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MINI-REVIEW

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Biodegradation of microbial and synthetic polyesters by fungi

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Abstract A variety of biodegradable polyesters have been developed in order to obtain useful biomaterials and to reduce the impact of environmental pollution caused by the large-scale accumulation of non-degradable waste plastics. Polyhydroxyalkanoates, poly(ε-caprolactone), poly(L-lactide), and both aliphatic and aromatic polyalkylene dicarboxylic acids are examples of biodegradable polyesters. In general, most aliphatic polyesters are readily mineralized by a number of aerobic and anaerobic microorganisms that are widely distributed in nature. However, aromatic polyesters are more resistant to microbial attack than aliphatic polyesters. The fungal biomass in soils generally exceeds the bacterial biomass and thus it is likely that fungi may play a considerable role in degrading polyesters, just as they predominantly perform the decomposition of organic matter in the soil ecosystem. However, in contrast to bacterial polyester degradation, which has been extensively investigated, the microbiological and environmental aspects of fungal degradation of polyesters are unclear. This review reports recent advances in our knowledge of the fungal degradation of microbial and synthetic polyesters and discusses the ecological importance and contribution of fungi in the biological recycling of waste polymeric materials in the biosphere.

Introduction

Nowadays, a wide variety of petroleum-based synthetic polymers are produced worldwide to the extent of approximately 140 million tonnes/year and remarkable amounts of these polymers are introduced in the ecosystem as industrial waste products (Shimao 2001). However, the majority of synthetic polymers are extremely resistant to microbial attack, due to their excessive molecular mass, high number of aromatic rings, unusual bonds, or halogen substitutions (Alexander 1981). For this reason, the large-scale accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution.

Over the past three decades, a great deal of intensive effort has been spent upon the preparation of environmentally friendly polymers, which can be easily degraded by microorganisms. As a result, many types of aliphatic polyesters, including polyhydroxyalkanoates (PHAs), poly(ε-caprolactone) (PCL), and poly(L-lactide) (PLA), have been developed as biologically recyclable green polymers (Scott 2000; Müller et al. 2001; Shimao 2001). Generally, the extent of polymer biodegradation in an ecosystem is affected by material processing, the inherent characteristics of the substrate to be degraded, and various microbiological and environmental factors (Brandl et al. 1995). These factors are all interdependent.

Up to this time, many reports on the bacterial degradation of microbial and synthetic polyesters in the environment have been published. However, reports on the fungal degradation of these polyesters and the related hydrolytic enzymes are relatively rare and not well documented. Considering that fungi play a significant role in degrading natural organic substances in the ecosystem, such as cellulose, hemicellulose, and lignin, the fungal contribution to the biodegradation of polyesters in the environment should be recognized along with the bacterial degradation of polyesters. In this paper, recent progress in our knowledge of the fungal degradation of biodegradable polyesters is reviewed.

Biodegradation of microbial polyesters

Polyhydroxyalkanoates

PHAs are versatile polyesters produced by numerous bacterial species as intracellular storage compounds of carbon and energy. PHAs are currently divided into two

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groups, short-chain-length (SCL)-PHAs and medium-chain-length (MCL)-PHAs, according to the carbon-chain-length of the constituents. SCL-PHAs consist of (*R*)-hydroxyalkanoates of C_3–C_5, while MCL-PHAs are comprised of aliphatic and/or aromatic (*R*)-hydroxyalkanoates of C_6–C_{14}. SCL-PHAs are thermoplastics with a high degree of crystallinity, while MCL-PHAs are elastomeric or tacky materials with properties that vary according to their composition (Madison and Huisman 1999). Poly(β-L-malic acid) (PMA) is a soluble anionic biopolyester produced only by eukaryotic microorganisms (Shimada et al. 1969; Holler et al. 1992; Liu and Steinbüchel 1996), which is now classified as a PHA due to its chemical structure (Steinbüchel and Valentin 1995). Recently, Steinbüchel (2001) reported that approximately 150 hydroxyalkanoates, detected as constituents of PHAs, are produced by microorganisms grown with carbon sources containing different types of chemical structures. Besides PHAs and PMA, the other types of polyester found in living organisms are cutin and suberin, which are water-insoluble polymeric materials occurring in higher plants (Kolattukudy 2001).

