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Screening of Microorganisms for Biodegradation of Poly(Lactic Acid) and Lactic Acid-Containing Polymers

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The ability of some microorganisms to use lactic acid stereocopolymers and copolymers with glycolic acid as sole carbon and energy sources was studied under controlled or natural conditions. First, 14 filamentous fungal strains were tested in liquid cultures, adopting total lactic acid consumption, nitrogen source exhaustion, and maximal biomass production as selection criteria. Two strains of *Fusarium moniliforme* and one strain of *Penicillium roqueforti* were able to totally assimilate DL-lactic acid, partially soluble racemic oligomers (M_w = 1,000), and the nitrogen source. Only one strain of *F. moniliforme* was able to grow on a poly(lactic acid)-glycolic acid copolymer (M_w = 150,000) after 2 months of incubation at 28°C on synthetic agar medium. Mycelium development was examined by scanning electron microscopy. *F. moniliforme* filaments were observed to grow not only at the copolymer surface but also through the bulk of the copolymer. In a second approach, plates made of a racemic poly(lactic acid) were buried in the soil before being incubated in petri dishes containing mineral agar medium under controlled conditions. Five strains of different filamentous fungi were isolated, and their ability to assimilate racemic poly(lactic acid) oligomers was tested in liquid cultures.

Polymer materials developed during the last 50 years are inert and resistant to microbial attack. Nowadays, the concepts of degradable and biodegradable polymers are of considerable interest with respect to solid waste accumulation. For many years, poly(hydroxy butyrate) and other bacterial polymers have been considered the best candidates to replace biostable polymers like polyethylene because of their bacterial biodegradability. Poly(lactic acid) (PLA), stereocopolymers (PLA_x), and copolymers with glycolic acid (PLA_xGA_y) are more and more regarded as competitors now that production costs tend to decrease rapidly, thanks newly engineered on-line production in the United States. In PLA_xGA_y acronyms, x stands for the percentage in L-lactyl units and y stands for the percentage in glycolyl units, the content in D-lactyl units being given by $100 - (x + y)$ (15).

The degradation of PLA_xGA_y polymers has been widely investigated in vitro and in vivo as well (11), primarily because of interest in their applications as bioresorbable polymers in surgery and in pharmacology, which are now at the level of everyday clinical exploitation. It has been established that PLA_xGA_y polymers do not biodegrade in mammalian bodies. They first degrade by abiotic hydrolysis to yield oligomers that can be released into the external aqueous medium when they become small enough to be soluble (7, 8, 16, 17). At this point, soluble oligomers can be metabolized or mineralized by cells and enzymes. PLA_xGA_y polymers are thus called bioresorbable in mammalian bodies and bioassimilable if outdoor microorganisms are involved (17). Whether PLA_xGA_y polymers can really be biodegraded by microorganisms in the wild environment has not been shown yet. Whether the by-products generated by abiotic hydrolysis can be bioassimilated by fungi and bacteria also has yet to be demonstrated.

This paper reports the results of a screening of microorganisms supposed to be able to use PLA_xGA_y polymers as sole carbon and energy sources. In a first approach, model compounds were selected. First, DL-lactic acid (DL-LA) was selected because LA is one of the ultimate intermediate products of PLAGA chemical degradation, the other one being glycolic acid, which is known to behave similarly from a microbial viewpoint. Second, DL-LA oligomers were selected as a model of chemical degradation by-products. The screening was carried out with a limited number of fungal strains under selected culture conditions. The 14 fungal strains tested belonged to the genera *Aspergillus*, *Rhizopus*, *Penicillium*, and *Trichoderma*, which are usually found in natural soils. On the other hand, *Aspergillus niger* is one of the microorganisms used in the American Society for Testing and Materials G21 standard method recommended to evaluate the resistance of polymers to microbial attack (1). Selection criteria were total LA consumption as monitored by high-performance liquid chromatography (HPLC), final nitrogen source consumption according to colorimetric assays, and final biomass production as deduced from weighing. The same strains were tested in solid culture by using a model member of the PLA_xGA_y family, namely, high-molecular-weight $PLA_{37.5}GA_{25}$ copolymer, as the sole carbon and energy sources. $PLA_{37.5}GA_{25}$ was selected because it is known from in vitro investigations of abiotic degradation that this compound is one of the most rapidly chemically degraded member of the PLAGA family (9). In a second approach, PLA_{50} plates were buried within a local soil. After recovery, they were incubated to show whether some wild fungal strains capable of growing on PLA_{50} oligomers could be isolated.

