JOURNAL OF BACTERIOLOGY, Apr., 1966
Vol 91, No. 4

Susceptibility and Resistance of Several Fungi to Microbial Lysis[1]

H. J. POTGIETER[2] AND M. ALEXANDER
Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York

Received for publication 18 November 1965

ABSTRACT

POTGIETER, H. J. (Cornell University, Ithaca, N.Y.), AND M. ALEXANDER. Susceptibility and resistance of several fungi to microbial lysis. J. Bacteriol. **91**:1526–1532. 1966.—Strains of *Streptomyces*, *Nocardia*, and *Pseudomonas* capable of lysing hyphae of *Fusarium solani* or *Neurospora crassa* were obtained by selective culture, but attempts to isolate an organism lysing *Rhizoctonia solani* failed. When provided with *F. solani* or *N. crassa* as carbon sources, the actinomycetes tested produced β-(1 $\rightarrow$ 3) glucanase and chitinase. A mixture containing purified chitinase and β-(1 $\rightarrow$ 3) glucanase induced spheroplast formation in *F. solani*, caused some morphological changes in *N. crassa*, but had almost no effect on *R. solani* hyphae. The polysaccharides in *R. solani* walls, which contain a large amount of glucose as well as galactose, mannose, and glucosamine, were not hydrolyzed appreciably by the two enzymes. Laminaribiose and laminaritriose were released by enzymatic hydrolysis of *F. solani* and *N. crassa* walls, and gentiobiose was liberated from *R. solani* and *N. crassa* walls. Melaninlike materials were found in *R. solani* walls, accounting for 8.50% of the wall weight. A role for melanin in protecting hyphae from microbial lysis is suggested.

A number of fungi are particularly susceptible to lysis by microorganisms. The mechanism of lysis of at least some of these fungi appears to involve the enzymatic hydrolysis of glucan and chitin components of the hyphal walls. Thus, chitinase and β-(1$\rightarrow$3) glucanase participate in the digestion by *Bacillus circulans* of *Penicillium chrysogenum* walls and in the destruction by a streptomycete of *Fusarium solani* hyphal walls, the surface structures of these fungi being rich in a glucose polymer (16; Horikoshi, Koffler, and Garner, Bacteriol. Proc., p. 95, 1961); the residue remaining after incubation of purified walls of *Aspergillus oryzae* with β-(1$\rightarrow$3) glucanase has been shown to be chitin on the basis of X-ray diffraction patterns (7).

Other fungi, by contrast, are resistant to microbial degradation. The chlamydospores and sclerotia of a number of species endure for surprisingly long periods in natural environments, even when exposed to large, diverse, and active microbial communities. Similarly, the vegetative filaments of certain species may show considerable resistance to degradation in natural ecosystems. Lockwood (9), for example, observed that the dark hyphae produced by *Helminthosporium sativum*, *Rhizoctonia solani*, and *Alternaria solani* were particularly resistant.

The present study was designed to determine the hyphal wall components that are associated with the resistance and susceptibility of fungi to enzymatic lysis.

MATERIALS AND METHODS

Neurospora crassa A74, *R. solani*, *Fusarium solani* f. *phaseoli*, and *Cladosporium* sp. were grown on a rotary shaker at 30 C for 1 to 3 days in 500-ml flasks containing 200 ml of the sucrose-yeast extract-Casamino Acids medium previously described (16). The inoculum was either a thick spore suspension in distilled water or a homogenized suspension of hyphae. The mycelium was collected on a sintered-glass filter and washed with distilled water.

Cell walls were prepared either by the method of Skujins, Potgieter, and Alexander (16) or by homogenizing 20 g of hyphae in 60 ml of water at 6 C in a Servall Omnimix at 16,000 rev/min for 2 min. This hyphal suspension was then treated in a 20-kc Bronwill ultrasonic disintegrator at a temperature not exceeding 10 C. The treatment was continued until no

[1] Agronomy Paper No. 696.

