Effect of the Growth of *Phanerochaete chrysosporium* in a Blend of Low Density Polyethylene and Sugar Cane Bagasse

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ABSTRACT: Thermogravimetry, differential scanning calorimetry, and wide-angle X-ray scattering techniques were used to study changes in the composition, relative heat of fusion, and mean crystal size of blends made from low-density polyethylene (LDPE) and sugar cane bagasse (SCB), before and after exposure to the fungus *Phanerochaete chrysosporium* for 32 days. The initial blends contained equal weights of each component. The composition of the blend LDPE/SCB at 32 days changed to the value (66 $\pm$ 3)/(34 $\pm$ 3). The relative heat of fusion of LDPE increased during the first 16 days, but then it showed a tendency to decrease and remain constant. The estimated mean crystal size of polyethylene decreased but then remained almost constant. These changes indicate that the microorganisms mainly digest SCB in the first stage of the experiment, but later they also digest LDPE. The crystalline morphology of the LDPE is modified; the crystalline domains are divided into smaller crystallites. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 105–111, 1997

Key words: degradation; crystalline morphology

INTRODUCTION

Because problems related to solid waste are increasing, it is necessary to find viable alternatives. Plastics are a visible part of the problem because of their extended use as packaging materials. Some polyolefins, such as polyethylene and polypropylene, have excellent physical properties and therefore are produced in high quantities; unfortunately, they are resistant to degradation. Fortunately, plastics can be recycled and incinerated, and some of them can be made to be degradable by introducing prodegradant additives into the matrix. A strategy to turn these materials into biodegradable forms is to mix them with natural polymers.[1–3] Polyolefin/starch mixtures are degraded in the natural environment by different mechanisms: chemical degradation, photodegradation, and biological degradation.

In particular, the biodegradation of low-density polyethylene (LDPE) films containing starch and pro-oxidants has been studied in aqueous media inoculated with bacteria and molds at room temperature.[4] The time at which degradation starts to occur in the LDPE matrix strongly depends both on the sample thickness and on the activity of the microbiological system. LDPE without starch and pro-oxidant is not affected by microorganisms. In the degradation of LDPE in the blend, the bacteria *Arthrobacter paraffineus* was slightly more efficient than a fungal mixture of *Verticillium lecanii* and *Verticillium migresens*. Biodegradation of polyethylene/starch composites, with different starch content and pro-oxidant additives, has also been carried out by composting.[5]

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Studies on composite materials have also been carried out with axenic cultures of the lignocellulosic degradators, bacteria *Streptomyces* species and fungus *Phanerochaete chrysosporium.*[6]

In this study, the effect of the growth of *P. chrysosporium* on a blend of LDPE and sugar cane bagasse (SCB) was characterized as a function of digestion time. The purpose is to study the changes in the crystalline morphology of polyethylene by using a variety of techniques. Thermogravimetry (TGA) was used to determine the composition, differential scanning calorimetry (DSC) was used to determine the changes in the melting enthalpy, and X-ray diffraction was used to estimate the mean crystal size of LDPE.

EXPERIMENTAL

Sample Preparation

The main chemical constituents of the SCB are cellulose (approximately 43%), hemicellulose (approximately 26%), and lignin (approximately 21%); the remainder (10%) corresponds to soluble components in organic solvents (no more than 3%), ashes (2–3%), and water-soluble components such as residual saccharose, other sugars, and polysaccharides. In order to eliminate easily degradable material, the SCB was thoroughly washed with hot water (80°C) and dried in a desiccator under a dry air current at 60°C and 1.8 kg/cm^2 for 12 h. Washed SCB was mixed with commercial LDPE (PX 17070, Pemex), in equal parts by weight (50 : 50), in a Banbury mixer at 150°C and 40 rpm for 10 min. The composite material was ground and sieved to obtain particle sizes between 0.8 and 2.4 mm. A culture medium was prepared containing, per liter of distilled water, the following substances (in g/L): saccharose, 50; KH_2PO_4, 0.312; K_2HPO_4, 0.187; $CaCl_2$, 0.484; NaCl, 0.320; $MgSO_47H_2O$, 0.380; $FeSO_47H_2O$, 0.010; $(NH_4)_2SO_4$, 2.000, and yeast extract, 0.200. Sixty milliliters of this culture medium was mixed with 90 g of the composite material and inoculated with 2×10^7 spores of *P. chrysosporium* per gram of dry matter. Initial pH and moisture content values were 7 and 41%, respectively. An aeration rate of 2 mL of air per minute per gram of wet matter was used. The inoculated material was incubated in glass columns[7] at 35°C for 32 days, during which samples were taken at intervals of 4 days to determine pH, moisture content, and some polyethylene characteristics. Those values reported at 0 days correspond to the control sample, to which microorganisms were not added.