MATERIALS AND METHODS

LA. DL-LA (85% aqueous solution) was obtained from Sigma and used as received.

Racemic PLA oligomers. Typically, 1 liter of DL-LA commercial solution was heated under normal pressure to gently distillate water. When the temperature reached 130°C, vacuum was applied and distillation was controlled as desired. The resulting oligomers, whose characteristics depended on reaction temperature and time, were dissolved in chloroform and then reprecipitated in basal

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TABLE 1. Filamentous fungal strains selected for the screening test based on utilization of PLA or its derivatives as sole sources of carbon and energy

Species	Code(s)[a]
Aspergillus awamori	Aa 20, NRRL 3112
Aspergillus foetidus	NRRL 341
Aspergillus nidulans	J1
Aspergillus niger	CH4, An 10
Aspergillus oryzae	CCM F172
Fusarium moniliforme	Fmm, Fm1
Penicillium roqueforti	RE 10
Penicillium sp.	C16
Rhizopus oligosporus	196
Trichoderma harzianum	TH 31
Trichoderma sp.	TH 15

[a] Collection of the Institut Français de Recherche Scientifique pour le Développement en Cooperation.

culture medium to eliminate any soluble fraction. Racemic oligomers appeared as a very sticky transparent material. One fraction of racemic oligomers (M_w = 1,000, $I = M_w/M_n = 1.3$) was used for the screening of filamentous fungi, and another one (M_w = 2,000, $I = 1.3$) was used for soil screening. Molecular weights were determined by size exclusion chromatography.

PLAGA copolymer. High-molecular-weight PLAGA copolymer (M_w = 150,000, $I = 1.4$) was prepared by bulk polymerization of a 75% DL-lactide and 25% glycolide feed (Purac), initiated with SbF_3 at 145°C for 96 h. Residual initiator and low-molecular-weight oligomers were separated by the dissolution-precipitation method using acetone-methanol as a solvent-precipitant.

Racemic PLA polymer. Racemic PLA was obtained by bulk polymerization of DL-lactide under vacuum at 140°C for 10 days. Zinc powder was used as the initiator. Removal of low-molecular-weight residual compounds was achieved by the dissolution-precipitation method. The resulting polymer (M_w = 40,000, I = 1.6) was compression molded as circular plates (75-mm diameter by 2-mm thickness). Parallelepiped specimens (10 by 10 by 2 mm) were then machined from these plates.

Culture medium. Basal culture medium was composed of $(NH_4)_2SO_4$ (0.6 g/liter), KH_2PO_4 (1.3 g/liter), $Na_2HPO_4 \cdot 2H_2O$ (0.12 g/liter), $MgSO_4 \cdot 7H_2O$ (0.3 g/liter), and KCl (0.3 g/liter). As recommended by Roussos (13), mineral salts and vitamins were added after sterilization. The initial pH was 5.6 ± 0.1.

Microorganisms. All strains were obtained from the collection of the ORSTOM Laboratory of Biotechnology (Table 1).

Size exclusion chromatography. Molecular weights were determined by using a Waters model 600 A gel permeation HPLC (stationary phase, PL Gel Mixed; mobile phase, dioxane; flow rate, 1 ml/min; injection volume, 20 μl; refractometry detection). Data were expressed considering polystyrene standards without universal calibration.

