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Phylloplane Fungal Enzyme Accelerate Decomposition of Biodegradable Plastic Film in Agricultural Settings

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Abstract

The rate at which biodegradable plastic (BP) mulch films decompose in agricultural fields depends on environmental conditions. If degradation of used mulch film is insufficient for plowing-down, they impede agricultural work and get entangled in farm equipment. We developed a new technique to accelerate the degradation of BP mulch films in agricultural fields by applying an enzyme from a *Paraphoma*-like phylloplane fungus (strain B47-9). Spray treatment of the enzyme solution alone significantly accelerated film degradation, and the additional application of a moisture-retaining agent, carboxymethyl cellulose sodium salt (CMC), further accelerated decomposition. Commercially available BP mulch films started to break down one day after treatment with the enzyme solution and CMC. Within seven days of treatment, small tears in the film turned into long cracks, covering 6.2% of the total film area.

Discipline: Agricultural environment
Additional key words: biodegradable mulch film, accelerated degradation, moisture-retaining agent

Introduction

Agricultural mulch films are widely used in vegetable farming to prevent weed growth, maintain stable soil temperatures, and improve the effectiveness of water and fertilizer. Commonly used plastic mulch films are made of non-biodegradable polyethylene (PE), although collecting these films after use is labor-intensive and recycling soil-contaminated films is difficult. Recently developed biodegradable plastic (BP) products are decomposed by soil microorganisms and can be plowed into fields (Brodhagen et al. 2015; Kijchavengkul et al. 2010). Still, BP mulch film is not widely used on farms due to its variable degradation rates (Kyrikou and Briassoulis 2007). Moreover, its strength decreases with the defragmentation of polymer chains, and may be too weak to stretch over soil beds by the middle of the growing season. However, if the film's strength was enhanced, it would decompose slowly and remain intact long after harvest. Thus, the degradation of BP mulch film after use must be accelerated to facilitate its incorporation into soil (Kyrikou and Briassoulis 2007). The strength of BP mulch film depends on the blended BP compounds, which are generally various polyesters, such as polybutylene succinate-*co*-adipate (PBSA), polybutylene succinate (PBS), and polybutylene adipate-*co*-terephthalate (PBAT).

We previously selected the *Paraphoma*-related fungal strain B47-9, isolated from leaves, as a strain that strongly degrades PBS and PBSA films (Koitabashi et al. 2012). When the fungus was applied to a commercially available mulch film (composed of a mixture of PBS, PBSA, and PBAT) that was placed on the surface of sterilized soil in a Petri dish, the film lost up to 99.8% of its weight after six days of incubation at 28°C, whereas the same film treated with water did not degrade (Koitabashi et al. 2012). Moreover, when the fungal strain was cultivated in a liquid medium containing emulsified PBSA as the only carbon source, it secreted a BP-degrading enzyme that was isolated and identified as a *Paraphoma*-related cutinase-like enzyme (PCLE). This enzyme degrades compounds at various rates, breaking down PBSA fastest, followed by PBS and PBAT (Suzuki et al. 2014). In this study, we determined whether application of the PCLE-containing culture filtrate (enzyme solution) could accelerate the degradation of BP mulch films in an agricultural setting. Moreover, we developed an efficient and practical method of applying the enzyme solu-

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tion to agricultural fields.

Materials and Methods

1. Experiment location

We used a pipe house (20 × 5.5 m) in an experimental field at the National Institute of Agro-Environmental Sciences (Kannondai, Tsukuba, Ibaraki, Japan) for the BP mulch film degradation experiment.

2. Mulch film

We used three types of BP mulch film colored with carbon powder: one was composed of PBSA (Bionolle 3001G); one of PBS (Bionolle 1001G); and one of PBS, PBSA and PBAT (at a monomer-based molar ratio of 49:37:14). In addition, we used a non-degradable PE mulch film (Noupori; Sekisui Chemical Co., Ltd., Osaka, Japan). Each piece of film was 20 μm thick, 135 cm wide, and cut as per the experimental plot length.

3. Preparation of enzyme solution

We prepared a culture broth of fungal strain B47-9 with enzymatic activity to degrade emulsified PBSA at 0.28 U/mL as described by Koitabashi et al. (2012). To prepare the enzyme solution, we removed mycelia from the broth by using a membrane filter with a pore size of 0.45 um (Advantec C045A090C; Toyo Roshi Kaisha, Ltd., Tokyo, Japan).