Studies on the bacterial degradation of PHAs have been intensively conducted and are well documented by several researchers (Brandle et al. 1995; Jendrossek et al. 1996). However, the metabolic capability to hydrolyze PHA is not restricted to bacteria; and many filamentous fungi and yeasts capable of degrading poly(3-hydroxybutyrate) (PHB) and its copolyesters with 3-hydroxyvalerate [poly(3HB-*co*-3HV)] have been isolated from various environments. Neumeier (1994) described a list of the taxa of PHA-degrading fungi isolated from soil and marine environments. Of the 95 genera listed, the overwhelming majority (97%) were members of the division Amastigomycota. Of these, the Basidiomycotina, Deuteromycotina, and Ascomycotina accounted for 48%, 27%, and 19% of the total, respectively; and the Zygomycotina accounted for only 2%, which indicates that higher fungi are the predominant degraders of PHAs in the environment. Until now, however, the occurrence of MCL-PHA-degrading fungi in the environment has been uncertain. Recently, an attempt to examine the distribution and diversity of MCL-PHA-degrading microorganisms in various soil samples was made by Nam et al. (2002). Approximately 60 different species belonging to 13 genera were isolated as MCL-PHA degraders, but the authors failed to isolate any MCL-PHA-degrading fungus.

Most studies on the environmental degradation of PHAs have shown that fungi belonging to the group, Deuteromycota, including mainly *Aspergillus* spp and *Penicillium* spp, contribute considerably to PHA breakdown in the biological system (Table 1). Apparently, a number of mesophilic fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Paecilomyces* are primarily responsible for degrading PHAs in soil and aquatic environments (Matavulj and Molitoris 1992; Mergaert et al. 1993, 1995; Kim et al. 2000c; Sang et al. 2002). In addition to mesophiles, many thermotolerant *A. fumigatus* strains that are capable of degrading PHB and poly(3HB-*co*-3HV) at high temperatures (above 40 °C) have been isolated from soil samples (Mergaert et al. 1993; Kim et al. 2000c) and compost (Mergaert et al. 1994). Recently, the fungal degradation of BIOPOL under a simulated deep-sea environment was investigated using the two deep-sea isolates of a filamentous fungus, *A. ustus*, and a yeast, *Rhodosporidium spaerocarpum* (Gonda et al. 2000). In that case, the marine isolate, *A. ustus*, efficiently disintegrated BIOPOL under a hydrostatic pressure of 20 MPa, while polymer degradation under a hydrostatic pressure of 30 MPa was significantly low. No fungal hydrolysis of the polymer occurred at hydrostatic pressures above 30 MPa, which indicates that increasing the hydrostatic pressure retards both polymer degradation and the metabolic activity of the *A. ustus* strain.

Biochemical properties of fungal PHB depolymerases

Extracellular PHA depolymerases are a class of serine hydrolases which are susceptible to serine esterase inhibitors, such as phenylmethylsulfonyl fluoride (PMSF) and diisopropyl fluorophosphate (DFP). Extracellular PHA depolymerases are divided into two classes based on their substrate specificity. One group contains the SCL-PHA depolymerases, which degrade only PHB and its copolyesters with 3HV or 4HB. The other group is composed of MCL-PHA depolymerases, which primarily decompose the aliphatic and aromatic PHAs consisting of 3-hydroxyalkanoates of C_6–C_{14}. The great majority of PHA-degrading microorganisms are known to produce only one type of PHA depolymerase, which acts upon either the SCL-PHAs or the MCL-PHAs (Jendrossek 1998, 2001).