Scanning electron microscopy. Typically, pieces of partially degraded material were fixed in 1.5% glutaraldehyde; dehydrated for 10 min each in 10, 20, 40, 60, 80, 95, and 100% ethanol; and mounted on aluminum stubs. Samples were coated with gold and examined with a J.S.M. 35 JEOL scanning electron microscope.

Preparation of culture media. DL-LA (10 g/liter) was added before sterilization of the culture medium, and the pH was adjusted to 6.6 ± 0.1 with a 10 N NaOH solution. For each experiment, 50 ml of culture medium was introduced into a 250-ml Erlenmeyer flask.

DL-Oligomers (10 g/liter) were added to the culture medium after sterilization to avoid chemical hydrolysis. No pH adjustment was realized before inoculation. For each experiment 50 ml of culture medium was placed into a 250-ml Erlenmeyer flask.

For the PLAGA copolymer in solid culture conditions, vacuum-popped copolymer pieces (0.6 to 1 mm) were placed into petri dishes containing a sterilized medium composed of the basal medium to which 15 g of agar per liter was added.

Inoculation. Inoculation with the various fungus strains was accomplished with a spore suspension (0.01% Tween solution) obtained from a potato dextrose agar culture (Difco Laboratories, Detroit, Mich.) after 7 days of incubation at 30°C. In all cases, inoculation was made with $2 \cdot 10^7$ spores per g of LA-type substrate.

Culture conditions. All culture media were incubated at 30°C. Liquid media were shaken under gentle rotation at 150 rpm for 7 days. In all cases, controls without inoculation were used.

pH measurement. The pH of the liquid media was measured at 25°C with a Knick digital pH meter equipped with an Ingold electrode.

Consumption of LA-type compounds. Final LA concentration in liquid media was measured by a Waters chromatograph equipped with a photodiode array UV detector model 996 (stationary phase, Shodex Ionpak KC-811; mobile phase, 0.02% H_3PO_4 solution; flow rate, 0.5 ml/min; injection volume, 20 μl; detection wavelength, 210 nm).

TABLE 2. Amounts of LA and dry biomass after 7 days in culture media inoculated with filamentous fungi in the presence of DL-LA or racemic LA oligomers as the sole carbon and energy sources

Strain	Final amt (mg/liter [mean ± SD]) of:			
	LA with:		Biomass with:	
	DL-LA	Oligomers	DL-LA	Oligomers
A. awamori Aa 20	7.8 ± 0.4	7.8 ± 0.2	0.1 ± 0.04	0.4 ± 0.02
A. awamori NRRL 3112	8.6 ± 0.6	7.6 ± 0.2	0.1 ± 0.07	0.3 ± 0.01
A. foetidus	3.3 ± 0.4	5.6 ± 0.2	0.7 ± 0.02	1.4 ± 0.03
A. nidulans	4.8 ± 0.3	5.6 ± 0.2	0.9 ± 0.03	0.9 ± 0.08
A. niger CH4	7.8 ± 0.2	7.7 ± 0.2	0.1 ± 0.01	0.5 ± 0.03
A. niger An 10	3.1 ± 0.8	5.9 ± 0.2	0.7 ± 0.02	2.0 ± 0.40
A. oryzae	3.3 ± 0.5	7.4 ± 0.5	0.2 ± 0.01	1.3 ± 0.30
F. moniliforme Fmm	0.0	0.0	2.8 ± 0.03	3.1 ± 0.02
F. moniliforme Fml	0.0	0.0	2.6 ± 0.02	2.9 ± 0.09
P. roqueforti	0.0	0.0	0.9 ± 0.05	2.8 ± 0.20
Penicillium sp.	6.1 ± 0.8	7.7 ± 0.3	0.1 ± 0.02	0.7 ± 0.06
R. oligosporus	7.5 ± 0.3	7.5 ± 0.4	0.1 ± 0.03	0.4 ± 0.08
T. harzianum	2.2 ± 0.6	7.8 ± 0.5	0.1 ± 0.01	1.8 ± 0.10
Trichoderma sp.	3.6 ± 0.9	5.5 ± 0.2	1.0 ± 0.03	0.3 ± 0.01
Control	9.2 ± 0.6	8.2 ± 0.5	0.0	0.0