Thermal Analysis

The effect of the mold growth on the thermal properties of the LDPE/SCB blend was examined by TGA and DSC techniques. Samples were run in triplicate in a TGA analyzer (951, TA instruments) from room temperature (25°C) to 600°C, using an atmosphere of nitrogen (50 mL/min) and a heating ramp of 10°C/min. The TGA thermograms were obtained only for pure materials (LDPE and SCB) and for the blends at the beginning and at the end of the experiment (0 and 32 days). Each sample was run twice in a DSC analyzer (910S, TA instruments). The unit was calibrated using indium. LDPE/SCB samples were encapsulated in standard aluminum pans and heated at a rate of 10°C/min from room temperature to 180°C in an atmosphere of nitrogen (50 mL/min).

X-Ray Determinations

The estimation of the crystallite size was determined in the standard way[8] by using the wide-angle X-ray scattering technique (WAXS). The WAXS patterns were recorded with a Philips horizontal goniometer model PW 1380/60 fitted with a scintillation counter, a pulse-height analyzer, and a graphite crystal monochromator placed in the scattered beam. CuKα radiation was used and the scattered radiation was registered in the angular interval (2θ) from 5 to 45°.

RESULTS AND DISCUSSION

P. Chrysosporium Growth

Because of the heterogeneity of the composite material, growth measurement was not directly carried out. However, substrate consumption, which is closely related to the mold metabolism, was estimated by the weight loss of dry matter. This loss of dry matter, the results of which are represented in Figure 1, started at the beginning of the culture and increased rapidly up to 12 days of cultivation; after this time, the dry matter remained almost constant, around 16%, indicating that the metabolic activity was reduced. This value of 16% in the loss of dry matter detected at 32 days corresponded to a mass ratio of LDPE/SCB = 60/40,

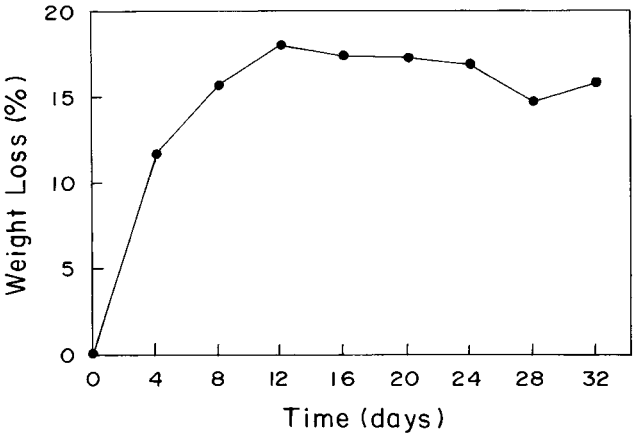

Figure 1 Weight loss of dry matter as a function of time.

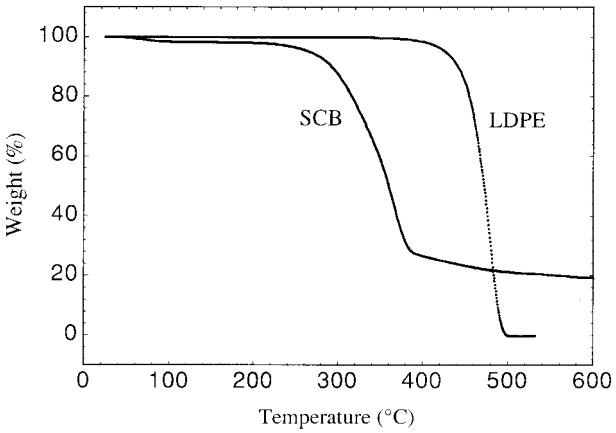

Figure 3 TGA thermograms for LDPE and SCB.

if the assumption is made that the weight loss of dry matter was only due to the SCB consumption.