4. Field design

(1) Enzyme-based degradation of BP mulch film

We established three square soil beds (1 × 1 m × 0.1 m deep) for each type of mulch film in the experimental field in September 2009. We initially sprayed 3 L of enzyme solution onto each soil bed using a manual sprayer (MH9D-1; Maruyama Mfg., Co. Inc., Tokyo, Japan). Then we laid the mulch films on the beds, covered the edges with soil, and sprayed 1 L of enzyme solution onto each film's surface. After 10 and 20 days, we covered each plot with a piece of simili paper and traced all the cracks in the film. Crack lengths were measured with a cotton thread, and the sum of all lengths was calculated to assess the degree of degradation.

(2) Enzyme-based degradation of BP mulch film supplemented with a moisture-retaining agent

Effect of treatment with a powdered moisture-retaining agent and enzyme solution: In September 2010, we established rows of PBSA, PBS, and commercial BP film (15 × 1 m) in a field, spaced 50 cm apart. One of two moisture-retaining agents—carboxymethyl cellulose sodium salt (CMC; Wako Pure Chemical Industries, Ltd., Osaka, Japan) or the Sanfresh ST-500D water-retentive polymer (SF; Sanyoukasei Co. Ltd., Aichi, Japan)—was wiped onto the surface of each row of film at 28 g/m^2. Then the enzyme solution was sprayed on the film surface at 400 mL/m^2 so that the surface was thoroughly wetted. Control plots received no moisture-retaining agents or enzyme solution. We replicated each treatment in the three plots. The film in each plot was photographed after incubation periods of one day and seven days, respectively, and the total crack length (cm) and hole area were measured using ImageJ processing software (Schneider et al. 2003). Degradation ratios were calculated as the ratio of the total area of holes to the total film area (expressed as percentages).

Effect of treatment with a moisture-retaining agent dissolved in the enzyme: In October 2010, we established plots of PBSA, PBS, and commercial BP films (60 × 50 cm). Plots received one of the following treatments: (a) enzyme solution application (sprayed on at 400 mL/m^2); (b) CMC powder and enzyme solution application (wiped on at 14 g/m^2 and sprayed on at 400 mL/m^2, respectively); or (c) application of CMC powder dissolved in the enzyme solution (dissolved at 1% w/v; sprayed at 400 mL/m^2). Control plots were either wiped with CMC powder (14 g/m^2) and then sprayed with sterilized distilled water (400 mL/m^2), or received no treatment. Treatments were not replicated. The film in each plot was photographed after incubation periods of one day and seven days, respectively.

Results

1. Evaluation of enzyme-based BP mulch film degradation

Both the PBSA and PBS mulch films had cracks 10 days after treatment with the enzyme solution (Fig. 1). Crack length on the PBSA film increased from 64 cm to 114 cm from days 10 to 20, whereas crack length on the PBS film increased from 19 cm to 48 cm during the same period (Fig. 2). Cracks on both types of film treated with the enzyme were significantly longer than those on untreated film at all observation times, indicating that the enzyme accelerated degradation. The non-biodegradable PE film did not show signs of degradation throughout the experiment (data not shown).

2. Enzyme-based degradation of BP mulch film supplemented with a moisture-retaining agent

For large-scale applications, the amount of enzyme we used per m^2 would not be practical; therefore, we considered ways to increase application efficiency. Because evaporation of the enzyme solution after its application would inhibit the enzymatic hydrolysis of the film, treatment with a moisture-retaining agent could improve film degradation. Figure 3 shows the images of three types of BP film one day and seven days after the films were wiped with moisture-retaining agents (CMC and SF), and then sprayed with the

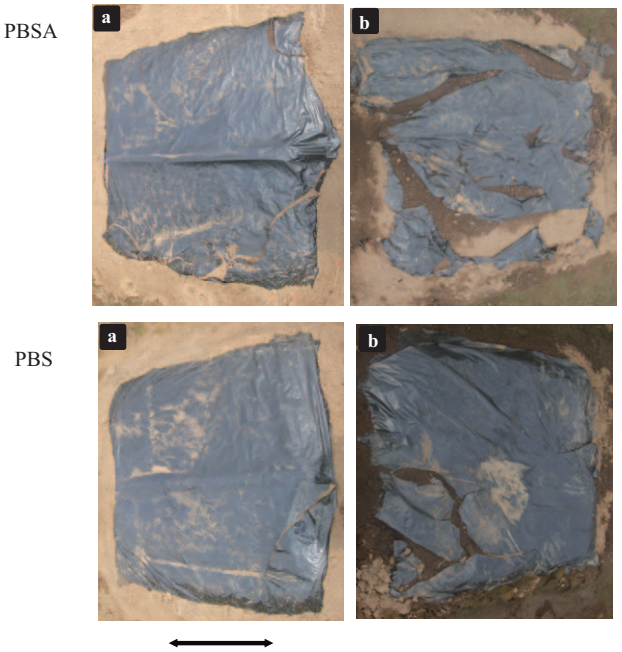

Fig. 1. Degradation of PBSA mulch film and PBS film over 10 days following enzyme application to the soil bed. Films were treated with (a) no treatment; (b) enzyme solution. The arrow indicates the film's drawing direction