Biomass estimation. Liquid cultures were filtered in a 0.45-μm-pore-size Millipore filter, and biomass production was estimated as the weight of dry matter. It is worth noting that the initial dry biomass composed of conidia was negligible.

Ammonium concentration. The colorimetric technique used to determine the ammonium concentration was based on the Berthelot reaction with indophenol blue, using $(NH_4)_2SO_4$ as the standard solution (2).

Screening test by soil burial. Racemic PLA plates were buried in soil (northern area of Montpellier, France). After 8 weeks, plates were recovered and placed into petri dishes containing the basal culture media added with 15 g of agar per liter. Incubation was at 30°C for 8 weeks in a hydrated environment. After a total of 16 weeks, plates were examined by scanning electron microscopy. Microorganisms developed at the surface of plates were isolated and cultivated into a potato dextrose agar culture for 7 days. Spores were recovered with a 0.01% Tween solution. All isolated strains were used to test the assimilation of racemic PLA oligomers, by measuring the final LA concentration in medium (by HPLC) after 5 days of incubation at 30°C and 150 rpm.

RESULTS AND DISCUSSION

Screening test using collection filamentous fungal strains. (i) DL-LA and racemic PLA oligomers. Tables 2 and 3 show the data collected after 7 days of incubation. In the case of the

TABLE 3. Amounts of NH_4^+ ions and pHs after 7 days in culture media inoculated with filamentous fungi in the presence of DL-LA or racemic LA oligomers as the sole carbon and energy sources

Strain	Final amt (mg/liter [mean ± SD] of NH_4 with:		Final pH with:	
	DL-LA	Oligomers	DL-LA	Oligomers
A. awamori Aa 20	135.5 ± 3.9	140.0 ± 5.0	4.7	3.6
A. awamori NRRL 3112	142.5 ± 2.3	165.8 ± 5.6	4.5	3.6
A. foetidus	81.6 ± 6.2	18.2 ± 2.3	7.2	4.7
A. nidulans	0.0	84.1 ± 6.9	7.7	3.9
A. niger CH4	141.0 ± 3.0	23.9 ± 5.1	4.5	3.7
A. niger An 10	67.4 ± 3.5	0.0	7.2	5.8
A. oryzae	124.6 ± 11.9	0.0	4.8	4.8
F. moniliforme Fmm	0.0	0.0	9.1	8.6
F. moniliforme Fml	0.0	0.0	9.1	8.2
P. roqueforti	52.3 ± 4.1	0.0	8.7	6.2
Penicillium sp.	133.6 ± 5.7	53.7 ± 2.3	4.7	3.7
R. oligosporus	131.1 ± 10.9	117.4 ± 10.0	4.6	3.7
T. harzianum	100.2 ± 3.0	0.0	4.6	6.2
Trichoderma sp.	0.0	65.4 ± 6.7	7.3	5.5
Control	141.6 ± 10.9	157.1 ± 12.9	3.6	4.5