[2] Present address: Department of Microbiology, University of the Orange Free State, Bloemfontein, South Africa.

cytoplasm could be observed upon microscopic examination of the hyphae. The walls were then washed 10 times with water and incubated with trypsin in 0.2 M phosphate buffer (*p*H 7.5) for 12 hr at 37 C in 0.01% Merthiolate. After 10 washings with the buffer and 5 with distilled water, the walls were lyophilized and stored at 4 C.

For the isolation of lytic organisms, the following basal medium was used: $MgSO_4$, 0.6 g; K_2HPO_4, 0.7 g; KH_2PO_4, 0.3 g; $CaCl_2$, 0.05 g; KNO_3, 4 g; the trace elements mixture of Skujins et al. (16), 0.1 ml; and distilled water, 1,000 ml. Either 0.2% mycelium or 0.1% cell walls (w/v) of a particular fungus was employed as carbon source in the enrichments. In some instances, the medium was supplemented with 0.001% yeast extract. A 2-g amount of soil was added to 200 ml of the medium, and the enrichment cultures were incubated on a shaker at 25 or 30 C. After several subcultures, the enrichments were plated on the hyphal wall medium which had been solidified with 1.5% agar. The plates were incubated at 30 C and examined for the formation of clear zones around the colonies.

Chitinase preparations were obtained in one of three ways: from commercial mushrooms (*Agaricus bisporus*) by the procedure of Carlisle (6) followed by dialysis of the preparation against deionized water; from *Streptomyces* 3 by the method of Skujins et al. (16); or from a commercial source (Worthington Biochemical Corp., Freehold, N.J.). Before use, 10 mg of the commercial enzyme preparation was dissolved in 1 ml of 0.05 M sodium phosphate (*p*H 8.4), and the solution was applied to a diethylaminoethyl (DEAE)-Sephadex column, from which the chitinase was eluted with 0.05 M sodium phosphate (*p*H 8.4). The eluate was collected in 2.0-ml fractions, and each fraction was tested for enzyme activity. Only the first chitinase-containing peak obtained from the commercial chitinase was used. The solution contained 63 milliunits of chitinase per ml.

Exo-β-D-(1→3) glucanase was obtained from *Streptomyces* 3 as described by Skujins et al. (16), only the first β-(1→3) glucanase fraction being utilized, or from Basidiomycete QM 806 that had been grown for 10 days on a rotary shaker at 30 C in 500-ml flasks containing 200 ml of the medium of Reese and Mandels (14). Proteins in the culture filtrate were precipitated with $(NH_4)_2SO_4$ at 0.70 saturation. The precipitate was dissolved in deionized water, dialyzed for 24 hr against deionized water, and lyophilized. DEAE-Sephadex A-50, 100/270 mesh, capacity 3.5 meq/g, was used to purify the glucanase. The ion exchanger was allowed to swell in an excess of water, after which it was treated with 0.5 N NaOH and again washed with water. The resin then was treated with 0.05 M phosphate buffer (*p*H 7.4) until it was in equilibrium with the buffer. The resulting slurry was poured into a column (40 cm by 1 cm). The lyophilized enzyme preparation (10 mg) was dissolved in 1.0 ml of 0.05 M phosphate buffer (*p*H 7.4) and added to the surface of the column; the enzyme was eluted by the addition of 0.05 M phosphate buffer (*p*H 7.4) at a pressure of 9 psi. Fractions of 3.5 ml were collected and assayed for activity.

For the production of endo-β-D-(1→3) glucanase, *Rhizopus arrhizus* QM 1032 was grown for 10 days on a rotary shaker at 30 C in 500-ml flasks containing 200 ml of the medium of Reese and Mandels (14). Proteins were precipitated from the culture supernatant fluid with $(NH_4)_2SO_4$ at 0.80 saturation. The precipitate was dissolved and dialyzed against deionized, distilled water, and then was lyophilized. The lyophilized material (10 mg) was dissolved in phosphate buffer (*p*H 7.4), and was purified by the procedure used for the exo-glucanase.