Until now, more than 80 extracellular PHA depolymerases from prokaryotic and eukaryotic microorganisms have been purified and characterized (Jendrossek 2001). However, most PHA depolymerases are prokaryotic enzymes and are specific for only SCL-PHAs. During the past decade, several bacterial MCL-PHA depolymerases exhibiting broad substrate specificities for structurally different aliphatic and aromatic MCL-PHAs were isolated and biochemically characterized (Schirmer et al. 1993; Kim et al. 2000a, 2000b, 2002a). However, there is no report on the isolation of a fungal MCL-PHA depolymerase.

Since Brucato and Wong (1991) purified PHB depolymerase from *Penicillium funiculosum* as the first eukaryotic enzyme, eight distinct fungal PHB depolymerases have been isolated from different filamentous fungi (Table 2). Nevertheless, there is no report on the preparation of PHA depolymerase from yeast. Fungal PHB depolymerases share several properties among these enzymes; and certain enzyme properties are very similar to those with bacterial origins. However, fungal depolymerases also show some particular characteristics, which are distinct from the common properties of bacterial PHB depolymerases. The following descriptions indicate the

Table 1 Biodegradation of microbial and synthetic polyesters by fungi or their hydrolases. *PBA* Polybutylene adipate, *PBS* polybutylene succinate, *PCL* poly(ε-caprolactone), *PEA* polyethylene adipate, *PES* polyethylene succinate, *PET* polyethylene terephthalate, *PHB* poly(3-hydroxybutyrate), *PHV* poly(3-hydroxyvalerate), *PLA* poly(L-lactide), *PMA* poly(β-L-malic acid), *PPA* polypropylene adipate, *Sky-Green* a polyester consisting of succinic acid, adipic acid, butanediol, and ethylene glycol