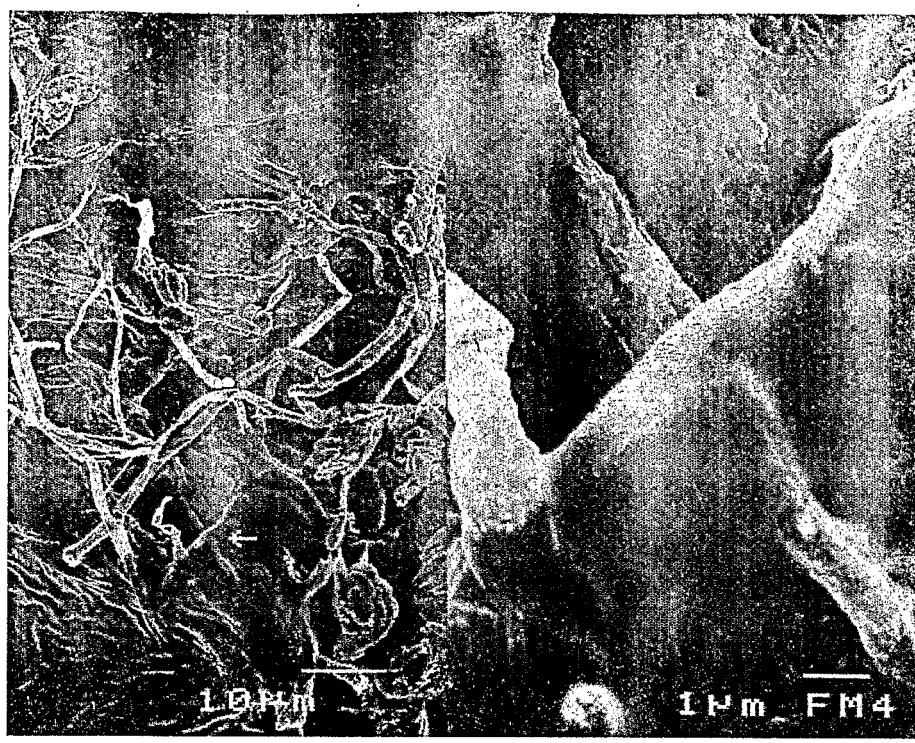

FIG. 1. Micrographs indicating the penetration of an *F. moniliforme* filament into the mass of a PLAGA copolymer allowed to age for 2 months at 30°C in a hydrated environment. (Right) Enlargement of the area indicated by the arrow in the left panel.

medium supplied in DL-LA, final LA concentration, biomass dry weight, NH_4 concentration, and pH varied depending on the fungal strains. Strain dependence was also observed when the oligomers were the sole carbon and energy sources. It can be seen from controls that, after 7 days of incubation, a high fraction of oligomers was transformed in soluble LA. However, only three strains were able to totally use DL-LA and DL-LA oligomers as sole carbon and energy sources (two strains of *Fusarium moniliforme* and one strain of *Penicillium roqueforti*). Some other strains were able to assimilate these compounds only partially within the selected aging period. Moreover, some fungi assimilated the LA itself more easily than the residual oligomers.

Table 3 shows that the strains which were able to totally assimilate LA and oligo-LA compounds also assimilated all the nitrogen source [$(NH_4)_2SO_4$]. For the other fungi, a partial nitrogen assimilation was observed, corresponding to the same degree of LA-type compound consumption. These trends agreed well with biomass productions and final pH values (Tables 2 and 3). In the case of *F. moniliforme* and *P. roqueforti* strains, the final biomass production was the highest (3 to 3.5 g of dry weight mass per liter), and for the other fungi, values were very poor (from 0 to 2.5 g/liter). Furthermore, final pH values were high with microorganisms able to assimilate LA-type compounds to some extent. For the strains mentioned before, pH values were above 8.5 in the case of LA consumption and were lower in oligomer-containing media. For controls, the final pH was lower in LA-based medium, confirming that not all the oligomers were transformed in LA. From these results, *F. moniliforme* strains were considered to have the more important metabolic activity.