Enzyme activities were determined by incubating enzyme, substrate, and 100 μmoles of acetate buffer at 37 C in a final volume of 4.0 ml. Substrate concentrations were 3.0 mg of cell walls, 5.0 mg of chitin, 6.0 mg of laminaran, or 3.0 mg of other polysaccharides (16) in the reaction mixture. At the end of the incubation period, the enzymes were inactivated by immersing the tubes in boiling water. Chitinase activity was determined by measuring the release of *N*-acetylhexosamine, and β-D-(1→3) glucanase was assayed by measuring the formation of glucose or reducing sugars (expressed as glucose). A unit of glucanase and chitinase was defined as the quantity of enzyme required to catalyze the formation of 1 μmole of reducing sugar, expressed as glucose, or *N*-acetylhexosamine, expressed as *N*-acetylglucosamine, per min at 37 C at *p*H 5.0 and 5.6 from β-D-(1→3) glucan or chitin, respectively. Activity is usually expressed in terms of milliunits.

Melanin or a melaninlike material was obtained from 24 g of walls according to the method of Nicolaus et al. (11), and the preparation was characterized by the procedures of Lingappa, Sussman, and Bernstein (8). "Readily extracted" and "bound" lipids were obtained by the methods of Al-Doory and Larsh (1).

To characterize the sugar monomers, 30 mg of wall material was hydrolyzed with 1 ml of 72% H_2SO_4 for 12 hr at room temperature; the mixture was then diluted to 1 N acidity and further hydrolyzed in sealed tubes at 105 C for 12 hr. The solution was neutralized with $BaCO_3$, passed through a Dowex 50 H^+ column, and concentrated in a flash evaporator to 4 ml. Sugars were identified by paper chromatography with butanol-pyridine-water (10:10:5) and isopropanol-ethyl acetate-water (7:1:2) solvent systems and aniline phthalate, *p*-dimethylaminobenzaldehyde-acetyl acetone, and Tollens reagent as sprays. R_G is defined as the movement of the spots relative to glucose.

Reducing sugars were analyzed with the low alkalinity copper reagent of Somogyi (17) and arsenomolybdate chromogen of Nelson (10). Glucose was determined by means of the glucose oxidase reagent Glucostat (Worthington Biochemical Corp.) according to the directions of the manufacturer. *N*-acetylhexosamine was determined with the *p*-dimethylaminobenzaldehyde reagent (15). Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

Light absorption spectra were measured in a Beckman DU spectrophotometer. Infrared spectra were obtained with a Beckman IR 10 spectrophotometer by use of 2 mg of the melanin preparation that had been pressed into a pellet with 6 mg of KBr.

RESULTS

Four actinomycetes and one bacterium capable of lysing the hyaline hyphae of *F. solani* or *N. crassa* were isolated. The bacterium, a strain of *Pseudomonas*, showed moderate lytic activity on agar plates containing *N. crassa* hyphae, but no zone of digestion was noted if *F. solani* hyphae were provided as the only carbon source. A *Nocardia* species produced a large zone of lysis on *N. crassa*-containing agar, but the zone was small as compared with that obtained with the *Fusarium*. *Streptomyces* strains 1 and 2 yielded large clear zones when grown on agar supplied with *F. solani*, but they were less active on *N. crassa*. Of the isolates, *Streptomyces* 3 gave the largest and clearest zones. Nevertheless, none of the isolates yielded totally transparent zones when grown on agar containing *N. crassa* cell walls as sole carbon source, and very thin wall material was observed to remain within the halo of lysis. By contrast, no organisms lytic to *R. solani* could be isolated.

F. solani and *N. crassa* walls contain chitin and a glucan with β-(1→3) linkages, and lysis of the former fungus apparently is effected by chitinase and β-(1→3) glucanase (13, 16). To investigate the production of these enzymes by lytic organisms, *Streptomyces* 2 and *Nocardia* species were grown on a shaker at 30 C for 10 days in the basal medium containing 0.4% mycelium as carbon source. The results in Fig. 1 demonstrate that the greatest glucanase activity appears with *N. crassa* as the substrate, but the activity rose with time when the nocardium was the lytic organism and it fell with the streptomycete cultured on *N. crassa* hyphae. The glucanase activity was generally less when *F. solani* mycelium was the carbon source.