Fungal strain	Polyesters hydrolyzed	Group	Reference
Acremonium sp.	PHB, Poly[3HB-*co*-(10 mol%) 3HV]	Deuteromycota	Mergaert et al. (1993)
Aspergillus fischeri	PCL	Deuteromycota	Benedict et al. (1983a)
A. flavus ATCC 9643	PCL	Deuteromycota	Benedict et al. (1983a)
A. flavus QM380	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
A. fumigatus M2A	PHB, Poly[3HB-*co*-(7–77 mol%) 3HV], PHV, Poly[3HB-*co*-(13–61 mol%) 4HB], PES, PEA, PBA, PES/A, PES, PBS/A	Deuteromycota	Scherer et al. (1999)
A. fumigatus	PHB, Poly[3HB-*co*-(10 mol%) 3HV]	Deuteromycota	Mergaert et al. (1993, 1994)
A. fumigatus LAR 9	PHB, Sky-Green	Deuteromycota	Kim et al. (2000c)
A. fumigatus ST-01	PHB, PCL, PBS, PBS/A	Deuteromycota	Sanchez et al. (2000)
A. fumigatus Pdf1	PHB, Poly(3HB-*co*-3HV), PHV	Deuteromycota	Iyer et al. (2000)
A. niger ATCC 9642	Sky-Green	Deuteromycota	Kim et al. (2000c)
A. niger QM386	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
A. penicilloides	PHB	Deuteromycota	Mergaert et al. (1992)
A. ustus T-221	PHB	Deuteromycota	Gonda et al. (2000)
A. ustus M-224	PHB	Deuteromycota	Gonda et al. (2000)
A. ustus LAR 25	Sky-Green	Deuteromycota	Kim et al. (2000c)
A. versicolor QM432	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
Aureobasidium pullulans	PCL	Deuteromycota	Fields et al. (1974)
A. pullulans QM279c	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
Candida guilliermondii	PHB	Deuteromycota	Gonda et al. (2000)
Cephalosporium sp.	PHB	Deuteromycota	Matavulj and Molitoris (1992)
Chaetomium globosum ATCC 6205	PCL	Ascomycota	Benedict et al. (1983a)
C. globosum QM459	PEA, PPA, PBA	Ascomycota	Darby and Kaplan (1968)
Cladosporium sp.	PHB	Deuteromycota	Matavulj and Molitoris (1992)
Cryptococcus laurentii	PCL	Basidiomycota	Benedict et al. (1983b)
Curvularia protuberata LAR 12	Sky-Green	Deuteromycota	Kim et al. (2000c)
Debaryomyces hansenii	PHB	Ascomycota	Gonda et al. (2000)
Emericellopsis minima W2	PHB, Poly[3HB-*co*-(30 mol%) 3HV]	Ascomycota	Kim et al. (2002b)
Eupenicillium sp. IMI 300465	PHB	Ascomycota	McLellan and Halling (1988)
Fusarium sp.	PCL	Deuteromycota	Benedict et al. (1983a)
F. moniiforme	PCL, cutin	Deuteromycota	Murphy et al. (1996)
F. oxysporium F1-3	Poly[3HB-*co*-(12 mol%) 3HV]	Deuteromycota	Sang et al. (2002)
F. solani LAR 11	PHB	Deuteromycota	Kim et al. (2000c)
F. solani strain 77-2-3	PCL, cutin	Deuteromycota	Murphy et al. (1996)
F. solani ATCC 38136	PCL, cutin	Deuteromycota	Murphy et al. (1996)
Mucor sp.	PHB	Zygomycota	Matavulj and Molitoris (1992)
Paecilomyces farinosus F4-7	Poly[3HB-*co*-(12 mol%) 3HV]	Deuteromycota	Sang et al. (2002)
P. farinosus LAR 10	PHB, Sky-Green	Deuteromycota	Kim et al. (2000c)
P. lilacinus D218	PHB, PCL	Deuteromycota	Oda et al. (1995)
P. lilacinus F4-5	Poly[3HB-*co*-(12 mol%) 3HV]	Deuteromycota	Sang et al. (2002)
P. marquandii	PHB	Deuteromycota	Mergaert et al. (1992)
Penicillium adametzii	PHB	Deuteromycota	Mergaert et al. (1992)
P. argillaceum IFO 31071	PCL	Deuteromycota	Sanchez et al. (2000)
P. chermisinum	PHB	Deuteromycota	Mergaert et al. (1995)
P. crysosporium	Poly[3HB-*co*-(7 mol%) 3HV]	Deuteromycota	Renstad et al. (1999)
P. daleae	PHB	Deuteromycota	Mergaert et al. (1992)
P. dupontii IFO 31798	PCL	Deuteromycota	Sanchez et al. (2000)
P. funiculosum ATCC 9644	PHB, PCL	Deuteromycota	Brucato and Wong (1991); Oda et al. (1995)
P. funiculosum IFO 6345	PHB, PHV, Poly[3HB-*co*-(7, 14%) 4HB], Poly[3HB-*co*-(7, 27, 45, 71%) 3HV]	Deuteromycota	Miyazaki et al. (2000)
P. funiculosum QM301	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
P. funiculosum ATCC 11797	PCL	Deuteromycota	Benedict et al. (1983a)
P. funiculosum LAR 18	PHB	Deuteromycota	Kim et al. (2000c)
P. janthinellum	PHB	Deuteromycota	Mergaert et al. (1995)
P. minioluteum LAR 14	PHB	Deuteromycota	Kim et al. (2000c)
P. orchrochloron	PHB	Deuteromycota	Mergaert et al. (1992)
P. pinophilium ATCC 9644	PHB	Deuteromycota	Han et al. (1998)
P. pinophilium LAR 15	PHB	Deuteromycota	Kim et al. (2000c)
P. restricum	PHB	Deuteromycota	Mergaert et al. (1992)
P. roqueforti	PLA	Deuteromycota	Torres et al. (1996)
P. simplicissimum IMI 300465	PHB	Deuteromycota	McLellan and Halling (1988)

Table 1 (continued)

