure to show cellulolytic activity is due a lack of growth factors, unless certain growth factors are required specially for the production of cellulase.

4) The "non-cellulolytic" soft rot fungi initially require an easily available carbon source ("start-glucose")

This hypothesis was tested (see Table 9) and it was found that no stimulation of the cellulase production could be ascertained when glucose was added in small amounts.

5) The cellulase is cell-bound.

According to Cowling and Brown (1969), the cavities formed in the wood cell walls by soft rot fungi are an example of a degradation pattern produced by cell-bound enzymes. However, the mere fact that the degradation is restricted to localized areas within the cell walls does not prove that the enzymes are cell-bound. The restriction of the attack is more probably due to the compact structure of the cell wall in which the enzymes are formed. If the enzymes are unable to diffuse through this compact structure they will migrate only with the simultaneous dissolution of the components of the cell wall.

In the present study it has been found that several of the fungi which produce soft rot cavities also produce diffusible cellulases as indicated by the clear zones formed in cellulose agar beneath the growing fungi. Several of these species also release cellulase into the nutrient solution when grown in liquid media. Most, but not all, of these species also cause an erosion of the wood cell walls. However, it might be possible that such species produce two different types of cellulases; one which is diffusible and produced by luminal hyphae, while the other is cell-bound and produced by hyphae within the cavities in the wood cell walls. The "non-cellulolytic" species which were all found to be unable to cause an erosion of the wood cell walls would thus only produce the cell-bound enzyme.

As stated previously, the possession of only cell-bound cellulases would still allow the "non-cellulolytic" species to degrade the celluloses used as substrates in the liquid cultures as well as the cellulose used in the agar media. Since none of these species were able to degrade cellulose under the conditions used, it is unlikely that cell-bound enzymes is an explanation of the anomalous behaviour of these fungi. Furthermore, the clear zones obtained around wood sections cut from wood blocks decayed by some of the "non-cellulolytic" species indicate that the cellulases of these species are not cell-bound.

6) Cellulase is not produced on pure cellulose.

The cellulose substrates which were used, viz. Avicel, cotton, cellulose N, Walseth cellulose and ball-milled cellulose, can be regarded as pure celluloses containing only small amounts of other substances. Wood, on the other hand, contains hemicelluloses and lignin in addition to cellulose. It might be possible that the hemicelluloses and/or lignin in some way stimulate the production of cellulase. Basu and Ghose (1960), have, for example, found that several species of microfungi were able to produce cellulase on jute holocellulose (which contains hemicelluloses) but not when grown on a pure cellulose like filter paper.

In the present study some of the "non-cellulolytic" soft rots were grown on birch wood meal and jute fibres. As mentioned previously, birch wood meal was not degraded by any of the "non-cellulolytic" soft rot fungi and the jute fibres were degraded only by one species, viz. *Ceratocystis albida*. This would seem to indicate that *C. albida* is able to degrade cellulose only when it is associated with hemicellulose and lignin. This is, however, not the case as will be shown in a later paper. As stated earlier, the use of wood meal and jute fibres in liquid media is an unsuitable method for assays of enzymatic activity since even a cellulolytic species like *Chrysosporium pannorum* failed to show any activity on birch wood meal. Thus, the experiments carried out in the present investigation are not suitable for studies of the influence of associated substances on the production of cellulase. These questions will be discussed more in detail in a later paper.

7) Cellulase production is regulated by the physical structure of the wood.

The formation of soft rot cavities in wood must be regarded as a rather unique type of degradation. The enzyme-producing hyphae of white and brown rot fungi and the hyphae of microfungi which cause erosion of the wood cell walls, are more or less randomly distributed within the wood. The distribution of the cavity-forming hyphae of the soft rot fungi are, however, closely related to the structure of the wood cell wall. These hyphae always appear to grow parallel with the direction of the cellulose microfibrils within the cell walls (cf. Bailey & Vestal 1937 and Nilsson 1974).

It was suggested in a previous paper concerning the formation of soft rot cavities in various cellulose fibres by *Humicola alopallonella* (Nilsson 1974) that the cellulase production was stimulated when the hyphae grow parallel to the cellulose microfibrils inside the wood cell wall. This idea was supported by the following observation: The fungus, when offered a thin transverse section of birch wood, failed to produce an erosion attack on the exposed secondary cell walls. This indicated a low cellulase activity of the hyphae which grew over the wood section. The only hyphae which exhibited cellulolytic activity were those which penetrated the secondary cell walls in the longitudinal direction of the wood fibres. These hyphae appeared to follow the direction of the cellulose microfibrils and they formed typical soft rot cavities in the cell walls.

The only exhibition of cellulolytic activity of the "non-cellulolytic" soft rot fungi which was found was the formation of soft rot cavities in the secondary cell walls of birch wood. The hyphae within the cell walls, which secrete cell wall degrading enzymes and give rise to the cavities, appear to follow closely the direction of the cellulose microfibrils in the same manner as *Humicola alopallonella*. Thus, there is a strong relationship between the enzyme-producing (= cavity-forming) hyphae and the physical structure of the wood cell walls. Based on these observations, the following hypothetical explanation is suggested for the anomalous behaviour of the "non-cellulolytic" soft rot fungi: Theses species are induced to produce cellulase when their hyphae are growing parallel to the cellulose microfibrils whithin the wood cell walls.

8) The physiological conditions of cultivation have not been suitable for cellulase production.

A common feature for all types of experiments carried out here is that the cellulose substrates have had a very high moisture content. The cellulose has either been immersed in liquid media or suspended in agar substrates. Since no experiments with cellulose substrates with lower moisture content have been carried out in the present study, it is impossible to discuss the influence of the moisture content at the present stage. It will, however, be shown in a later paper that the moisture content of the cellulose substrates has a decisive influence on the degradation of these substrates by certain soft rot fungi.

If the last part of the discussion is summarized, the following conclusions can be drawn:

a) The inability of the "non-cellulolytic" soft rot fungi to degrade pure cellulose is *not* due to 1) weak cellulolytic activity; 2) temperature-dependent cellulase in the sense that this enzyme is produced only within a certain temperature range; 3) lack of growth factors; 4) lack of "start-glucose"; 5) cell-bound enzymes.

b) The failure of the "non-cellulolytic" soft rot fungi to degrade pure cellulose might be due to: 1) The absence of substances such as hemicellulase and lignin which are associated with the cellulose in wood; 2) specific regulation of the cellulase production by the physical structure of the wood; 3) unsuitable physiological conditions.

The data from the experiments carried out in the present investigation are evidently insufficient to provide a solution of the problem discussed. It is obvious that much work remains to be done before the decay mechanisms of the soft rot fungi can be understood.

Summary

The production of the enzymes cellulase, xylanase, mannanase and amylase have been studied for thirty-six different species of wood-attacking microfungi. The ability of the fungi to degrade wood was already known. Three of the species lacked the ability to degrade birch wood while the remainder gave rise to the following three patterns of attack in birch wood: 1) soft rot cavities in the secondary cell walls (= Type 1 attack); 2) erosion of the cell walls caused by fungal hyphae in the cell lumina (= Type 2 attack); and 3) simultaneous attacks of Type 1 and Type 2.