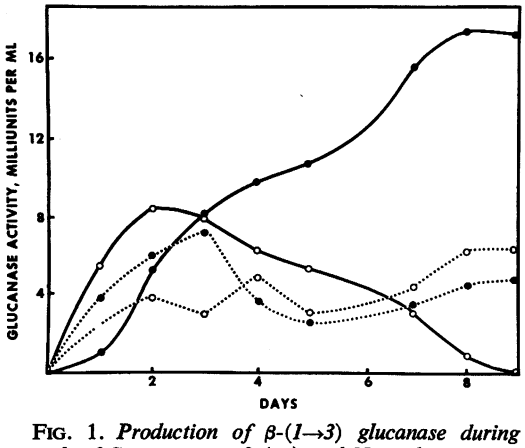

FIG. 1. *Production of β-(1→3) glucanase during growth of Streptomyces 2 (○) and Nocardia sp. (●) on Fusarium solani (dashed line) or Neurospora crassa (solid line) mycelium.*

The maximal glucanase and chitinase activity in each of the cultures examined is shown in Table 1. The three actinomycetes excreted β-(1→3) glucanase when grown on either of the fungi, but *Streptomyces* 2 and *Nocardia* sp. produced only traces of chitinase. Upon dialysis of crude extracts of *A. bisporus*, a precipitate was formed in the dialysis tubing. The precipitate was discarded, and the remaining portion was found to be rich in glucanase and chitinase. The most active of the organisms in glucanase production, however, was Basidiomycete QM 806.

Aspergillus niger was also examined as a possible source of β-(1→3) glucanase. The organism was grown at 30 C on a shaker in 500-ml flasks containing 200 ml of the medium of Reese and Mandels (14) with 0.5% starch as carbon source. The enzyme level increased rapidly after the 6th day of incubation and reached a maximal activity of 46.0 milliunits per ml of culture supernatant liquid at 9 to 10 days, after which time the activity decreased (Fig. 2). This glucanase activity is much higher than those of the actinomycetes tested. The culture filtrate did not hydrolyze carboxymethyl cellulose, β-(1→6) glucan (pustulan), chitin, galactan, mannan, xylan, polygalacturonic acid, amylose, dextran, inulin, arabinogalactan, guar gum, polyglucuronic acid, or algin.

Filtrates from 10-day cultures of Basidiomycete QM 806, the most active of the glucanase producers, contained 3.5 units of β-(1 → 3) glucanase. The enzyme was purified by the addition of $(NH_4)_2SO_4$ to 0.70 saturation, dissolving the precipitate that had formed in water, dialyzing the solution, and fractionating it on the DEAE-Sephadex column. The tube with the highest β-D-(1→3) glucanase activity contained 165 units of enzyme per ml. The enzyme, after dialysis against deionized water for 24 hr, did not form the appropriate sugar monomers from carboxymethyl cellulose, pustulan, chitin, araban, galactan, mannan, xylan, polygalacturonic acid, amylose, dextran, inulin, arabinogalactan, guar gum, polyglucuronic acid, or algin.

To determine the morphological changes induced by the two enzymes degrading polysaccharides in hyphal walls, living mycelium obtained from 1-day-old cultures of *N. crassa* and *F. solani* was treated with β-(1→3) glucanase (3.0 units/ml) and chitinase (22 milliunits per ml) prepared from Basidiomycete QM 806, *Streptomyces* 3, or a commercial chitinase. The hyphae were suspended in 0.1 M acetate buffer (*p*H 5.0) in 20% sucrose solution. Within 4 hr, spheroplasts of *F. solani* were obtained. The first noticeable effect was the withdrawal of cytoplasm from

TABLE 1. *Enzyme production by microorganisms grown in basal medium with starch, Fusarium solani, or Neurospora crassa mycelium as sole carbon source*

Organism	Carbon source	Age of culture	Activity in culture supernatant fluid*	
			Glucanase	Chitinase
		days		
Streptomyces 2	*N. crassa*	2	8.3	Trace
Streptomyces 2	*F. solani*	8	7.2	Trace
Nocardia sp	*N. crassa*	8	17.2	Trace
Nocardia sp	*F. solani*	3	6.1	Trace
Streptomyces 3	*F. solani*	3	4.7	0.0409
Agaricus bisporus	—		74.4	2.45
Aspergillus niger	Starch	9	46.0	0
Basidiomycete QM 806	Starch	10	3,500	0

* Expressed as milliunits per milliliter.