Fungal strain	Polyesters hydrolyzed	Group	Reference
P. simplicissimum LAR 13	PHB, Sky-Green	Deuteromycota	Kim et al. (2000c)
P. simplicissimum	PHB	Deuteromycota	Mergaert et al. (1995)
P. simplicissimum	Poly[3HB-*co*-(7 mol%) 3HV]	Deuteromycota	Renstad et al. (1999)
Penicillium sp. strain 14-3	PEA, PCL, polyalkylene dicarboxylic acids	Deuteromycota	Tokiwa and Suzuki (1977a)
Penicillium sp. strain 26-1	PHB, PCL, polyalkylene dicarboxylic acids	Deuteromycota	Tokiwa et al. (1976)
P. verruculosum LAR 17	Mater-Bi	Deuteromycota	Kim et al. (2000c)
Phanerochaete chrysosporium	Poly[3HB-*co*-(7 mol%) 3HV]	Basidiomycota	Renstad et al. (1999)
Physarum polycephalum	PMA	Myxomycota	Korherr et al. (1995)
Polyporus circinatus	PHB	Basidiomycota	Matavulj and Molitoris (1992)
Pullularia pullulans QM279c	PEA, PPA, PBA	Deuteromycota	Darby and Kaplan (1968)
Rhizopus delemar	PPA, PET copolymers with dicarboxylic acids	Zygomycota	Walter et al. (1995); Nagata et al. (1997)
R. arrhizus	PCL, polyalkylene dicarboxylic acids	Zygomycota	Tokiwa et al. (1986)
Rhodosporidium sphaerocarpum	PHB	Basidiomycota	Gonda et al. (2000)
Thermoascus aurantiacus IFO 31910	PHB, PCL, PBS	Ascomycota	Sanchez et al. (2000)
Tritirachium album ATCC 22563	PLA	Deuteromycota	Jarerat and Tokiwa (2001a)
Verticillium leptobactrum	PHB	Deuteromycota	Mergaert et al. (1994)

Table 2 Biochemical characteristics of purified fungal PHB depolymerases. *DFP* Diisopropyl fluorophosphate, *DTT* dithiothreitol, *NEM N*-ethylmaleimide, *PMSF* phenylmethylsulfonyl fluoride, *PNP para*-nitrophenol

Characteristics	PhaZ$_{Emi}$	PhaZ$_{Pfu}$ATCC 9644	PhaZ$_{Ppi}$	PhaZ$_{Pli}$	PhaZ$_{Afu}$M2A	PhaZ$_{Afu}$Pdf1	PhaZ$_{Psi}$	PhaZ$_{Pfu}$ IFO 6345
Quaternary structure	Monomer	Monomer	Monomer		Monomer		Monomer	
M_r (SDS-PAGE)	48,000	37,000	35,000	48,000	57,000	40,000	36,000	33,000
pI	4.4	5.8			7.2	Alkaline		6.5
Carbohydrate content		+ (12%)			+ (<5%)	+	+ (<5%)	+
Optimum pH	9.0	6.0	6.0	7.0	8.0	8.5	5.0	6.5
Optimum temperature (°C)	55		50	45	70	45–60	45	
Sensitivity to:								
Sodium azide	–					–		
DTT	+	+		+	+	+	+	+
PMSF	–	–		+	+	–	+	–
DFP	+	+						+
NEM	–	–		+				–
EDTA				+		–		
Triton X-100	+	+			+			
Tween 80	+	+		+	+			
Hydrolysis of:								
PNP-C_2	+[a]					–		+[a]
PNP-C_4	+				–	+		+
PNP-C_6	–			+				
PNP-C_8	–			+[a]				
PNP-C_{10} to PNP-C_{14}	–			+				
PNP-C_{16}	–			–				
Main hydrolysis product of PHB	Dimer				Monomer	Monomer		
Reference	Kim et al. (2002b)	Brucato and Wong (1991)	Han et al. (1998)	Oda et al. (1997)	Scherer (1996)	Iyer et al. (2000)	Han and Kim (2002)	Miyazaki et al. (2000)

[a] This enzyme shows the highest esterase activity for the corresponding PNP-alkanoate

characteristics that are common among the fungal depolymerases:

1. PHB depolymerases consist of one single polypeptide chain with a molecular mass (M_r) ranging over 33.0–57.0 kDa.
2. In contrast to the general property of bacterial PHB depolymerases, fungal hydrolases possess an acidic or neutral pI value. It has been also demonstrated that some bacterial SCL- and MCL-PHA depolymerases have an acidic pI value (Kita et al. 1995; Kim et al. 2000b, 2002a).