It was presumed *a priori* that the wood-degrading microfungi produced all of the three cell wall degrading enzymes, cellulase, xylanase and mannanase. No effort was made to obtain an absolute measurement of the various enzyme activities. The investigations were therefore concentrated on an endeavour to demonstrate the presence of the different enzymes.

The enzyme activity of the fungi was studied by means of the following methods: 1) measuring the clearing zones under fungal cultures growing on various substrates on agar columns in test tubes, Rautela-Cowling technique (cellulase, xylanase, mannanase and amylase activity); 2) the growth, formation of clearing zones and production of cellulase in fungal cultures on cellulose agar plates; 3) determination of the occurrence of cellulase and xylanase in birch wood which had been attacked by the test organisms; 4) determination of the weight losses in various cellulose substrates during culture in nutrient solutions and 5) determination of the occurrence of cellulase and xylanase in culture filtrates from cultures on cellulose substrates in liquid solutions.

The influence of various factors on the cellulase activity of the fungi was studied. Among other factors, studies were carried out to determine the effects of the composition of different media, additives of small amounts of glucose (as "start-glucose"), the type of cellulose substrate, different cultivation conditions such as shake and stationary cultures and different incubation temperatures. The results showed that the composition of the cultivation media was of decisive importance for certain fungi. Small amounts of yeast extract had an inhibiting effect on cellulase activity during cultivation on cellulose agar media. This effect was not noticeable during cultivation on a cellulose substrate in liquid solutions. The largest clearing zones were obtained on Walseth cellulose and ball-milled cellulose. Significantly smaller clearing zones were obtained on Avicel and on cellulose treated with hydrochloric acid. The different species of fungi reacted individually when cultivated in shake or stationary cultures. Some species caused the greatest losses in weight during cultivation in shake cultures while others showed greater activity during cultivation in stationary cultures.

All of the fungi studied, with the exception of *Phialophora verrucosa*, could degrade starch. Among the wood-degrading fungi, all of the three cell wall degrading enzymes, cellulase, xylanase and mannanase, could be determined in twenty species. Four of the twenty species only gave rise to Type 1 attacks (cavities), while the remainder caused some attack of Type 2 (erosion). In five of the wood-degrading fungi, all of which gave rise to attacks of Type 1, none of the enzymes mentioned could be found. Only one or two of the enzymes could be noted in the remainder of the wood-degrading fungi. Among the fungi which lacked the ability to degrade birch wood, xylanase, could be found in connection with one species while cellulase and mannanase could not be discovered in relation to either of these fungi.

The fact that one or more of the three cell wall degrading enzymes could not be detected in all of the wood-degrading fungi is considered to be a result of deficiencies in the test methods employed and not to the inability of the fungi to produce the enzyme.

Twelve of the wood-degrading fungi appeared to be totally unable to degrade pure cellulose substrate under the conditions prevailing in the tests described. All of these fungi caused attacks of Type 1 in birch wood. This attack was the only expression of cellulase activity which could be demonstrated. These fungi were therefore called "non-cellulolytic" soft rot fungi. The fungi could not degrade any of the pure cellulose substrates which were tested in agar media or in liquid cultures. Some of the species were cultivated on birch wood meal in liquid cultures but no degrading of this substrate was obtained. The degradation of cotton and jute fibres in liquid cultures was studied in a smaller test. It proved that one of the "non-cellulolytic" soft rot fungi, *Ceratocystis albida*, achieved a significant degradation of jute fibres while cotton was not attacked. The fungus formed soft rot cavities in the jute fibres but evidently lacked the ability to accomplish this in cotton fibres.

During cultivation on a pure cellulose substrate in a liquid culture, small amounts of glucose (as "start-glucose") had no stimulating effect on the cellulase production of the "non-cellulolytic" soft rot fungi.

Various explanations of the anomalous behavior of "non-cellulolytic" soft rot fungi are discussed. The results obtained demonstrate that their inability of degrade pure cellulose substrate is probably not due to 1) weak cellulolytic activity, 2) temperature factors, 3) the lack of growth factors, 4) the requirement of "start-glucose" or 5) a cell-bound enzyme. Possible explanations may be 1) that the production of cellulase is induced by substances such as hemicellulase and lignin, which are associated with the cellulose in wood; 2) that the production of cellulase is specifically regulated by the physical structure of the wood; 3) that the cultivation conditions employed are unsuitable for the production of cellulase.

A compilation of the results obtained by the various test methods is shown in Table 10. The advantages and disadvantages of the different methods for the determination of cellulase acitivity in microfungi are discussed. Among other things, it is shown that growth on a cellulose agar medium is an uncertain criterium for cellulase activity. The method employing the Rautela-Cowling technique appears to be superior to the other methods.

The present study shows that it is not possible to prove the existance of cellulase in certain species of cellulolytic microfungi by means of several generally employed methods. In studies of a group of microfungi by means of conventional methods it may therefore be anticipated that a number of cellulolytic species will not be detected. This work proposes a procedure, consisting of two methods, for the determination of cellulase activity in microfungi. The ability of the fungi to produce clearing zones in cellulose agar is studied first with the Rautela-Cowling technique. All species which do not show cellulolytic activity with this method are then tested on birch wood for the ability to form soft rot cavities. Since the wood cellulose is degraded during the formation of cavities, all species which are able to form soft rot cavities in the birch wood must be regarded as cellulolytic. The mentioned procedure significantly increases the possibilities of discovering cellulolytic species.

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Sammanfattning

Produktionen av enzymerna cellulas, xylanas, mannanas och amylas har studerats hos trettiosex olika arter av vedangripande mikrosvampar. Svamparnas vednedbrytande förmåga var tidigare känd. Tre av arterna saknade förmåga att bryta ned björkved medan de övriga förorsakade följande tre angreppsmönster i björkved: 1) soft rot (mögelröta) kaviteter i de sekundära cellväggarna (= angrepp av Typ 1); 2) erosion av cellväggarna förorsakade av svamphyfer i cellumina (= angrepp av Typ 2); och 3) samtidigt angrepp av Typ 1 och Typ 2.

Det förutsattes *a priori* att de vednedbrytande mikrosvamparna bildade samtliga av de tre cellväggsnedbrytande enzymerna, cellulas, xylanas och mannanas. Några absoluta mått på de olika enzymaktiviteterna eftersträvades inte. Undersökningarna inriktades därför på att försöka påvisa närvaron av de olika enzymerna.

Svamparnas enzymaktivitet studerades med följande metoder: 1) Mätning av klarningszoner i olika substrat under svampkulturer växande på agarpelare i provrör, Rautela-Cowling teknik (cellulas-, xylanas-, mannanas- och amylsaktivitet), 2) tillväxt, bildning av klarningszoner och produktion av cellulas vid odling av svamparna på cellulosa-agarplattor, 3) bestämning av förekomsten av cellulas och xylanas i björkved som angripits av testorganismerna, 4) bestämning av viktsförluster hos olika cellulosasubstrat vid odling i närlösningar, och 5) bestämning av förekomsten av cellulas och xylanas i kulturfiltrat från odlingar på cellulosasubstrat i närlösningar.