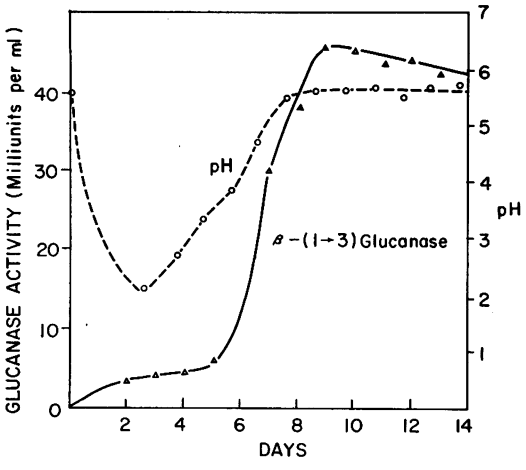

FIG. 2. *Production of β-(1→3) glucanase by Aspergillus niger.*

the cell wall to impart to the hyphae a somewhat plasmolyzed appearance. Both hyphal and macroconidial walls of *F. solani* were affected by the two enzymes. When the macroconidia were incubated with β-(1→3) glucanase and chitinase, the terminal cells were separated from the rest of the conidium after 3 hr, and the whole conidium had disintegrated after 6 hr. With *N. crassa* hyphae, on the other hand, no spheroplasts were obtained even after 6 hr, but the plasmolyzed appearance noted was identical to that of *F. solani*. The morphology of the hyphae could still be recognized, although the walls appeared much thinner when compared with untreated walls.

With *R. solani* hyphae obtained from 1-day cultures, by contrast, little effect of glucanase and chitinase on the mycelium was noticeable even after 12 hr of incubation, although some plasmolysis of the cells was observed. The lack of morphological change in *R. solani* and the failure to find any soil organisms able to lyse *R. solani* suggest that the chemistry of its wall differs markedly from that of walls of *N. crassa* and *F. solani*. Consequently, *R. solani* was investigated further.

R. solani walls (3.0 mg) were incubated with 139 milliunits of the β-(1→3) glucanase prepared from Basidiomycete QM 806 or *Streptomyces* 3 with and without 6.3 milliunits of chitinase obtained from either the commercial preparation or from *Streptomyces* 3. The same number of enzyme units was used in the reaction mixture regardless of the enzyme source. *N*-acetylhexosamine was released by action of a mixture of the two enzymes only during the initial phase of incubation, whereas the glucose release was prolonged (Fig. 3). The total amount of *N*-acetylhexosamine and glucose liberated by the enzyme mixture after 12 hr of incubation was 0.26 and 2.6%, respectively, of the wall weight. Glucose release was enhanced when chitinase and glucanase were incubated together with the walls as compared with the sugar release when the glucanase was used alone. Neither chitinase nor the glucanase alone released detectable quantities of *N*-acetylhexosamine from *R. solani* walls.

After hydrolysis of *R. solani* walls with H_2SO_4 and before neutralization of the hydrolysates, a distinct black residue appeared. Paper chromatography of the hydrolysates revealed the presence of glucose, galactose, mannose, glucosamine, and a spot similar to that given by glucuronic or galacturonic acid. Reducing sugars recovered in the acid hydrolysate accounted for 31.6% and glucose accounted for 22.4% of the total wall weight.