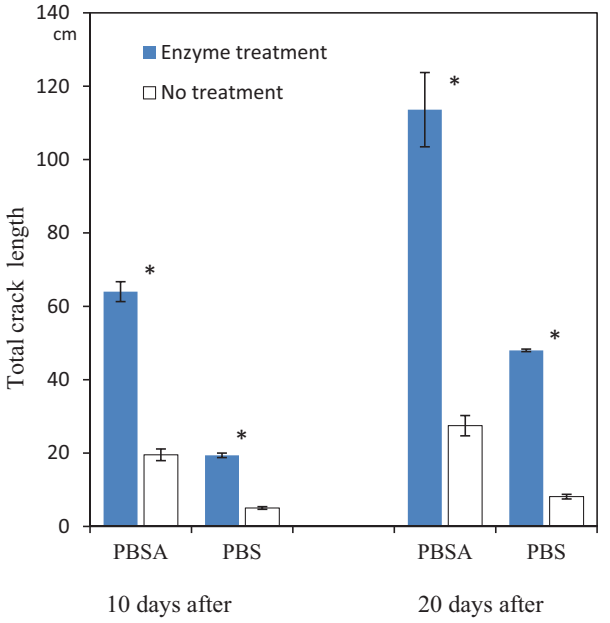

Fig. 2. Cracks lengths (cm) on mulch films after application of enzyme solution
*** P < 0.05 according to the Student's *t*-test. Data represents the means of three replicates**
Error bars represent standard errors

enzyme solution. Table 1 shows the total crack length and degradation ratio one day and seven days after the enzyme solution (supplemented with the moisture-retaining agents) was applied to all the BP films. The degree to which films degraded differed between the combinations of film type and moisture-retaining agent treatments (Table 1). One day after treatment with enzyme solution containing either CMC or SF, the total crack length in PBSA and PBS films was greater than 60 cm, and the cracks were increased after seven days of incubation. Enzyme treatment with CMC powder also accelerated the degradation of commercial BP films, which had a total crack length of 45.8 cm and a degradation ratio of 1.9% one day after treatment. Seven days after treatment, the crack length and degradation ratio increased to 74.0 cm and 6.2%, respectively. However, no cracks were observed on commercial films treated with the enzyme and SF and no degradation was observed in control films.

We further investigated CMC as a supplemental treatment to accelerate film degradation, as its application increased degradation more than SF. Figure 4 shows

Table 1. Degradation of BP films treated with the enzyme solution and moisture-retaining agents

Moisture-retaining agents	CMC[1]		S F[2]	
Days after treatment	1 day	7 days	1 day	7 days
Total crack length (cm)				
P B S A	60.7 ± 10.5*	82.3 ± 22.3	64.8 ± 5.5	100.6 ± 20.0
P B S	96.1 ± 21.6	159.0 ± 65.9	67.7 ± 5.0	97.8 ± 25.9
Commercial BP film	45.8 ± 23.4	74.0 ± 45.2	0.0	0.0
Degradation ratio (%)				
P B S A	4.4**	10.3	2.6	10.6
P B S	3.1	9.2	1.6	11.6
Commercial BP film	1.9	6.2	0.0	0.0