(ii) PLAGA copolymer. During the first weeks postinoculation, spores remained almost unchanged in all cases. This suggests that none of the tested fungal strains was able to attack the copolymer from the very beginning under the selected conditions. However, after 2 months, one strain of *F. moniliforme* (Fmm) started growing on the specimens. This observation showed that this strain was able to take advantage of the degradation products resulting from the initial abiotic degradation phase. Scanning electron microscopy revealed the development of a mycelium at the surface of the specimens which appeared swollen and deformed. *F. moniliforme* filaments not only invaded the copolymer surface but also penetrated into its bulk, as shown in Fig. 1. In contrast, controls remained apparently homogeneous.

The ability of *F. moniliforme* to grow on the PLAGA copolymer was tentatively related to the way in which this microorganism attacks cutin from plants. Indeed, some *Fusarium* species are known to infect plants by degrading their structural polymer, the cutin. This compound consists of a mixture of polyesters in which monomeric units are hydroxy acids of 16 to 18 carbon atoms (6). It has been demonstrated that some *Fusarium* species are able to use cutin as their sole carbon and energy source, and this capacity has been related to the production of carboxyl-esterases, namely, cutinases (14). On the other hand, it has been reported that *F. moniliforme* is able to degrade poly(ε-caprolactone) and poly(ester amide) films by the production of extracellular cutinases (3–5, 10, 12). Anyhow, we know that abiotic degradation did occur from the beginning and that it is able to lead to the release of water-soluble LA oligomers (9). The results of the screening with racemic PLA oligomers and the late attack of partially degraded $PLA_{37.5}GA_{25}$ agree well with the characteristics of the mechanism of abiotic hydrolytic degradation and allowed us to conclude that high-molecular-mass $PLA_{37.5}GA_{25}$ is a bioassimilable polymer. Whether *F. moniliforme* did attack the PLAGA surface from the very beginning is still questionable.

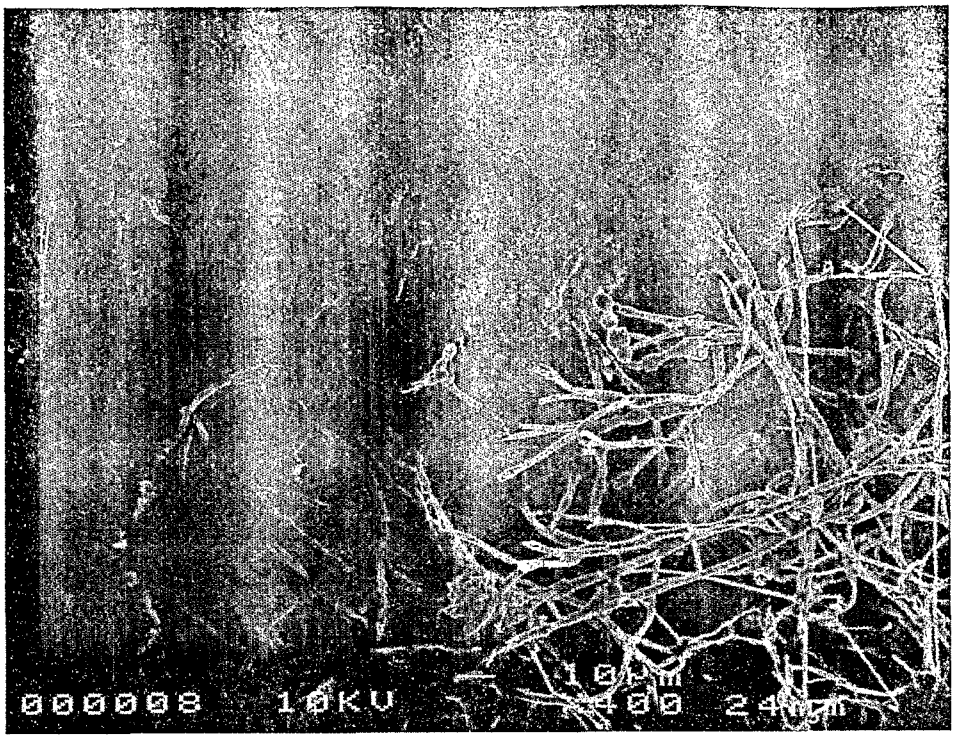

FIG. 2. Micrograph showing the growth of filamentous fungi at the surface of a racemic PLA plate buried for 8 weeks in a local natural soil and then allowed to age for 8 more weeks at 30°C in a hydrated environment.

