APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1992, p. 731–733
0099-2240/92/020731-03$02.00/0

Vol. 58, No. 2

Production of an Extracellular Polyethylene-Degrading Enzyme(s) by *Streptomyces* Species†

ANTHONY L. POMETTO III,* BYUNGTAE LEE, AND KENNETH E. JOHNSON

Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011-1060

Received 14 August 1991/Accepted 15 November 1991

Extracellular culture concentrates were prepared from *Streptomyces viridosporus* T7A, *Streptomyces badius* 252, and *Streptomyces setonii* 75Vi2 shake flask cultures. Ten-day-heat-treated (70°C) starch-polyethylene degradable plastic films were incubated with shaking with active or inactive enzyme for 3 weeks (37°C). Active enzyme illustrated changes in the films' Fourier transform infrared spectra, mechanical properties, and polyethylene molecular weight distributions.

There is a growing interest in the development of degradable plastics to enhance biodegradability of plastic products in landfills and composts. One type of these novel materials consists of starch-polyethylene-prooxidant degradable plastics, which are used in the composting of lawn, garden, and shrub litter. Recently, Lee et al. (3) demonstrated the ability of lignocellulose-degrading *Streptomyces badius* 252, *Streptomyces setonii* 75Vi2, and *Streptomyces viridosporus* T7A to attack polyethylene in heat-treated degradable plastics in pure shake flask culture studies. In this paper, we describe the presence of an extracellular enzyme(s) produced by these bacteria that is capable of attacking and modifying the polyethylene portion of 10-day-heat-treated degradable plastics.

S. badius 252, *S. setonii* 75Vi2, or *S. viridosporus* T7A was incubated separately in medium containing 0.6% yeast extract (Difco Laboratories, Detroit, Mich.) medium (4) at 37°C with shaking (125 rpm) for 48 to 72 h until the medium pH was ≥8.0. Cell mass was removed by filtration through glass wool, and the filtrate was concentrated threefold by dialysis against high-molecular-weight polyethylene glycol at 4°C (6). The final volume of each bacterial culture concentrate was then adjusted to 300 ml with deionized water and then divided in half. Inactive enzyme (control) was prepared by autoclaving three 50-ml samples in 250-ml Erlenmeyer flasks fitted with cotton plugs. The remaining 150 ml of culture concentrate was filter sterilized (0.45-μm-pore-size polycarbonate filter), 50 ml was dispensed into three sterile flasks, and then 1.3 ml of sterile antibiotic solution (5,000 U of penicillin, 5 mg of streptomycin, and 10 mg of neomycin per ml of a 0.9% NaCl solution) (Sigma Chemical Co., St. Louis, Mo.) was added.

The 6% starch–polyethylene–prooxidant degradable plastic was prepared commercially with POLYCLEAN masterbatch (Archer-Daniels-Midland Inc., Decatur, Ill.). A sheet of degradable plastic was heat treated (70°C) in a forced-air oven for 10 days and then cut in machine direction into strips (4 by 1 in. [10.16 by 2.54 cm]; 0.06 to 0.07 mm thick) (3). Films were then disinfected by using the procedure of Lee et al. (3). Sterility checks were conducted by aseptically adding each film to 100 ml of sterile 0.6% yeast extract medium and then incubating with shaking (125 rpm) at 37°C for 24 h. Properly disinfected films (no turbidity) were then stored at 4°C. Disinfected films were then aseptically added to each bacterial culture concentrate flask containing active or inactive enzyme (replicates of three). The films were then incubated with shaking at 37°C. Once each week, the films were aseptically removed from each flask and added to a fresh bacterial culture concentrate. After a 3-week incubation, the films were washed in 70% ethanol, dried overnight at 45°C, and then evaluated for changes. Data analyses were determined by using an analysis of variance to ascertain differences between corresponding films treated with active and inactive bacterial culture concentrates by SAS program (7). Values for which P was <0.05 were considered significantly different.