Inverkan av olika faktorer på svamparnas cellulasaktivitet undersöktes. Bland annat studerades effekten av sammansättningen av olika media, tillsatser av små mängder glukos (som "start-glukos"), typ av cellulosasubstrat, olikartade odlingsbetingelser som skak- eller stationära kulturer och olika inkuberingstemperaturer. Resultaten visade att odlingsmediets sammansättning var av avgörande betydelse för vissa svampar. Vid odling på cellulosaagarmedia hade små mängder av jästextrakt en hämmande effekt på cellulasaktiviteten. Denna effekt var ej märkbar vid odling på cellulosasubstrat i närlösningar. De största klarningszonerna erhölls på Walseth cellulosa och s k "ball-milled" cellulosa. Betydligt mindre klarningszoner erhölls på Avicel och saltsyrabehandlad cellulosa. De olika svamparterna reagerade individuellt vid odling i skak- eller stationär kultur. Några arter förorsakade de högsta viktsförlusterna vid odling i skakkultur medan andra uppvisade större aktivitet vid odling i stationära kulturer.

Samtliga undersökta svampar, med undantag av *Phialophora verrucosa,* kunde bryta ned stärkelse. Bland de vednedbrytande svamparna kunde samtliga av de tre cellväggsnedbrytande enzymerna, cellulas, xylanas och mannanas påvisas hos tjugo arter. Fyra av de tjugo arterna förorsakade angrepp endast av Typ 1 (kaviteter), medan övriga förorsakade något angrepp av Typ 2 (erosion). Hos fem vednedbrytande svampar, vilka samtliga förorsakade angrepp av Typ 1, kunde ingetdera av de nämnda enzymerna påvisas. Hos resten av de vednedbrytande svamparna kunde endast ett eller två av enzymerna påvisas. Bland de svampar som saknade förmåga att bryta ned björkved, kunde xylanas påvisas hos en art, medan cellulas och mannanas inte kunde påvisas hos någondera av dessa svampar.

Det förhållandet att ett eller flera av de tre cellväggsnedbrytande enzymerna inte kunde påvisas hos samtliga vednedbrytande svampar, anses bero på bristfälligheter i de använda testmetoderna och inte på att svamparna saknar förmåga att producera enzymen.

Tolv av de vednedbrytande svamparna tyck-

tes helt oförmögna att bryta ned rena cellulosasubstrat under de betingelser som rådde i de beskrivna försöken. Samtliga dessa svampar förorsakade angrepp av Typ 1 i björkved. Detta angrepp var den enda yttring av cellulasaktivitet som kunde påvisas. Dessa svampar kallades därför "icke-cellulolytiska" soft rot svampar. Svamparna kunde ej bryta ned någon av de rena cellulosasubstraten som prövades i agarmedia eller i närlösningar. Några arter odlades på mald björkved i närlösningar men någon nedbrytning av detta substrat erhölls ej. I ett mindre försök jämfördes nedbrytningen av bomull och jutefibrer vid odling i närlösningar. Det visade sig att en av de "icke-cellulolytiska" soft rot svamparna, *Ceratocystis albida*, åstadkom en kraftig nedbrytning av jutefibrerna medan bomullen ej angreps. Svampen bildade soft rot kvaiteter i jutefibrerna men saknade tydligen förmåga att göra detta i bomullsfibrerna. Vid odling på ett rent cellulosasubstrat i närlösning, hade små mängder glukos (som "start-glukos") ingen stimulerande effekt på cellulasproduktionen hos dessa svampar.

Olika förklaringar till fenomenet med de "icke-cellulolytiska" soft rot svamparna diskuteras. De resultat som erhållits visar att deras oförmåga att bryta ned rena cellulosasubstrat troligen inte beror på 1) svag cellulasaktivitet, 2) temperaturfaktorer, 3) avsaknad av tillväxtfaktorer, 4) krav på "start -glukos" eller 5) cellbundna enzym. Möjliga förklaringar kan vara 1) att cellulasproduktionen induceras av substanser såsom hemicellulosa och lignin, vilka är associerade med cellulosan i ved, 2) att cellulasbildningen specifikt regleras av vedens fysikaliska struktur, eller 3) att de odlingsbetingelser som använts är olämpliga för produktion av cellulas.

En sammanställning av de resultat som erhållits med de olika testmetoderna ges i Tabell 10. För- och nackdelar med de olika metoderna för att bestämma cellulasaktivitet hos mikrosvampar diskuteras. Bland annat visas att tillväxt på ett cellulosaagarmedium är ett osäkert kriterium på cellulasaktivitet. Den metod där Rautela-Cowling tekniken utnyttjas framstår som överlägsen de andra metoderna.

Föreliggande arbete visar, att man med flera allmänt använda metoder för bestämning av cellulasaktivitet hos svampar, inte kan påvisa cellulas hos vissa arter av cellulolytiska mikrosvampar. Vid undersökningar av en grupp mikrosvampar med konventionella metoder, kan det därför befaras att ett antal cellulolytiska arter undgår att upptäckas. I detta arbete föreslås ett förfarande, bestående av två olika metoder, för bestämning av cellulasaktiviteten hos mikrosvampar. Svamparnas förmåga att åstadkomma klarningszoner i cellulosaagar studeras först med Rautela-Cowling tekniken. De svampar, som ej uppvisar någon aktivitet med denna metod, undersökes sedan med avseende på förmågan att bilda soft rot kaviteter i björkved. Eftersom vedens cellulosa brytes ned vid bildandet av dessa kaviteter, måste alla arter som har förmåga att bilda kaviteter i björkved betraktas som cellulolytiska. Detta förfarande ökar avsevärt möjligheterna att upptäcka cellulolytiska arter.

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Table 1. List of organisms

	Strain
Acremonium atro-griseum (Panasenko) W. Gams	SP35–7 (= CBS 981.70)
Bispora betulina (Corda) Hughes	P175–26
Catenularia heimii Mangenot	CBS 141.53
Ceratocystis albida (Mathiesen-Käärik) Hunt	B–23
Ceratocystis olivacea (Mathiesen) Hunt	B–57
Ceratocystis stenoceras (Robak) C. Moreau	B–104
Chrysosporium pannorum (Link) Hughes	SP47–16
Cladorrhinum sp. A	600–2
Cladosporium resinae (Lindau) de Vries	13385
Cladosporium sp. A	SP78–4
Coniothyrium fuckelii Sacc. var. sporulosum W. Gams & Domsch	CBS 218.68
Cordana pauciseptata Preuss	B63–A–25
Dictyosporium elegans Corda	SP5–16 (= CBS 946.70)
Gonatobotrys sp. A	SP37–6
Graphium sp. A	B68–A–16
Humicola alopallonella Meyers & Moore	CBS 207.60
Humicola grisea Traaen	SP37–22
Mollisia sp. A	T694C
Petriellidium boydii (Shear) Malloch	SP31–4
Phialocephala dimorphospora Kendrick	CBS 300.62
Phialocephala sp. A	P152–55 (= CBS 390.71)
Phialocephala sp. C [1]	71257–13
Phialophora fastigiata (Lagerb. & Melin) Conant	731–1–3b
Phialophora hoffmannii (van Beyma) Schol-Schwarz	SP33–4
Phialophora verrucosa Medlar	P152–8 (= CBS 839.69)
Phialophora sp. A	SP35–1 (= CBS 882.73)
Pseudeurotium zonatum van Beyma	SP17–2
Rhinocladiella anceps (Sacc. & Ellis) Hughes	SP35–15
Rhinocladiella sp. A	P160–14
Scytalidium lignicola Pesante	H97–1
Scytalidium sp. B	721009–2
Wardomyces inflatus (March.) Hennebert	P180–62 (= CBS 412.68)
Xylogone sphaerospora v. Arx & Nilsson	E7–2 (= CBS 186.69)
Fungus A	A40–1
Fungus B	SP3–10
Fungus D	B77–A–23

Strain number within parenthesis refer to species which have been included in the culture collection at "Centraalbureau voor Schimmel-cultures" in Baarn.