Screening test by natural soil burial. To consider the possibility of degrading PLA-type polymers by wild microbial strains, it was decided to extend screening to microorganisms present in a local natural soil. Racemic PLA (PLA_{50}) processed as plates was selected as the experimental material. First, plates were buried for 8 weeks. After recovery, the plates looked intact with no fungal nor bacterial growth at the surface. Therefore, the plates were allowed to incubate under controlled culture conditions for 8 more weeks. The partially degraded samples were then examined by scanning electron microscopy. Figure 2 shows a view of the filamentous fungi which were growing on the surface of the plates. It can be observed that some filaments were very well attached to this surface and that, in some places, filaments penetrated the polymer mass, others being even completely embedded. The growth of these microorganisms was significant. Therefore, the recovery of mycelium was easy. Five wild strains of filamentous fungi were isolated selectively on different culture media. These strains were all different, as shown by microscopic morphology analyses. However, they were not precisely identified. Recovery of spores from the potato dextrose agar cultures used to isolate the five strains was realized in order to verify the ability of the spores to grow on PLA_{50} oligomers. For this study, a fraction of oligomers with a relative molecular mass in the range of 2,000 Da instead of 1,000 Da as in the screening of collection strains was used. Only the LA concentration was evaluated after a 5-day incubation. Compared with data in Table 1, isolated strains appeared less active than *F. moniliforme* and *P. roqueforti* strains, since after 5 days there was still 2.5 to 3.5 g of residual LA per liter present in the culture medium. This lower activity might be related to the inherent activity of these strains or to the shorter culture time. However, it is likely that the higher molecular mass of the selected oligomers contribute also. Indeed, a mixed culture of the wild strains in the presence of 1,000-Da oligomers appeared more efficient than a mixed culture of *F. moniliforme* and *Pseudomonas putida*. On the other hand, only 40% of the 2,000-Da oligomers initially present appeared as LA in the culture medium, as seen from the controls. Anyway, one can conclude that microorganisms which are able to assimilate the low-molecular-mass by-products issued from the abiotic degradation of a high-molecular-mass PLA can be found in nature. As in the case of the PLAGA polymer, it is still not clear whether these microorganisms can attack virgin PLA plates; however, one can conclude that if biodegradation can start from the very beginning, it has to be a slow process with a comparably lower rate than abiotic hydrolysis.

Conclusion. From two different screening tests, we have shown that there are some microorganisms able to utilize LA acid and low-molecular-weight polymeric derivatives as sole carbon and energy sources. From our first approach, an *F. moniliforme* strain appeared the most active among the 14 filamentous fungi tested. The methodology used for this screening appeared to be very efficient to demonstrate PLA-type polymer bioassimilation and to select a suitable microorganism. From our second approach, five different strains were isolated from a local wild soil. They all appeared capable of assimilating water-soluble racemic PLA oligomers. Therefore, the bioassimilation of PLAGA, but not its biodegradability, can be considered demonstrated. Indeed, in both screenings, microorganisms were able to grow at the surface of high-molecular-mass PLAGA-type polymers, provided that these polymers were already partially degraded by abiotic chemical hydrolysis. Further studies with the selected microorganisms will help us to better understand their actual potential to biodegrade poly(α-alkanoates).