After incubation of *R. solani* walls with the endo-β-(1→3) glucanase preparation obtained from *R. arrhizus*, paper chromatography revealed

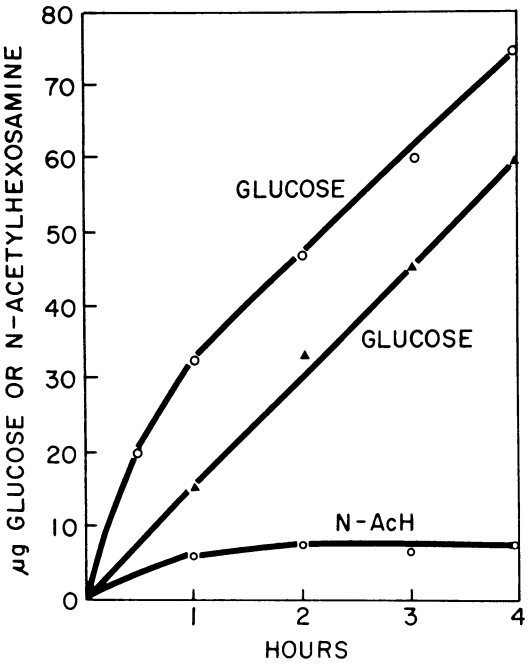

FIG. 3. *Release of glucose and N-acetylhexosamine (N-AcH) from Rhizoctonia solani walls incubated with β-(1→3) glucanase alone (▲) and with glucanase and chitinase (○).*

the presence of glucose, a substance with an R_G value of 0.45 (corresponding to gentiobiose), and two unidentified compounds with R_G values of 0.27 and 0.17. On the other hand, when *F. solani* walls were incubated with the endoglucanase, glucose and compounds with R_G values of 0.70 (laminaribiose), 0.40 (laminaritriose), and 0.23 were released. *N. crassa* walls yielded glucose, laminaribiose (R_G, 0.69), gentiobiose (R_G, 0.45), and laminaritriose (R_G, 0.41) under identical conditions.

When *R. solani* walls were extracted for 1 hr with boiling distilled water, material equivalent to 7.0% of the wall weight was removed. Readily extracted lipids and bound lipids constituted 5.2 and 7.2%, respectively, of the walls. After extracting the lipids from 500 mg of the walls of *R. solani*, *N. crassa*, and *F. solani*, the walls were treated with 138 milliunits of β-(1 → 3) glucanase and 6.3 milliunits of chitinase. There were, however, no significant differences in the rate of release of the sugar monomers from lipid-extracted walls as compared with the original preparations.

Since polyaromatics like lignin and humic acids are resistant to enzymatic degradation, it was considered possible that the presence of melanin, another polyaromatic material, in the walls might be responsible for the resistance of *R. solani* to lysis. A large amount of a black, melaninlike substance was isolated from walls of *R. solani* and *Cladosporium* sp., another fungus producing dark hyphae. The isolated material from *R. solani* showed the properties usually used to characterize melanins, namely, solubility in 0.5 N NaOH and in cold 1 N Na_2CO_3; insolubility in alcohol, ether, chloroform, and acetone; formation of a heavy brown precipitate when the preparation was treated with a small amount of Fe_2CO_3, the precipitate disappearing upon the addition of more salt; bleaching of the preparation by oxidizing agents such as H_2O_2 and 2% $KMnO_4$; and the characteristic absorption curve between 400 and 600 mμ. The isolated melanin (3.0 mg) was dissolved in 10 ml of 0.5 N NaOH, and the absorbance was determined. The line obtained by plotting the logarithm of absorbancy versus wavelength for the material obtained from *R. solani* had a slope of −0.0037; such plots are useful in the characterization of melanins.

The melaninlike material recovered after acid hydrolysis of *R. solani* and *Cladosporium* sp. walls accounted for 8.50 and 12.83% of the weight of the walls, respectively. The purified *R. solani* preparations contained 62.0% carbon, 4.8% hydrogen, 3.3% nitrogen, and 0.59% methoxyl groups, and the purified material from *Cladosporium* sp. contained 60.7% carbon, 4.0% hydrogen, 2.2% nitrogen, and 1.58% methoxyl groups. The infrared spectrum of the materials released upon acid hydrolysis of *R. solani* hyphal walls with 72% H_2SO_4 was compared with the melaninlike fraction isolated from *R. solani* and *Cladosporium* sp. walls by the procedure of Nicolaus et al. (11). The results shown in Fig. 4 reveal characteristics typical of melanin preparations.