1) Recently identified as *Phialocephala fusca* Kendrick.

Table 2. Depth of clearing (mm) of Walseth cellulose, ball-milled cellulose, Avicel, HCl-treated cellulose, larch xylan, pine glucomannan and starch in agar columns (Rautela-Cowling technique).

Species	Type of attack on birch wood [1]	Walseth cellulose				Ball-milled cellulose	
		R–C medium		B–VII medium		B–VII medium	
		21 days	42 days	21 days	42 days	21 days	42 days
Acremonium atro-griseum	1	<1		10		10(D)	
Bispora betulina	1	0	0	0	0	0	0
Catenularia heimii	1	0	0	0	0	0	0
Ceratocystis albida	1	0	0	0	0	0	0
Ceratocystis olivacea	0	0	0	0	0	0	0
Ceratocystis stenoceras	1	0	0	0	0	0	0
Chrysosporium pannorum	1+2	8		14		1	
Cladorrhinum sp. A	1+2	<1		12		1	
Cladosporium resinae	0	0	0	0	0	0	0
Cladosporium sp. A	2	0	0	0	+	8(D)	
Coniothyrium fuckelii var. sporulosum	1+2	4		12		2	
Cordana pauciseptata	1+2	9		15		10	
Dictyosporium elegans	1+2	<1		13		+	1
Gonatobotrys sp. A	1	0	0	0	0	0	0
Graphium sp. A	1	0	0	0	0	0	0
Humicola alopallonella	1	0	0	+	7	2(D)	10(D)
Humicola grisea	1+2	3		7		1	
Mollisia sp. A	1	0	0	0	0	0	0
Petriellidium boydii	1	7		12		4	
Phialocephala dimorphospora	1	0	0	+	+	0	0
Phialocephala sp. A	1	0	0	0	0	0	0
Phialocephala sp. C	1	0	0	0	0	0	0
Phialophora fastigiata	1+2	<1		12		5	
Phialophora hoffmannii	1+2	11		12		6	
Phialophora verrucosa	0	0	0	0	0	0	0
Phialophora sp. A	1	0	0	0	0	0	0
Pseudeurotium zonatum	2	7		11		4	
Rhinocladiella anceps	2	3		8		6	
Rhinocladiella sp. A	1	0	0	0	0	0	0
Scytalidium lignicola	2	7		10		3	
Scytalidium sp. B	2	0	0	3		5	
Wardomyces inflatus	1+2	<1		9(D)		9(D)	
Xylogone sphaerospora	2	8		28		8	
Fungus A	1	0	0	0	0	0	0
Fungus B	1	0	0	0	0	0	0
Fungus D	2	0	0	5		3	

Notes

1) Data from Nilsson (1973) except for *Ceratocystis albida, Ceratocystis stenoceras, Cladosporium* sp. A and Fungus D. These fungi were tested for type of wood attack according to the methods described previously.

2) Cellulose powder (Whatman CF 11) treated with concentrated hydrochloric acid according to Bose (1963).

Type of attack

0 no attack, 1 Type 1 attack (cavities), 2 Type 2 attack (erosion).

Cellulolytic activity

+ definite clearing, but measurements not possible due to diffuse zonal fronts.

D diffuse clearing.

Substrate											
Avicel				HC1-treated cellulose 2)		Xylan		Glucomannan		Starch	
R–C medium		B–VII medium		B–VII medium		B–VII medium		B–VII medium		B–VII medium	
21 days	42 days	21 days	42 days	21 days	42 days	21 days	42 days	21 days	42 days	21 days	42 days
0	0	0	+	0	+	20		13		15	
0	0	0	0	0	0	0	+	0	0	28	
		0	0	0	0	0	0	0	0	18	
				0	0	0	0	0	0	23	
				0	0	0	0	0	0	12	
				0	0	0	0	0	0	25	
0	+	0	7	0	7	35		33		12	
0	0	0	6	0	5	25		14		19	
		0	0	0	0	19		0	0	37	
				0	0	18		18		25	
0	0	0	4	0	9	35		15		20	
0	0	0	+	0	+	25		10		9	
0	0	0	0	0	+	23		0	6	19	
0	0	0	0	0	0	0	0	0	0	21	
		0	0	0	0	28		0	0	19	
0	0	0	+	0	+	3	7	0	0	7	
0	0	0	0	0	+	24		28		8	
0	0	0	0	0	0	0	+	0	0	23	
0	0	0	0	0	0	18		10		7	
				0	0	0	0	8		25	
0	0	0	0	0	0	0	+	0	0	26	
				0	0	0	0	0	0	8	
0	0	0	0	0	+	19		9		20	
0	0	0	+	+	10	31		18		30	
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	+	+	7	28	
0	0	0	0	0	0	33		11		25	
0	0	0	11(D)	0	0	24		8		21	
0	0	0	0	0	0	0	0	0	0	26	
0	0	0	+	0	+	27		8		25	
				0	0	0	+	0	+	19	
0	0	0	0	0	0	28		18		6	
0	0	4		0	4	28		14		31	
0	0	0	0	0	0	19		+	11	25	
0	0	0	0	0	0	15		0	10	20	
				0	0	+	+	18		18	

Table 3. Relative growth on two cellulose agar media, clearing of cellulose in agar plates and demonstration of cellulolytic activity by the use of an agar-plate diffusion technique with mycelium-agar plugs.

Species	Time (days)	Relative growth on cellulose agar [1]		Clearing of cellulose in the agar test plates [2]		Clearing of cellulose agar around transferred agar plugs [2]	
		Medium F6A	Medium B–VII	Medium F6A	Medium B–VII	Plugs from medium F6A	Plugs from medium B–VII
Acremonium atro-griseum	21	2		–		+	
Bispora betulina	21	2	1	–	–	–	–
	42	2	1	–	–	–	–
Catenularia heimii	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Ceratocystis albida	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Ceratocystis olivacea	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Ceratocystis stenoceras	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Chrysosporium pannorum	21	2		–		+	
Cladorrhinum sp. A	21	3		+		+	
Cladosporium resinae	21	2	1	–	–	–	–
	42	2	1	–	–	–	–
Cladosporium sp. A	21	2	1	–	–	–	–
	42	2	1	–	+	+	+
Coniothyrium fuckelii var. sporulosum	21	3		+		+	
Cordana pauciseptata	21	1		–		+	
Dictyosporium elegans	21	2		–		+	
Gonatobotrys sp. A	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Graphium sp. A	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Humicola alopallonella	21	2		–		–	
	42	2	2	–	–	–	+
Humicola grisea	21	3	2	+	–	+	+
Mollisia sp. A	21	2	1	–	–	–	–
	42	2	1	–	–	–	–