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REFERENCES

1. **American Society for Testing and Materials.** 1980. ASTM method G21-70. Determining resistance of synthetic polymeric materials to fungi. American Society for Testing and Materials, Villanova, Pa.
2. **Boissezon, C.** 1992. Personal communication.
3. **Chen, X., K. E. Gonsalves, and J. A. Cameron.** 1993. Further studies on biodegradation of aliphatic poly(ester-amides). J. Appl. Polymer Sci. **50:** 1999–2006.
4. **Gonsalves, K. E., X. Chen, and J. A. Cameron.** 1992. Degradation of non-alternating poly(ester-amides). Macromolecules **25:**3309–3312.
5. **Jarret, P., C. V. Benedict, J. P. Bell, J. A. Cameron, and S. J. Huang.** 1983. Mechanism of the biodegradation of polycaprolactone, p. 181–192. *In* S. W. Shalaby, A. S. Hoffman, B. D. Ratner, and T. A. Horbett (ed.), Polymers as biomaterials. Plenum Publishing Corp., New York.
6. **Kolattukudy, P. E.** 1984. Cutinases from fungi and pollen, p. 473–503. *In* B. Borgstrom and L. Brockman (ed.), Lipases. Elsevier Science Publishers, B.V., Amsterdam.
7. **Li, S. M., H. Garreau, and M. Vert.** 1990. Structure-property relationships in the case of the degradation of massive aliphatique poly(α-hydroxy acids) in aqueous media. Part 1. Poly (DL-lactic acid). J. Mater. Sci. Mater. Med. **1:**123–130.
8. **Li, S. M., H. Garreau, and M. Vert.** 1990. Structure-property relationships in the case of the degradation of massive aliphatique poly(α-hydroxy acids) in aqueous media. Part 2. Degradation of lactide-glycolide copolymers: PLA37.5GA25 and PLA75GA25. J. Mater. Sci. Mater. Med. **1:**131–139.
9. **Li, S. M., and M. Vert.** 1995. Biodegradation of aliphatic polyesters, p. 44–87. *In* G. Scott and D. Gilead (ed.), Degradable polymers: principles and applications. Chapman & Hall, Ltd., London.
10. **Martinez, C., P. De Gens, M. Lauwereys, G. Matthyssens, and C. Cambillau.** 1992. *Fusarium solani* cutinase is a lipolytic enzyme with a catalytic serine accessible to solvent. Nature (London) **356:**615–618.
11. **Masusue, Y., T. Yamamuro, M. Oka, Y. Shikinami, S. H. Hyon, and Y. Ikada.** 1992. In vitro and in vivo studies on bioabsorbable ultra high strength poly(L-lactide) rods. J. Biomed. Mater. Res. **26:**1553–1567.
12. **Nishida, H., and Y. Tokiwa.** 1993. Distribution of poly(β-hydroxybutyrate) and poly(ε-caprolactone) aerobic degrading microorganisms in different environments. J. Environ. Polym. Degrad. **1:**227–233.
13. **Roussos, S.** 1982. Mise au point d'une méthode pour l'étude des caractères morphologiques, biochimiques et nutritionnels des champignons imparfaits. Cah. O. R. S. T. O. M. Ser. Biol. **45:**25–34.
14. **Sebastiao, M. J., J. M. S. Cabral, and M. R. Aires-Barros.** 1993. *Fusarium solani pisi* recombinant cutinase partitioning in PEG/potassium phosphate aqueous two-phase systems. Biotechnol. Tech. **7:**631–634.
15. **Vert, M., F. Chabot, J. Leray, and P. Christel.** 1981. Bioresorbable polyesters for bone surgery. Makromol. Chem. **5**(Suppl.)**:**30–41.
16. **Vert, M., S. M. Li, and H. Garreau.** 1991. More about the degradation of LA-GA derived matrices in aqueous media. J. Controlled Release **16:**15–26.
17. **Vert, M., A. Torres, S. M. Li, S. Roussos, and H. Garreau.** 1994. The complexity of the biodegradation of poly(α-hydroxyacid)-type aliphatic polyesters, p. 11–23. *In* Y. Doi and K. Fukuda (ed.), Biodegradable plastics and polymers. Elsevier, Amsterdam.

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