1 CMC: Carboxymethylcellulose sodium salt; 2 SF: Sanfresh ST-500D
* Standard error; ** Average

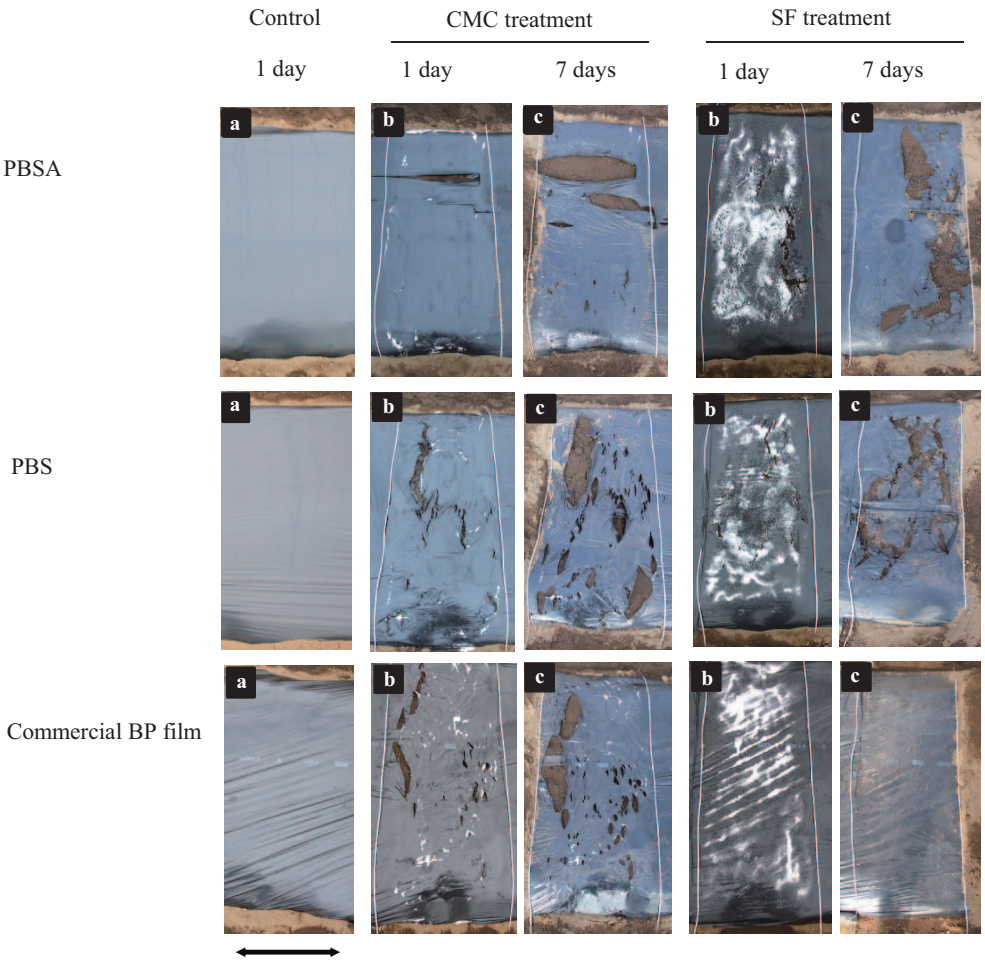

Fig. 3. Images of BP mulch film degradation with (a) no treatment; (b) one day; and (c) seven days after films were wiped with CMC and SF, and then sprayed with enzyme solution. The arrow indicates the film's drawing direction

images of PBS film degradation 10 days after various treatments. Treatment with the enzyme solution degraded PBSA and PBS films, but did not degrade the commercial film (Fig. 4 a). Consistent with the previous test done in September 2010 (when tested films were wiped with CMC prior to applying the enzyme solution), numerous cracks were observed on the surfaces (Fig. 4 b). In addition, the enzyme solution containing dissolved CMC powder (1% w/v) also accelerated film degradation (Fig. 4 c). In contrast, film degradation was not accelerated by the treatment of CMC powder with water (Fig. 4 d). The stability of film treated with CMC powder was the same as that of films without treatment (Fig. 4 e).

Discussion

Spraying the fungal enzyme solution onto the surface of BP mulch films placed in agricultural fields accelerated the degradation of the films. Previous studies have examined the capacities of purified enzymes secreted by various fungi to degrade several BP materials (Murphy et al. 1996; Maeda et al. 2005; Baker et al. 2012;). However, agricultural BP mulch films are blends of several polymers and additives, such as an ultraviolet scattering agent, and no previous studies have investigated the enzymatic degradation of these films in agricultural fields (Brodhagen et al. 2015).