Petriellidium boydii	21	3		+		+	
Phialocephala dimorphospora	21	2	1	–	–	–	–
	42	2	1	–	–	–	–
Phialocephala sp. A	21	2		–		–	
	42	2	1	–	–	–	–
Phialocephala sp. C	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Phialophora fastigiata	21	3		–		+	
Phialophora hoffmannii	21	3	1	+	–	+	+
Phialophora verrucosa	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Phialophora sp. A	21	2	1	–	–	–	–
	42	2	1	–	–	–	–
Pseudeurotium zonatum	21	2		–		+	
Rhinocladiella anceps	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Rhinocladiella sp. A	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Scytalidium lignicola	21	3		+		+	
Scytalidium sp. B	21	1		–		–	
	42	1	3	–	+	–	+
Wardomyces inflatus	21	3		–		+	
Xylogone sphaerospora	21	3	2	+	–	+	+
Fungus A	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Fungus B	21	1	1	–	–	–	–
	42	1	1	–	–	–	–
Fungus D	21	1	1	–	–	–	+
	42	1	2	–	+	–	+

Notes

1) The growth has been estimated and compared with growth on 2.5 percent malt extract agar
1 = Very sparse growth
2 = Moderate growth
3 = Equal or more growth than on malt extract agar
2) + clearing observed
– no clearing observed

Table 4. Cellulase and xylanase activity in birch wood blocks attacked by 30 species of the test fungi.

Species	Cellulase	Xylanase	Agar medium (1)
Acremonium atro-griseum	+	+	C
Bispora betulina	+	–	M
Catenularia heimii	–	–	M
Ceratocystis albida	–	–	M
Ceratocystis stenoceras	–	+	M
Chrysosporium pannorum	+	+	C
Cladorrhinum sp. A	+	+	C
Cladosporium resinae	–	–	M
Coniothyrium fuckelii var. sporulosum	+	+	C
Cordana pauciseptata	+	+	C
Dictyosporium elegans	–	–	C
Gonatobotrys sp. A	–	–	M
Graphium sp. A	–	–	M
Humicola alopallonella	–	–	C
Humicola grisea	+	+	C
Mollisia sp. A	–	–	M
Petriellidium boydii	–	–	C
Phialocephala sp. A	+	+	M
Phialophora fastigiata	+	+	C
Phialophora hoffmannii	+	+	C
Phialophora verrucosa	–	–	M
Phialophora sp. A	+	+	M
Pseudeurotium zonatum	+	–	C
Rhinocladiella anceps	+	+	C
Rhinocladiella sp. A	–	–	M
Scytalidium lignicola	+	+	C
Wardomyces inflatus	–	–	C
Xylogone sphaerospora	+	+	C
Fungus A	+	+	M
Fungus B	–	–	M

Notes
1) The agar medium used in the decay test
C = Cellulose agar F6A
M = 2.5 percent malt extract agar

Table 5. Growth on the nutrient solutions EP and B–VII–L with added glucose.

Species	Time (days)	EP (Control. No glucose added)		EP + glucose (20 g/l)		B–VII–L + glucose (20 g/l)	
		Weight of mycelium (mg)	Final pH	Weight of mycelium (mg)	Final pH	Weight of mycelium (mg)	Final pH
Bispora betulina	15	28.1	7.6	57.3	3.6	31.0	2.8
	23	4.5	7.3	51.4	3.7	55.0	2.6
	28	6.2	7.4	51.1	3.7	37.4	2.7
Catenularia heimii	14	6.7	6.7	23.6	3.1		
	21	21.0	6.7	32.1	3.0	36.1	2.5
	28	2.5	6.9	43.4	4.2		
Ceratocystis albida	14	14.3	7.6	103.0	2.5		
	21	4.1	7.5	90.6	2.6	87.5	2.3
	28	3.2	7.2	77.8	2.6		
Ceratocystis olivacea	14	2.1	7.7	40.7	3.2		
	21	5.1	7.6	54.1	2.8	19.8	2.6
	28	4.2	7.5	57.9	2.6		
Ceratocystis stenoceras	14	26.0	7.6	63.6	2.6		
	21	4.4	7.5	81.7	2.6	53.4	2.4
	28	3.5	7.4	83.0	2.6		
Cladosporium resinae	14	15.3	6.7	89.8	2.3		
	21	5.1	6.9	79.4	3.0	51.2	2.3
	28	3.5	6.9	64.3	3.6		
Cladosporium sp. A	14	4.7	7.7	172.0	2.6		
	21	4.5	7.6	171.9	2.5	95.9	2.4
	28	4.0	7.5	156.7	2.5		
Gonatobotrys sp. A	14			49.7	2.7		
	21			66.8	2.6	36.5	2.4
	28			64.1	2.6		
Graphium sp. A	14			28.4	4.8		
	21			37.5	3.9	40.5	2.4
	28			42.2	3.0		
Mollisia sp. A	14			84.6	2.9		
	21			123.8	2.6	72.3	2.4
	28			139.3	2.6		
Phialocephala dimorphospora	14			114.8	2.5		
	21			122.4	2.5	59.2	2.4
	28			109.3	2.6		

Table 5. (Continued).

Species	Time (days)	EP (Control. No glucose added)		EP + glucose (20 g/l)		B–VII–L + glucose (20 g/l)	
		Weight of mycelium (mg)	Final pH	Weight of mycelium (mg)	Final pH	Weight of mycelium (mg)	Final pH
Phialocephala sp. A	14			97.2	2.6		
	21			112.4	2.6	32.0	2.5
	28			120.1	2.7		
Phialocephala sp. C	14			13.2	3.7		
	21			20.2	4.9	44.2	2.5
	28			28.4	6.2		
Phialophora verrucosa	14			42.4	3.6		
	21			56.8	3.8	22.6	2.6
	28			75.1	3.0		
Phialophora sp. A	14			49.0	3.4		
	21			93.0	3.0	32.2	2.6
	28			126.3	2.7		
Rhinocladiella sp. A	14			16.5	3.1		
	21			28.4	2.9	43.9	2.5
	28			39.2	2.8		
Scytalidium sp. B	14			102.9	2.9		
	21			129.9	2.6	82.9	2.4
	28			105.2	2.9		
Fungus A	7			19.3	3.6	15.6	3.0
	14			52.9	3.5	30.4	2.7
	21			84.1	3.5	62.5	2.4
	28			122.4	3.0	94.0	2.4
Fungus B	14			29.9	3.5		
	21			97.9	3.4	32.6	2.7
	28			107.8	3.1		
Fungus D	14			47.0	3.2		
	21			68.4	2.9	30.4	2.5
	28			96.1	2.7		

Table 6. Degradation of cellulose N, Avicel and cotton, and the production of cellulase and xylanase in liquid cultures with medium EP.