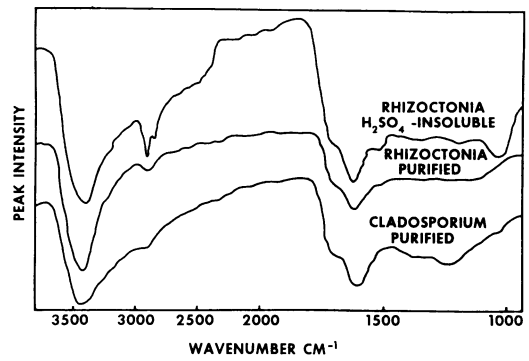

FIG. 4. *Infrared spectra of Rhizoctonia solani and Cladosporium sp. melanin.*

DISCUSSION

On the basis of the susceptibility of fungal hyphae to microbial lysis and of their walls to enzymatic digestion, three general types of mycelial wall materials are apparent. *F. solani* hyphae are readily converted to spheroplasts by purified chitinase and β-(1→3) glucanase preparations. Skujins, Potgieter, and Alexander (16) earlier showed that purified preparations containing the same two enzymes digested 94% of the wall material of this organism, leaving only a small quantity of insoluble residue containing mannose, galactose, and a uronic acid. On the basis of these studies, the major wall components of the fusarium seem to be chitin and a glucan with β-(1→3) linkages, and enzymatic digestion releases *N*-acetylhexosamine equivalent to 47% and glucose equivalent to 14% of the wall. The chitin appears to exist, at least in part, in an internal shielded layer masked by a glucan component.

In contrast to the readily lysed walls of *F. solani* is the surface structure of *N. crassa*. Although the chitinase and glucanase preparations effected some changes in the hyphae of the latter organism, spheroplasts were not produced. Nevertheless, Potgieter and Alexander (13) demonstrated that *N. crassa* hyphal walls contain chitin and a glucose polymer which was hydrolyzed to the monomer by β-(1→3) glucanase preparations. Hence, it is likely that the walls of this fungus contain components, in addition to chitin and the glucan, which permit the maintenance of morphological integrity in the presence of chitinase and the glucanase. Yet, strains of *Pseudomonas*, *Nocardia*, and *Streptomyces* capable of lysing these hyphae were isolated, although the lysis was not as complete as that observed with *F. solani*. Bachman and Bonner (2) observed that spheroplasts of juvenile hyphae of *N. crassa* could be obtained by incubating the organism with snail digestive juice, but these investigators also observed that the walls were not completely dissolved.

The composition of the walls of *R. solani* is completely different. No organisms using these walls as carbon sources could be isolated by elective culture techniques, and no significant morphological changes occurred when the hyphae were incubated with chitinase and β-(1→3) glucanase preparations. Further, despite the fact that reducing sugars account for 31.6% of the walls, most being glucose, only a small quantity of monosaccharides was released when the walls were treated with the glucanase and chitinase. Lockwood (9) and Papavizas (12) observed some lysis of *R. solani* in soil, but the mycelium of this organism was more resistant to degradation under natural conditions than those of the other fungi tested.

The reason for the resistance of *R. solani* hyphae to lytic degradation is not yet clear. The most striking difference between *R. solani* and *N. crassa* or *F. solani* is the presence in its walls of a significant amount of an acid-insoluble, melaninlike material. The results indicate that lipids do not interfere with wall degradation by chitinase and glucanase. It is tempting to suggest that melaninlike compounds, most of which are considered as random polymers (4, 18), are the structural components serving to protect certain fungal forms from microbial lysis in nature. Blois (4) found no evidence for the degradation of melanin by mixed bacterial cultures, and Lockwood (9) noted that the dark mycelium of *H. sativum* and *A. solani* was more resistant to lysis than the hyaline filaments. *R. solani* does not form spores profusely, and a surface-localized, resistant polyaromatic material may, therefore, be associated with its survival in nature.