The pH value decreased from its initial value of 7 to 4 during 12 days of culture (see Figure 2), after which it remained almost constant, increasing slightly at the end of the experiment because of the proteolysis of the mold. During the first 12 days of culture, the decrease of pH as well as the weight loss of dry matter reflected maximal metabolic activity of the mold due to the saccharose added in the culture medium. The reduction of the metabolic activity was due to the presence of carbon sources difficult to metabolize such as hemicellulose, cellulose, lignin, or the polyethylene. Figure 2 also shows that the moisture increased rapidly from the very beginning of the experiment from 41 to 53% in 12 days, after which it presented small changes. The increase of the moisture content during the culture reflects some level of degradation of the fiber present in the SCB.

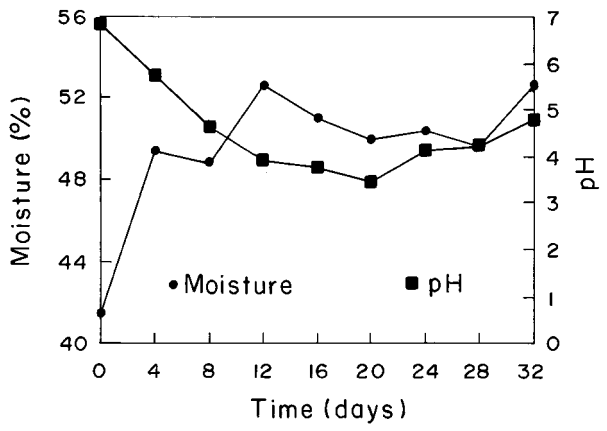

Figure 2 Time dependence of moisture (circles) and pH (squares).

Thermal Properties

Typical TGA thermograms obtained for the blend components are reproduced in Figure 3. Thermal degradation of pure polyethylene started around 380°C and finished at 500°C with 0.13% of the initial weight. On the other hand, pure SCB had a slight loss of weight in the temperature range from 40 to 85°C due to the water content in the material. In the range from 85 to 198°C, the weight remained almost constant (96%), but in the range from this last temperature to 520°C, the SCB was degraded and its weight was reduced up to 18% of the initial value. Thus, the temperature of processing (150°C) of the LDPE/SCB blend in the Banbury mixer did not contribute to its thermal degradation.

With a selection of adequate temperature intervals (far from the large slope changes) in the TGA thermograms of the pure materials and of the biologically treated mixture, it was possible to determine the homogeneity in the composition of the initial blend sample and to quantify its composition after the microbiological treatment. For example, 50% reduction of the pure SCB weight was obtained at 351 ± 5°C. At this temperature, the weight percentages of the blend sample at the beginning and after 32 days of culture were 75 ± 3 and 82 ± 1, respectively. Thermograms for the blends at 0 and 32 days are shown in Figure 4.

In order to determine the composition of the blend, the weight of each component was calculated. From the TGA thermograms, the weight of the component materials may be calculated in the

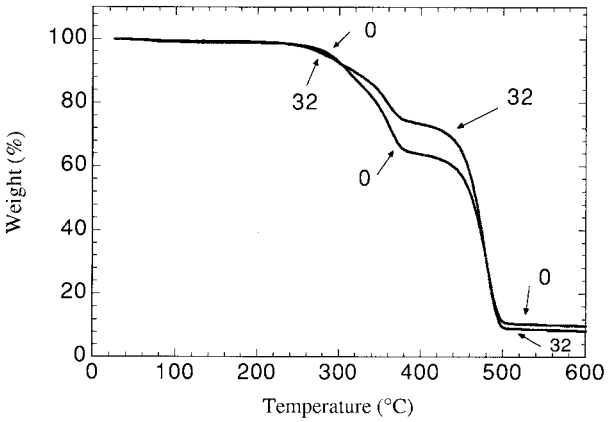

Figure 4 TGA thermograms for the blend LDPE/SCB without the effect of microorganisms (0 days) and after 32 days of fermentation.