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Control (not inoculated)	Avicel	21	0.8			5.6	5.1			5.0
	Cotton	21	0.7			5.1	4.0			4.9
	Cellulose N	21	1.0			5.1	2.1			5.1
Acremonium atro-griseum	Avicel	21	35.0	+	+	5.6				
	Cotton	21	4.1	+	–	7.5				
	Cellulose N	21	48.4	+	–	6.4				
Bispora betulina	Avicel	21	0	–	–	6.6	0	–	–	6.4
		42	0	–	–	6.3	0	–	–	6.5
	Cotton	21	0	–	–	6.6	0	–	–	6.6
		42	0	–	–	6.1	0	–	–	6.5
	Cellulase N	21	0	–	–	6.6	0	–	–	6.7
		42	0	–	–	6.4	0	–	–	6.6
Catenularia heimii	Cellulose N	21					2.1	–	–	6.6
		42	5.3	–	–	6.5	0.8	–	–	6.7
Ceratocystis albida	Cellulose N	21					1.8	–	–	7.3
		42	0	–	–	7.1	0	–	–	7.2
Ceratocystis olivacea	Cellulose N	21	0.6	–	–	7.4	0	–	–	7.5
		42	1.2	–	–	7.1	2.0	–	–	7.2
Ceratocystis stenoceras	Cellulose N	21					0	–	–	7.4
		42	3.1	–	–	7.2	0	–	–	7.2
Chrysosporium pannorum	Avicel	21	61.4	+	+	6.3				
	Cotton	21	9.3	+	–	6.1				
	Cellulose N	21	49.5	+	+	6.4				
Cladorrhinum sp. A	Avicel	21	82.6	+	+	6.8				
	Cellulose N	21	81.5	+	+	6.9				
Cladosporium resinae	Cellulose N	21	0	–	–	6.4	1.1	–	–	7.1
		42	0	–	–	6.9	3.2	–	–	7.1
Cladosporium sp. A	Cellulose N	21	0	–	–	7.5	0	–	–	7.6
		42	0	–	–	7.1	0.4	+	–	7.3
Coniothyrium fuckelii var. sporulosum	Avicel	21	30.6	+	+	6.5				
	Cellulose N	21	53.5	+	+	5.2	44.0	+	+	6.0

Table 6. (Continued).

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Cordana pauciseptata	Avicel	21	45.8	+	+	6.3				
	Cotton	21	15.0	+	–	7.5				
	Cellulose N	21	66.6	+	–	6.5				
Dictyosporium elegans	Avicel	21	2.9	+	–	7.6	0.2	+	+	7.6
		42					6.4	+	+	7.2
	Cotton	21	1.4	+	–	7.4	2.7	+	+	7.5
		42					1.7	+	+	7.4
	Cellulose N	21	4.9	+	–	7.4	17.1	+	+	7.6
		42					42.4	+	+	7.1
Gonatobotrys sp. A	Avicel	21	0	–	–	7.2				
		42	0	–	–	6.6	0.4	–	–	7.2
	Cotton	21	0	–	–	7.2				
		42	0	–	–	7.1	0.4	–	–	7.2
	Cellulose	21	0	–	–	7.2				
		42	0	–	–	7.0	0.6	–	–	7.1
Graphium sp. A	Avicel	42	0	–	–	7.0	0	–	–	7.1
	Cotton	42	0	–	–	7.0	0	–	–	7.1
	Cellulose N	42	0	–	–	7.0	0	–	–	7.1
Humicola alopallonella	Avicel	21	0	–	–	7.4				
		42	0	–	–	7.1				
	Cellulose N	21	0	–	–	7.3	16.4	+	–	6.5
		42	10.8	–	–	6.8	36.6	+	–	6.6
Humicola grisea	Avicel	21	49.1	+	+	6.4				
	Cotton	21	41.6	+	+	6.3				
	Cellulose N	21	47.7	+	+	6.5				
Mollisia sp. A	Avicel	21	0	–	–	6.4				
		42	0	–	+	6.5	0	–	–	6.6
	Cotton	21	0	–	–	6.2				
		42	0	–	+	6.5	0	–	–	6.6
	Cellulose N	21	0	–	–	6.5				
		42	0	–	+	6.5	0	–	–	6.8

Table 6. (Continued).

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Petriellidium boydii	Avicel	21	55.4	+	+	6.5				
	Cotton	21	48.8	+	+	6.4				
	Cellulose N	21	55.0	+	+	5.2	49.6	+	–	6.1
Phialocephala dimorphospora	Cellulose N	21					0	–	–	7.4
		42	0	–	–	7.1	0	–	–	7.2
Phialocephala sp. A	Avicel	42	0	–	–	6.9	0	–	–	7.0
	Cotton	42	0	–	–	7.0	0	–	–	7.1
	Cellulose N	42	0	–	–	7.0	0	–	–	7.0
Phialocephala sp. C	Cellulose N	21	2.7	–	–	6.8	0.8	–	–	7.0
		42	3.1	–	–	6.8	0.5	–	–	7.0
Phialophora fastigiata	Avicel	21	0	–	–	7.3	0.7	+	–	7.4
		42	0	–	–	6.9	1.0	–	–	7.0
	Cotton	21	0	–	+	7.3	4.2	+	–	7.0
		42	1.0	+	+	6.1	38.3	+	+	5.6
	Cellulose N	21	30.8	+	–	6.5	38.2	–	+	6.2
		42	43.9	+	–	6.5	57.8	+	+	5.7
Phialophora hoffmannii	Avicel	21	71.3	+	+	6.0				
	Cellulose N	21	73.2	+	+	6.6				
Phialophora verrucosa	Avicel	21	0	–	–	6.4				
		42	0	–	–	6.6				
	Cotton	21	0	–	–	6.5				
		42	0	–	–	6.5				
	Cellulose N	21	2.2	–	–	6.5				
		42	0	–	–	6.6				
Phialophora sp. A	Avicel	21	0	–	–	7.5	0	–	–	7.3
		42	0	–	–	7.0	0	–	–	7.0
	Cotton	21	0	–	–	7.5	0	–	–	7.4
		42	0	–	–	7.2	0	–	–	7.3
	Cellulose N	21	0.6	–	–	7.5	0	–	–	7.4
		42	0	–	–	7.2	0	–	–	7.3
Pseudeurotium zonatum	Avicel	21	28.9	+	–	7.0				
	Cellulose N	21	65.2	+	–	6.3	42.1	+	+	6.2

Table 6. (Continued).

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Rhinocladiella anceps	Avicel	21	0	–	–	7.3				
		42	0	–	–	7.1	0	–	–	7.0
	Cotton	21	0	–	–	7.3				
		42	0	–	–	7.1	0	–	–	7.1
	Cellulose N	21	0	–	–	7.3				
		42	1.6	–	–	7.0	0	–	–	7.0
Rhinocladiella sp. A	Avicel	42	0	–	–	7.0				
	Cotton	42	0	–	–	7.0				
	Cellulose N	21					0	–	–	7.4
		42	2.4	–	–	7.1	0	–	–	7.2
Sytalidium lignicola	Avicel	21	26.7	+	+	5.9				
	Cellulose N	21	44.9	+	–	6.4	22.4	+	+	7.0
Sytalidium sp. B	Cellulose N	21					2.5	–	–	6.9
		42					0.5	–	–	6.9
Wardomyces inflatus	Avicel	21	5.9	+	+	7.1				
	Cotton	21	0	–	–	7.4				
	Cellulose N	21	23.4	+	+	6.6				
Xylogone sphaerospora	Avicel	21	74.6	+	+	6.7				
	Cotton	21	58.0	+	+	4.3				
	Cellulose N	21	74.0	+	–	7.0				
Fungus A	Avicel	21	4.8	–	–	7.2	0	–	–	6.9
		42	0	–	–	7.2	0	–	–	6.9
	Cotton	21	5.8	–	–	7.2	0	–	–	7.2
		42	0	–	–	7.2	0	–	–	7.2
	Cellulose N	21	4.0	–	–	7.3	2.6	+	+	7.2
		42	0	–	–	7.2	45.9	+	+	5.2
Fungus B	Cellulose N	21	0	–	–	7.0	1.6	–	–	6.5
		42	0	–	–	7.1	0	–	–	6.7
Fungus D	Cellulose N	21	60.8	+	+	7.0	41.6	+	+	5.5
		42	64.1	+	+	6.5	59.3	+	+	5.9

Table 7. Degradation of cellulose N, Avicel and cotton, and the production of cellulase and xylanase in liquid cultures with medium B–VII–L.