While enzyme treatment accelerated the degradation of PBSA and PBS films in our initial experiment (Figs. 1, 2), a large volume of enzyme solution (4 L/m^2 in total) was treated for film degradation. Most of the enzyme solution applied to the film surface went over the film and was adsorbed into the surrounding soil. However, most of the soil under the film did not directly contact the film. The enzyme degraded the film when adsorbed into the film surface (Shinozaki et al. 2013). Therefore, we speculate that most of the enzymes used in the initial experiment did not contribute to film degradation. In addition, the amount of enzyme solution must be reduced for practical use. Because the solution evaporates after application, we applied moisture-retaining agents to prevent its desiccation. As a result, treatment with a reduced amount of enzyme solution (400 mL/m^2) accelerated PBSA and PBS film

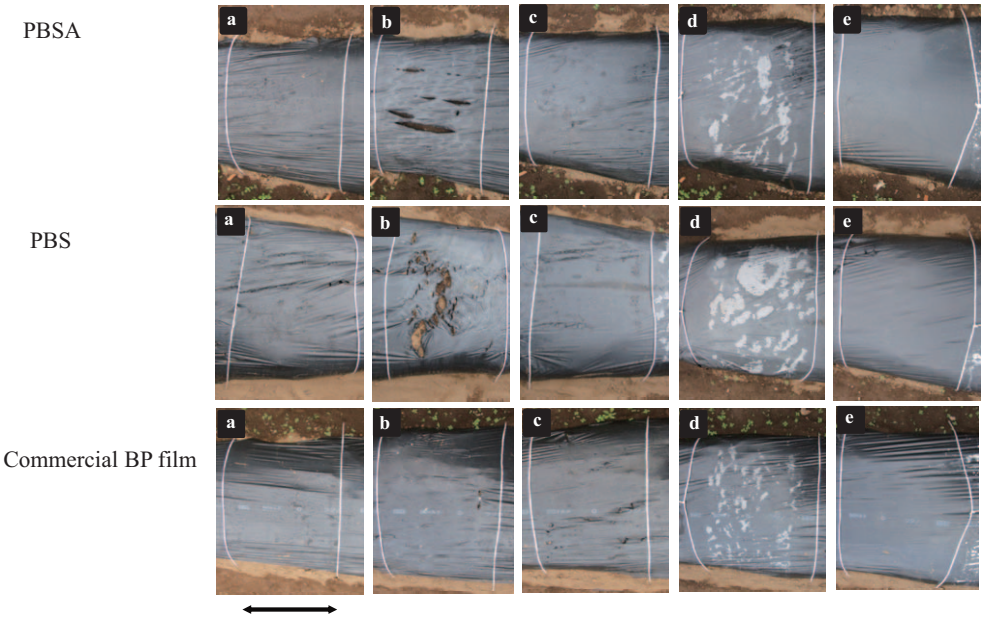

Fig. 4. Images of three types of film degradation with various treatments more than 10 days later. Films were treated with (a) enzyme solution; (b) 14 g/m^2 CMC, and then 400 mL/m^2 enzyme solution; (c) 400 mL/m^2 enzyme solution containing dissolved CMC powder (1% w/v); (d) 14 g/m^2 CMC, and then sterilized distilled water; or (e) no treatment. The arrow indicates the film's drawing direction

degradation, but no obvious degradation was observed on commercial film (Fig. 4 a). All enzyme treatments supplemented with moisture-retaining agents initiated film degradation within one day of application, with the exception of SF on commercial BP mulch film (Fig. 3; Table 1).

Deterioration was evident by the cracking, and most cracks in the films were vertical to their drawing directions (Fig. 3). Molecular chains of polymers in both crystalline and amorphous phases of thin films are well oriented along the drawing direction of the film (Iwata 2005). Thus, our results suggest that the enzyme easily degraded the amorphous structures of polymer chains, thereby creating visible cracks parallel to the films' drawing direction. Furthermore, as scissions in polymer chains decreased the mechanical strength of the films, crack lengths increased with duration following enzyme treatments. Moreover, the molecular structure of PBSA degrades more easily than PBS (Abe et al. 2010, Baker et al. 2012), but application of the moisture-retaining agent and enzyme solution enhanced the degradation of PBS more than other types of film. This may have been partially due to PBS having lower flexibility than PBSA film (Xu and Guo 2010), and tensile stress caused by desiccation and shrinkage of the treated moisture-retaining agents that adhered to the film may have increased the physical deterioration of the PBS film.

No mulch films composed solely of PBSA or PBS are available in the market. Commercial BP mulch films are composed with PBSA, PBS and other compounds to enhance their strength, such as PBAT. Consequently, because CMC treatment best accelerated the degradation of commercial BP film, it is currently the most viable option for agricultural use. Moreover, CMC is safe for use on farmland, as it is widely used as a food thickener and an emulsion stabilizer in products such as ice cream (Robert et al. 2003). Therefore, the application of CMC with the BP-degrading enzyme should be further pursued for large-scale agricultural use.

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