following way. At the beginning (at room temperature), the weights in the blend add up to 100%, i.e., $M_P + M_B = 1$, where M_P and M_B represent the weights of polyethylene and of SCB, respectively. In the temperature range $T < 380$°C, where only SCB degrades (the thermal degradation of LDPE started to occur at 380°C), the weight of SCB remaining in the sample at temperature T is $M(T) = xM_B$, where x, obtained from the thermogram of pure SCB, is the fraction of M_B that has not been degraded at this temperature. The fraction (y) of the total weight of the blend that, at temperature T, has not been degraded (taken from the TGA thermogram of the blend) is $y = [M_P + M(T)]/(M_P + M_B) = M_P + M(T)$. Combining these three relations, M_B is expressed in terms of the experimentally measured parameters y and x; the result is

$$M_B = (1 - y)/(1 - x). \qquad (1)$$

Following the above procedure and selecting temperatures at which the reduction of the weight of the pure SCB had fixed values, average LDPE/SCB weight ratios of 51/49 at 0 days and (66 ± 3)/(34 ± 3) at 32 days of culture were obtained. The observed ratio value at day 0 was as expected because the initial blend contained 50% of LDPE and 50% of SCB. However, the result obtained at 32 days of culture differs from the previous value (60/40), evaluated from the assumption that weight loss was due to the SCB fraction only, and indicates that the LDPE fraction was also modified by the action of the mold.

Some difference was observed in the shape of the DSC thermograms corresponding to the two runs of the same sample. In the region where the SCB produced a signal, the difference was due to the water content present in the sample during the first run. Because the contributions of the LDPE and the SCB in the DSC thermogram were difficult to separate, a geometrical strategy was followed in order to determine thermal differences in the materials treated with the mold. For that purpose, straight lines converging to the apex and being tangent to both sides of the endotherm were drawn and a triangle was completed by prolonging the base line from the high temperatures side. We assume, to a first approximation, that the area of the triangle is proportional to the heat of fusion of the LDPE fraction. It is important to emphasize that, because the first contributions to the melting endotherm come from the smallest or less perfect crystals, with this geometrical method, the contribution to the heat of fusion from the smallest crystallites is not taken into account. Kinetics of the heat of fusion differed in the two runs; values from the first run were more dispersed than those from the second run. The melting temperature of the LDPE fraction, taken at the apex of the endotherm, remained almost constant at 109°C.

The second run values of the heat of fusion, relative to that of the control sample, are shown in Figure 5. If the heat of fusion of the control sample is taken as unity, i.e., ΔHrel = 1 for the sample at 0 days, this figure shows that the heat of fusion at any time is greater than that of the reference. In the curve, there are two intervals separated by day 16, which could mean that the dominant mechanism causing the increase in ΔHrel is different in these intervals. In the initial interval, the *P. chrysosporium* digests predomi-

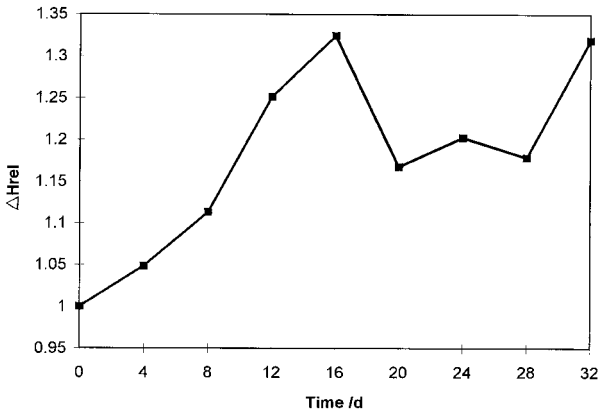

Figure 5 Heat of fusion of the LDPE relative to that of the sample at 0 days.