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Acremonium atro-griseum	Avicel	21	15.2	–	–	3.6				
	Cotton	21	33.2	–	–	5.0				
	Cellulose N	21	13.5	–	–	5.0				
Bispora betulina	Avicel	21	3.4	–	–	4.9	3.4	–	–	4.6
		42	3.1	–	–	5.3	1.8	–	–	4.7
	Cellulose N	21	4.2	–	–	4.9	6.0	–	–	4.6
		42	4.7	–	–	5.2	4.7	–	–	4.5
Catenularia heimii	Cellulose N	21	2.2	–	–	6.0	5.4	–	–	4.5
		42	3.2	–	–	6.0	4.8	–	–	5.2
Ceratocystis albida	Cellulose N	21					3.6	–	–	4.5
		42	4.1	–	–	4.8	4.0	–	–	4.6
Ceratocystis olivacea	Cellulose N	21	2.8	–	–	4.6	3.0	–	–	4.5
		42	2.1	–	–	4.7	0	–	–	4.5
Ceratocystis stenoceras	Cellulose N	21					4.5	–	–	4.5
		42	6.2	–	–	4.6	7.0	–	–	4.4
Cladosporium resinae	Cellulose N	21	3.8	–	–	4.6	3.0	–	–	4.5
		42	4.6	–	–	4.6	0	–	–	4.5
Cladosporium sp. A	Cellulose N	21	3.4	–	–	4.1	3.4	–	–	3.9
		42	4.0	–	–	3.8	4.3	–	–	3.9
Cordana pauciseptata	Avicel	21	37.1	–	–	2.8				
	Cotton	21	24.0	–	–	2.9				
	Cellulose N	21	44.7	–	–	2.9				
Dictyosporium elegans	Avicel	21	7.1	–	–	4.6				
	Cotton	21	6.5	–	–	4.9				
	Cellulose N	21	6.1	–	–	4.5				
Gonatobotrys sp. A	Cellulose N	21					4.7	–	–	4.3
		42	2.0	–	–	4.4	3.1	–	–	4.3
Graphium sp. A	Cellulose N	21					3.4	–	–	4.6
		42	5.6	–	–	4.6	5.3	–	–	4.5
Humicola alopallonella	Cellulose N	21	5.9	–	–	3.7	9.0	–	–	3.5
		42					9.3	–	–	3.4

Table 7. (Continued).

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Humicola grisea	Avicel	21	12.2	+	–	3.1				
	Cotton	21	3.5	–	–	3.9				
	Cellulose N	21	16.2	–	–	3.0				
Mollisia sp. A	Cellulose N	21					2.8	–	–	4.5
		42					1.8	–	–	4.0
Phialocephala dimorphospora	Cellulose N	21					7.2	–	–	3.8
		42	5.4	–	–	4.3	9.4	–	–	3.7
Phialocephala sp. A	Avicel	21	1.0	–	–	5.3	1.4	–	–	5.1
		42	3.0	–	–	5.2	2.0	–	–	5.1
	Cellulose N	21	2.6	–	–	5.2	3.8	–	–	4.7
		42	4.4	–	–	5.2	4.4	–	–	4.7
Phialocephala sp. C	Cellulose N	21					4.0	–	–	4.4
		42					3.1	–	–	4.2
Phialophora verrucosa	Cellulose N	21					4.1	–	–	4.2
		42	2.4	–	–	4.6	3.1	–	–	4.1
Phialophora sp. A	Avicel	21	6.0	–	–	4.6	5.8	–	–	4.0
		42	3.4	–	–	4.7	5.0	–	+	3.6
	Cotton	21	7.0	–	–	4.9	5.4	–	–	4.3
		42	4.6	–	–	4.6	3.3	–	–	4.0
	Cellulose N	21	7.7	–	–	4.7	8.6	–	–	3.5
		42	4.4	–	–	4.2	7.4	–	–	3.4
Rhinocladiella anceps	Avicel	21	16.1	+	+	3.5				
	Cellulose N	21	26.8	+	–	3.5				
Rhinocladiella sp. A	Cellulose N	21					2.6	–	–	4.5
		42	2.4	–	–	4.3	2.8	–	–	4.3
Scytalidium lignicola	Cellulose N	21	18.3	–	–	3.2				
Scytalidium sp. B	Cellulose N	21					46.0	+	–	2.7
		42					53.9	–	–	2.8

Table 7. (Continued).

Species	Substrate	Time (days)	Culture conditions							
			Shake cultures				Stationary cultures			
			Weight loss (%)	Cellulase	Xylanase	Final pH	Weight loss (%)	Cellulase	Xylanase	Final pH
Fungus A	Avicel	21	0	–	–	4.7	0	–	–	4.2
		42	1.9	–	–	4.6	3.2	–	–	3.8
	Cotton	21	0	–	–	4.7	0	–	–	3.6
		42	1.8	–	–	4.6	7.3	–	–	3.3
	Cellulose N	21	0	–	–	4.6	0	–	–	3.5
		42	2.6	–	–	4.6	7.5	–	–	3.5
Fungus B	Cellulose N	21	1.6	–	–	4.7	4.7	–	–	4.0
		42	1.4	–	–	3.9	3.5	–	–	4.2
Fungus D	Cellulose N	21	20.2	+	–	3.0	20.4	+	+	2.9
		42	25.6	+	–	3.0	31.2	+	+	2.9

Table 8. Degradation of cotton and jute fibres, and the production of cellulase and xylanase in liquid cultures with medium EP.

Species	Time (days)	Cotton				Jute			
		Weight loss %	Cellulase	Xylanase	Final pH	Weight loss %	Cellulase	Xylanase	Final pH
Control (not inoculated)	15	4.0			4.5	6.0			4.4
	28	7.0			4.3	6.2			4.2
	42	3.5			4.1	7.6			4.2
Bispora betulina	15	3.4	–	–	7.1	6.2	–	–	6.9
	28	2.7	–	–	6.9	8.5	–	–	6.9
	49	1.8	–	–	6.9	5.5	–	–	6.6
Ceratocystis albida	15	5.0	–	–	7.0	21.3	–	–	3.4
	28	6.2	–	–	6.8	28.2	–	–	3.5
	42	2.4	–	–	6.8	30.8	–	–	3.5
Phialophora sp. A	15	2.0	–	–	7.1	5.8	–	–	6.9
	28	3.8	–	–	6.8	5.7	–	–	6.7
	49	2.8	–	–	6.7	5.0	–	–	6.7
Wardomyces inflatus	15	5.5	+	–	7.0	16.1	–	–	5.4
	28	10.6	+	–	6.5	19.8	–	–	5.3
	42	12.9	+	+	6.0	19.8	–	–	5.3
Xylogone sphaerospora	15	31.7	+	–	2.8	12.1	+	+	6.0
	28	46.8	+	–	2.6	21.0	+	+	3.9
	42	59.5	+	–	2.7	27.2	+	+	3.5

Table 9. Effect of various amounts of glucose added to medium EP, on the production of cellulase and xylanase in liquid cultures with cellulose N.