The Fourier transform infrared (FT-IR) spectrometer used was a Bruker Instruments (Billerica, Mass.) Model IR 113V controlled by Bruker IFS version 12/87 software. Polyethylene films were affixed directly to standard FT-IR sample plates, which were made from 1-mm-thick aluminum plate. Wrinkles in the mounted films were avoided. The spectrum was performed from 600 to 4,000 wave numbers cm^{-1} for each sample. *S. setonii* and *S. viridosporus* active-enzyme-treated films illustrated an increase in the 900-to-1,200 cm^{-1} region, whereas their corresponding control films demonstrated no change (Fig. 1). The FT-IR spectrum for *S. badius*-treated films showed no changes (Fig. 1). The same FT-IR spectra were exhibited for all three replicate films. The identical spectral pattern observed for the films treated with inactive enzyme was observed for the zero-time 10-day-heat-treated material. This region of increased peak absorbance (900 to 1,200 cm^{-1}) of the FT-IR spectrum correlates with primary and secondary alcohols (8). The FT-IR spectrum for starch also absorbs in this region (Fig. 1). However, when the same heat-treated degradable plastic film was treated with exogenous α-amylase (*Bacillus* sp.) for 1 week in sodium acetate buffer (pH 6.0; 37°C), the FT-IR spectrum was unchanged despite starch hydrolysis. Accordingly, these changes in FT-IR spectra are not associated with extracellular amylase activity. Furthermore, Albertsson (1) demonstrated a similar infrared spectrum with an increase in the 1,100 cm^{-1} region for polyethylene films containing photooxidants exposed to UV light after a 2-year treatment with soil microorganisms.

Changes in tensile strength, percent elongation, and strain energy were determined with an Instron model 4502 Univer-

* Corresponding author.

† Journal paper no. J-14622 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (project no. 0178 and 2889).