The solubility characteristics of the *R. solani* preparations, its susceptibility to bleaching by oxidizing agents, and the characteristic light and infrared absorption spectra all indicate the identity of the dark material with melanin. The slope obtained by plotting the logarithm of absorbancy versus wavelength, which is used to characterize melanins, is −0.0037 for the *R. solani* material, a value comparable to the extracellular melanin of *Aureobasidium pullulans* (8), but different from that of *Mucor rouxii* spore walls (3), and the infrared spectra for the *R. solani* and *Cladosporium* sp. melanins are comparable to those obtained for other melanins (4, 5). The elemental analysis of these two preparations suggests similarities with the melanins of animal and plant tissues (11).

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant EF-00295 from the Division of Environmental Engineering and Food Protection, and by grants from the Maize Industry Control Board and the Department of Agricultural Technical Services, Republic of South Africa.

We thank A. M. Srb, D. F. Bateman, and E. T. Reese for gifts of cultures, and P. Klein for able technical assistance.

LITERATURE CITED

1. AL-DOORY, Y., AND H. W. LARSH. 1962. Quantitative studies of total lipids of pathogenic fungi. Appl. Microbiol. **10**:492–495.
2. BACHMANN, B. J., AND D. M. BONNER. 1959. Protoplasts from *Neurospora crassa*. J. Bacteriol. **78**:550–556.

3. BARTNICKI-GARCIA, S., AND E. REYES. 1964. Chemistry of spore wall differentiation in *Mucor rouxii*. Arch. Biochem. Biophys. **108**: 125–133.
4. BLOIS, M. S. 1965. Random polymers as a matrix for chemical evolution, p. 19–38. *In* S. W. Fox [ed.], The origins of prebiological systems and of their molecular matrices. Academic Press, Inc., New York.
5. BONNER, T. G., AND A. DUNCAN. 1962. Infra-red spectra of some melanins. Nature **194**:1078–1079.
6. CARLISLE, D. B. 1960. Softening chitin for histology. Nature **187**:1132–1133.
7. HORIKOSHI, K., AND K. ARIMA. 1962. X-ray diffraction patterns of the cell wall of *Aspergillus oryzae*. Biochim. Biophys. Acta **57**:392–394.
8. LINGAPPA, Y., A. S. SUSSMAN, AND I. A. BERNSTEIN. 1963. Effect of light and media upon growth and melanin formation in *Aureobasidium pullulans* (de By.) Arn. (= *Pullularia pullulans*). Mycopathol. Mycol. Appl. **20**:109–128.
9. LOCKWOOD, J. L. 1960. Lysis of mycelium of plant-pathogenic fungi by natural soil. Phytopathology **50**:787–789.
10. NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**:375–380.
11. NICOLAUS, R. A., M. PIATTELLI, AND E. FATTORUSSO. 1964. The structure of melanins and melanogenesis. IV. On some natural melanins. Tetrahedron **20**:1163–1172.
12. PAPAVIZAS, G. C. 1963. Microbial antagonism in bean rhizosphere as affected by oat straw and supplemental nitrogen. Phytopathology **53**: 1430–1435.
13. POTGIETER, H. J., AND M. ALEXANDER. 1965. Polysaccharide components of *Neurospora crassa* hyphal walls. Can. J. Microbiol. **11**:122–125.
14. REESE, E. T., AND M. MANDELS. 1959. β-D-1,3 Glucanases in fungi. Can. J. Microbiol. **5**:173–185.
15. REISSIG, J. L., J. L. STROMINGER, AND L. F. LELOIR. 1955. A modified colorimetric method for the estimation of N-acetylamino sugars. J. Biol. Chem. **217**:959–966.
16. SKUJINS, J. J., H. J. POTGIETER, AND M. ALEXANDER. 1965. Dissolution of fungal cell walls by a streptomycete chitinase and β-(1$\rightarrow$3) glucanase. Arch. Biochem. Biophys. **111**:358–364.
17. SOMOGYI, M. 1952. Notes on sugar determination. J. Biol. Chem. **195**:19–23.
18. SWAN, G. A. 1963. Chemical structure of melanins. Ann. N.Y. Acad. Sci. **100**:1005–1019.