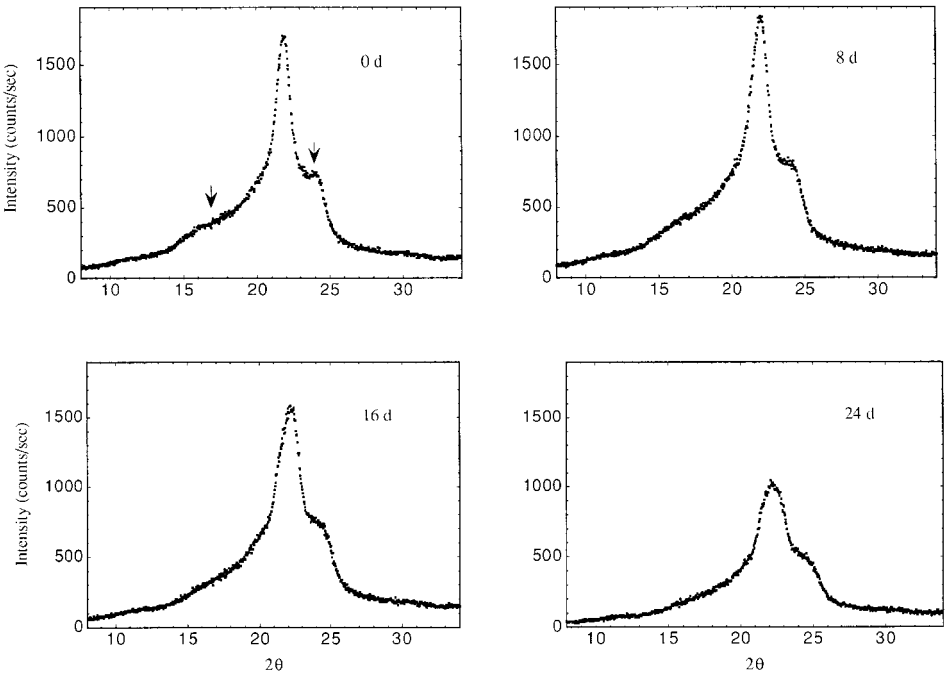

Figure 6 WAXS spectra for the blend LDPE/SCB at four fermentation times: 0, 8, 16, and 24 days. The left arrow indicates the signal coming from the SCB, and the right arrow indicates the 200 reflection of polyethylene.

nantly the SCB fiber because this material is more easily biodegradable than polyethylene. In this way, the weight ratio LDPE/SCB increases, i.e., more than half of the weight of the sample is of polyethylene. In other words, the apparent increase in time of the crystallinity of the sample is mainly because the whole amorphous phase in the blend is decreasing by the effect of the microorganisms.

The relative heat of fusion (ΔHrel) after day 16 has the tendency to decrease and remain constant, but it is still higher than that of the reference sample. This effect may be caused by the combination of two possible mechanisms. In addition to the consumption of SCB, the *P. chrysosporium* may also digest the polyethylene, mainly in its amorphous phase, which is less resistant to enzymatic attack than the crystalline phase.[2] This amorphous phase has two components: one that surrounds the crystalline particles, and the other that defines the boundaries and separates the crystalline blocks in the crystalline mosaic.[9,10] With the consumption of part of this latter amorphous component by the effect of the microorganisms, the crystalline particles may be divided into particles of smaller size. Because of the mixing method used here, it is more reasonable to expect the incorporation of the SCB component predominantly in the amorphous phase of the polyethylene, thus increasing the amorphous phase surrounding the crystalline regions, rather than in the amorphous region between the crystalline blocks. The microbial attack is mainly limited to surface or near-surface accessible particles, except in the case of hyphal penetration from fungi, which can occur deeper into the sample.[4] This does not immediately lead to disintegration of the LDPE matrix but weakens it and increases the permeability. Thus, the value of the relative heat of fusion decreases because the particle size decreases.

WAXS Results

The pure polyethylene spectrum shows the two sharp characteristic peaks at 21.7° and at 23.9° of the angular position 2θ; they are the reflections 110 and 200, respectively, from the crystalline domains.[11] In comparison, the SCB fiber shows two broad low-intensity peaks at 11.7° and 17.5° 2θ. The spectra of the blend samples change during fermentation time. The effect of the *P. chrysosporium* on the crystalline portion of both components in the blend is illustrated in the X-ray spectra shown in Figure 6. The intensity of the SCB fiber peaks decreased during fermentation and disap-