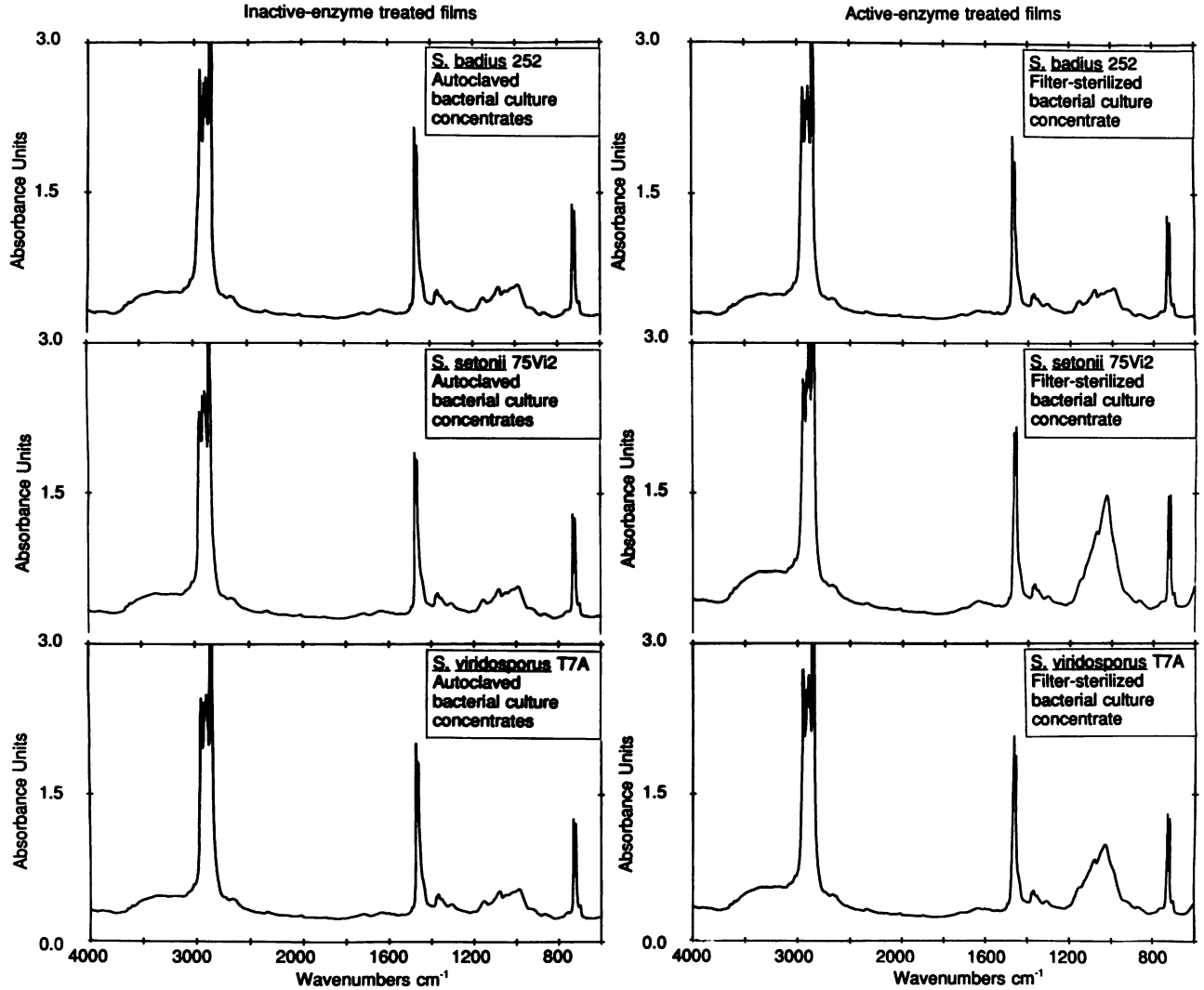

FIG. 1. FT-IR spectra for 10-day-heat-treated degradable plastic films after a 3-week incubation with active and inactive enzymes from each bacterial culture concentrate.

sal Tester (Instron Corporation, Canton, Mass.) at room temperature and 500 mm/min with a 5-cm gap (3). Tensile strength and percent elongation measure the stress at fracture of the specimen and the extension of the material under load, respectively (2). Strain energy represents the area under the stress-strain curve and is related to the toughness of the material (2). In every instance, the tensile strength, percent elongation, and strain energy of the specimens are reduced in the presence of active-enzyme concentrate compared with their corresponding control values (Table 1). Significant reduction ($P < 0.05$) in mechanical properties was demonstrated by films treated with active enzyme from

TABLE 1. Mechanical-properties measurements for 10-day-heat-treated (70°C) degradable plastic film[a]

Culture concentrate	Tensile strength (kg/mm^2)		% Elongation		Strain energy (kg · mm)	
	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme
S. badius 252	1.56	1.26[b]	638	551	519	392[b]
S. setonii 75Vi2	1.51	0.99[b]	611	506[b]	486	341[c]
S. viridosporus T7A	1.60[c]	1.16[b]	658[c]	576	536[c]	413

[a] After a 3-week incubation with active and inactive enzyme from each bacterial culture concentrate. Each value represents an average for three replicate samples. Each sample had a zero-time tensile strength, percent elongation, and strain energy of 1.77 kg/mm^2, 609%, and 465 kg · mm, respectively (averages of three replicates).

[b] Significantly different ($P < 0.05$) compared with the mean for the corresponding control value.

[c] One flask was lost because of contamination, so this value is the average for two replicate samples.

TABLE 2. Molecular-weight-distribution measurements for 10-day-heat-treated (70°C) degradable plastic film[a]

Culture concentrate	$\overline{M}_w$		$\overline{M}_n$		$\overline{M}_w/\overline{M}_n$	
	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme
S. badius 252	219,790	198,060	64,060	53,730[b]	3.45	3.69
S. setonii 75Vi2	226,750	224,400	63,270	62,540	3.60	3.59
S. viridosporus T7A	232,140[c]	208,490	71,360[c]	64,720	3.26[c]	3.21

[a] After a 3-week incubation with active and inactive enzyme from each bacterial culture concentrate. Each value represents an average for three replicate samples. Each sample had a zero-time $\overline{M}_w$ of 246,620, zero-time $\overline{M}_n$ of 64,720, and zero-time $\overline{M}_w/\overline{M}_n$ ratio of 3.72.
[b] Significantly different ($P < 0.05$) compared with the mean for the corresponding control value.
[c] One flask was lost because of contamination, so this value is the average for two replicate samples.

S. setonii 75Vi2, which also produced the greatest absorbance change in FT-IR spectra at the 1,000 cm^{-1} region (Fig. 1).