Species	Time (days)	Glucose concentration (per cent) 0 C	X	Final pH	0.005 C	X	Final pH	0.01 C	X	Final pH	0.025 C	X	Final pH	0.05 C	X	Final pH	0.1 C	X	Final pH	0.25 C	X	Final pH	0.5 C	X	Final pH
Bispora betulina	7	–	–	7.3	–	–	7.4	–	–	7.3	–	–	7.2	–	–	7.0	–	–	6.7						
	14	–	–	7.5	–	–	7.4	–	–	7.4	–	–	7.4	–	–	7.2	–	–	7.0						
	21	–	–	7.3	–	–	7.4	–	–	7.3	–	–	7.3	–	–	7.2	–	–	7.1						
Ceratocystis albida	7	–	–	7.5	–	–	7.5	–	–	7.5	–	–	7.5	–	–	7.4	–	–	7.3						
	14	–	–	7.4	–	–	7.4	–	–	7.4	–	–	7.5	–	–	7.5	–	–	7.4						
	21	–	–	7.4	–	–	7.4	–	–	7.4	–	–	7.4	–	–	7.3	–	–	7.2						
Cladorrhinum sp. A	5	+	–	6.8				+	–	6.9				–	–	6.6	–	–	4.7	–	–	3.5	–	–	3.5
	9	+	+					+	–					+	–		–	–		–	–		–	–	
	15	+	+	6.0				+	+	6.4				+	+	5.9	+	+	5.6	–	–	5.4	–	–	4.9
	21	+	+	6.6				+	+	6.4				+	+	6.6	+	+	6.4	+	–	6.5	–	–	3.0
Phialocephala sp. A	7	–	–	7.1	–	–	7.0	–	–	7.1	–	–	7.1	–	–	6.9	–	–	6.9						
	14	–	–	7.1	–	–	7.1	–	–	7.2	–	–	7.2	–	–	7.1	–	–	7.2						
	21	–	–	7.1	–	–	7.1	–	–	7.1	–	–	7.2	–	–	7.2	–	–	7.1						
Xylogone sphaerospora	5	+	–	6.4				+	–	6.5				+	–	6.7	+	–	6.8	+	–	5.6	–	–	3.1
	9	+	–					+	–					+	–		+	–		+	–		+	–	
	15	+	+	6.7				+	–	6.8				+	+	6.7	+	+	6.6	+	–	6.5	+	–	3.0
	21	+	+	5.6				+	+	5.7				+	+	5.6	+	+	3.8	+	–	4.3	+	–	3.8
Fungus A	7	+	–	6.7	+	–	6.7	+	–	6.6	–	–	6.4	–	–	6.1	–	–	5.8						
	14	+	+	6.9	+	+	6.9	+	+	6.8	+	+	6.7	+	+	6.2	+	+	5.9						
	28	+	+	6.2	+	+	5.8	+	+	4.7	+	+	4.3	+	+	3.6	+	+	3.5						

Notes
C = cellulase
X = xylanase

Table 10. Summation of the results obtained with the different test methods.

Species	Cellulase					Xylanase			Mannanase	Amylase
				Liquid cultures				*Liquid cultures*		
	Test tubes	Mycelium-agar plugs	Wood blocks	Weight loss[1)]	Culture filtrate	Test tubes	Wood blocks	Culture filtrate	Test tubes	Test tubes
Acremonium atro-griseum	+	+	+	+	+	+	+	+	+	+
Bispora betulina	–	–	+	–	–	+	–	–	–	+
Catenularia heimii	–	–	–	–	–	–	–	–	–	+
Ceratocystis albida	–	–	–	–	–	–	–	–	–	+
Ceratocystis olivacea	–	–	NT	–	–	–	NT	–	–	+
Ceratocystis stenoceras	–	–	–	–	–	–	+	–	–	+
Chrysosporium pannorum	+	+	+	+	+	+	+	+	+	+
Cladorrhinum sp. A	+	+	+	+	+	+	+	+	+	+
Cladosporium resinae	–	–	–	–	–	+	–	–	–	+
Cladosporium sp. A	+	+	NT	–	+	+	NT	–	+	+
Coniothyrium fuckelii var. sporulosum	+	+	+	+	+	+	+	+	+	+
Cordana pauciseptata	+	+	+	+	+	+	+	+	+	+
Dictyosporium elegans	+	+	–	+	+	+	–	+	+	+
Gonatobotrys sp. A	–	–	–	–	–	–	–	–	–	+
Graphium sp. A	–	–	–	–	–	+	–	–	–	+
Humicola alopallonella	+	+	–	+	+	+	–	–	–	+
Humicola grisea	+	+	+	+	+	+	+	+	+	+
Mollisia sp. A	–	–	–	–	–	+	–	+	–	+
Petriellidium boydii	+	+	–	+	+	+	–	+	+	+
Phialocephala dimorphospora	+	–	NT	–	–	–	NT	–	+	+
Phialocephala sp. A	–	–	+	–	–	+	+	–	–	+
Phialocephala sp. C	–	–	NT	–	–	–	NT	–	–	+
Phialophora fastigiata	+	+	+	+	+	+	+	+	+	+
Phialophora hoffmannii	+	+	+	+	+	+	+	+	+	+
Phialophora verrucosa	–	–	–	–	–	–	–	–	–	–
Phialophora sp. A	–	–	+	–	–	+	+	+	+	+
Pseudeurotium zonatum	+	+	+	+	+	+	–	+	+	+
Rhinocladiella anceps	+	–	+	+	+	+	+	+	+	+
Rhinocladiella sp. A	–	–	–	–	–	–	–	–	–	+
Scytalidium lignicola	+	+	+	+	+	+	+	+	+	+

Table 10. (Continued).

Species	Cellulase			Xylanase					Mannanase	Amylase
				Liquid cultures				*Liquid cultures*		
	Test tubes	Mycelium-agar plugs	Wood blocks	Weight loss[1]	Culture filtrate	Test tubes	Wood blocks	Culture filtrate	Test tubes	Test tubes
Scytalidium sp. B	+	+	NT	+	+	+	NT	–	+	+
Wardomyces inflatus	+	+	–	+	+	+	–	+	+	+
Xylogone sphaerospora	+	+	+	+	+	+	+	+	+	+
Fungus A	–	–	+	+	+	+	+	+	+	+
Fungus B	–	–	–	–	–	+	–	–	+	+
Fungus D	+	+	NT	+	+	+	NT	+	+	+
Positive results	20	18	16[2]	19	20	27	14[2]	18	22	35
Negative results	16	18	14[2]	17	16	9	16[2]	18	14	1

Notes
1) Significant weight loss of cellulose N
+ Activity present
– Activity absent
NT Not tested
2) Only 30 species tested