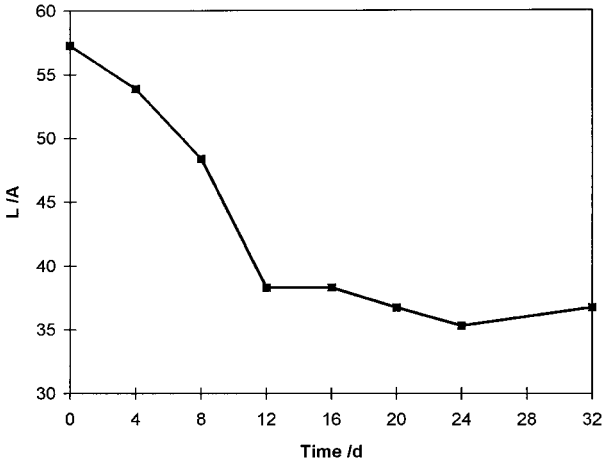

Figure 7 Mean crystal size of polyethylene as a function of time. Size in Angstrom unit.

peared at day 20. The intensity of the LDPE peaks also diminished. The 200 peak decreased to become a shoulder; the intensity of the peak 110 was also reduced, and it became wider during the fermentation time. This broadening of the peak 110 could be due to a reduction in the size of the crystallites.

The estimation of the mean size of the crystalline particles of polyethylene was made by using the WAXS data and the Scherrer equation, which is given by[8]

$$L_{hkl} = K\lambda/\beta \cos \theta \qquad (2)$$

where L_{hkl} represents the mean crystal dimension normal to the corresponding *hkl* plane, β is the half-height width of the scattering peak, 2θ is the scattering angle, λ stands for the radiating wavelength, and K is a constant of the order of 1. This equation does not consider instrumental broadening corrections, but for the purpose of this study, it is used as a practical reference to estimate the average dimension of the crystalline particles. With $\lambda = 1.5418$ Å, θ corresponding to that of the 110 plane, and β measured from the angular position of this peak, the crystal mean size as a function of time was estimated.

The half-height width (β) of the main peak increased up to day 20 of fermentation and remained practically constant up to the end of the experiment. The Scherrer equation applied to the obtained data demonstrated that the crystal mean size decreased from 57 Å, in the sample without mold, to 37 Å, in the sample biologically treated during 20 days. These results are shown in Figure 7. This value for the crystal size of the reference sample falls in the range of sizes (40–300 Å) reported for polyethylene.[12] The effect of the microorganisms in semicrystalline polymers is expected to occur in the amorphous phase rather than in the crystals because the latter are more resistant to enzymatic attack.[2] As mentioned above, in the polyethylene, two amorphous regions can be distinguished. In one, the crystalline particles are imbedded; these crystalline particles are formed by crystalline blocks, which are separated from each other by the other amorphous region. This second amorphous region is smaller than that surrounding the crystalline particles.[9,10] The reduction observed in the mean size of the crystalline particles is a consequence of the effect of the *P. chrysosporium* present in the blend. The WAXS technique cannot detect if the rupture of chains is taking place in the amorphous region surrounding the crystalline particles or in the amorphous region separating the crystalline blocks. However, if the rupture of chains is made in the amorphous region between the blocks, the effect can be detected because the mean size of the crystallites reduces, as illustrated in Figure 8. This could indicate that the effect of these microorganisms is able to penetrate and attack the interblock amorphous phase. These results confirm the information obtained from the DSC results given above.

CONCLUSION

TGA is an adequate technique that allowed us to calculate the content of each component in the LDPE/SCB blend after microbial treatment with the fungus *P. chrysosporium*. The changes in the relative heat of fusion of polyethylene obtained by DSC indicate that the microorganisms mainly digest SCB at the beginning of the experiment, but later they also digest polyethylene. X-ray results showed that one effect of the growth of *P. chrysosporium* is to modify the crystalline mor-

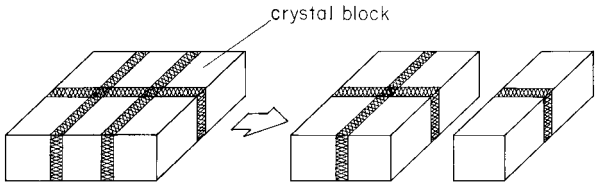

Figure 8 Schematic representation of the mechanism producing the reduction in the mean size of the crystalline structure. The shadowed regions represent the amorphous boundaries of the blocks.

phology of the LDPE by dividing the crystalline domains into smaller crystals. This division is possibly due to the breakdown of molecules in the amorphous phase that bind the crystalline blocks. From the results presented, it can be considered that there is strong evidence to support reduction in the polyethylene integrity caused by the fungus *P. chrysosporium*.

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