A Waters model 150-C (Waters/Millipore Co., Milford, Mass.) high-temperature gel permeation high-pressure liquid chromatograph was used to determine changes in polyethylene molecular weight distribution. The procedure of Lee et al. (3) was used for sample preparation and chromatographic evaluation. Changes in the $\overline{M}_w$ correspond directly to high-molecular-weight polymers found in linear-low-density polyethylene, whereas changes in $\overline{M}_n$ correspond to number of molecules per sample (2). $\overline{M}_w/\overline{M}_n$ corresponds to polydispersity or chromatographic peak width. Films treated with *S. badius* and *S. viridosporus* active enzyme demonstrated a slight reduction in $\overline{M}_w$s and $\overline{M}_n$s compared with their corresponding controls, whereas *S. setonii*-treated films demonstrated no change (Table 2). A significant difference ($P < 0.05$) between $\overline{M}_n$s for *S. badius* active-enzyme treated films and for those treated with inactive enzyme was observed.

Veratryl alcohol and 2,4-dichlorophenol lignin peroxidase activity was determined for each bacterial culture concentrate after filter sterilization by using the methods of Ramachandra et al. (5, 6). To measure enzyme activity, increase in A_{470} and A_{510} was monitored for 1 min at 37°C on a Beckman DU-50 UV-VIS spectrophotometer. The bacterial concentrates for all three bacteria demonstrated some veratryl alcohol but no 2,4-dichlorophenol lignin peroxidase. We can then assume that each of the bacterial cultures was ligninolytic (5).

This preliminary study confirms the presence of an extracellular enzyme(s) capable of attacking 10-day-heat-treated polyethylene of degradable plastics with 6% starch plus prooxidant. All three bacterial extracellular enzyme concentrates caused detectable changes in the degradable plastic as determined by the FT-IR spectrometer, Instron Universal Tester, and/or high-temperature gel permeation high-pressure liquid chromatography. No special enzyme inducer in the medium was required to produce this extracellular enzyme(s). To our knowledge, this is the first report describing extracellular-enzyme activity on polyethylene.

This research was supported by the Iowa State University Center for Crops Utilization Research, the U.S. Department of Agriculture, the Iowa State Legislature General Assembly, the State of Iowa Department of Natural Resources, and the Iowa Agriculture and Home Economics Experiment Station.

We thank Wei Sung for the α-amylase study and Al Fratzke for performing the FT-IR analyses.

REFERENCES

1. **Albertsson, A. C., S. O. Andersson, and S. Karlsson.** 1987. The mechanism of biodegradation of polyethylene. Poly. Degrad. Stability **18**:73–87.
2. **Chanda, M., and S. K. Roy.** 1986. Plastic technology handbook. Marcel Dekker, Inc., New York.
3. **Lee, B., A. L. Pometto III, A. Fratzke, and T. B. Bailey, Jr.** 1991. Biodegradation of degradable plastic polyethylene by *Phanerochaete* and *Streptomyces* species. Appl. Environ. Microbiol. **57**:678–685.
4. **Pometto, III, A. L., and D. L. Crawford.** 1986. The effects of pH on lignin and cellulose degradation by *Streptomyces viridosporus*. Appl. Environ. Microbiol. **52**:246–250.
5. **Ramachandra, M., D. L. Crawford, and G. Hertel.** 1988. Characterization of an extracellular lignin peroxidase of the lignocellulolytic actinomycete *Streptomyces viridosporus*. Appl. Environ. Microbiol. **54**:3057–3063.
6. **Ramachandra, M., D. L. Crawford, and A. L. Pometto III.** 1987. Extracellular enzyme activities during lignocellulose degradation by *Streptomyces* spp.: a comparative study of wild-type and genetically manipulated strains. Appl. Environ. Microbiol. **53**: 2754–2760.
7. **SAS Institute Inc.** 1985. SAS user's guide: statistics, version 5 ed. SAS Institute Inc., Cary, N.C.
8. **Weast, R. C., and M. J. Astle (ed.).** 1982. CRC handbook of chemistry and physics, 62nd ed. CRC Press, Boca Raton, Fla.