3. The fungal PHB depolymerases are most likely glycoproteins.
4. Fungal enzymes are susceptible to DFP or PMSF, which is a serine esterase inhibitor, as shown in the cases of bacterial PHA depolymerases.
5. Fungal PHB hydrolases are very sensitive to dithiothreitol, indicating that the disulfide bridges in the active sites play an essential role in the formation of the tertiary structure of the enzymes. Similar effects are also found in most bacterial SCL-PHA depolymerases, while the MCL-PHA depolymerases from *Pseudomonas*spp are not inactivated by this inhibitor (Schirmer et al. 1993; Kim et al. 2000b, 2002a).
6. Fungal PHB depolymerases do not hydrolyze tributyrin, olive oil, or *p*-nitrophenylpalmitate, which are substrates for lipases.

Recently, Miyazaki et al. (2000) reported that the PHB depolymerase from *Penicillium funiculosum* IFO6345 contained a lipase box sequence (GLSSG) in the N-terminus of the protein, suggesting that the catalytic domain is probably located in this area. Considering that the catalytic domain and the substrate-binding domain of all the PHB depolymerases investigated constitute the N-terminus and C-terminus of the enzymes, respectively, it is believed that the composite structure of fungal PHB depolymerases also corresponds to one of the composite structures of the bacterial PHB depolymerases (Jendrossek 1998). However, there is still a need to clarify the apparent composite structure of the fungal enzymes via molecular characterization of the PHB depolymerase genes. In contrast, MCL-PHA depolymerases consist of a substrate-binding domain in the N-terminus and a catalytic domain in the C-terminus (Schirmer and Jendrossek 1994; Kim et al. 2002c).

The majority of bacterial PHB depolymerases exhibit a pH optimum in the alkaline range of pH 7.5–9.8 (Jendrossek 2001). Similarly, it has been also reported that PHB depolymerases from *Emericellopsis minima* (Kim et al. 2002b) and two *A. fumigatus* species (Scherer 1996; Iyer et al. 2000) are alkaline enzymes, showing a maximum activity at pH 8.0–9.0. However, all PHB depolymerases from *Penicillium* spp are most active in the acidic range of pH 5.0–6.5, as shown in Table 2. The PHB depolymerase from *A. fumigatus* M2A is a thermotolerant enzyme, with an optimum temperature of 70 °C, while all the other fungal enzymes exhibited their highest activity at 45–60 °C.

Compared with many bacterial PHB depolymerases, some fungal PHB depolymerases are known as relatively nonspecific hydrolases that are capable of giving rise to chain scission, acting upon various types of polymer substrates. For example, the PHB hydrolase from *A. fumigatus* M2A catalyzes synthetic polyesters, such as polyethylene adipate (PEA), polyethylene succinate (PES), and polybutylene adipate (PBA), in addition to poly(3HB-*co*-3HV) and poly(3HB-*co*-4HB) over the entire range of compositions (Scherer et al. 1999). However, it does not decompose PLA, polyvalerolactone, or PCL. In particular, the hydrolytic enzyme from *A. fumigatus* efficiently degrades bacterial and synthetic polyesters in the following order: PEA >PES >PBA >PHB >PHV. The extracellular PHB depolymerases showing a broad substrate specificity towards PHB, PHV, and poly(3HB-*co*-3HV) were also prepared from cultures of *A. fumigatus* Pdf1 (Iyer et al. 2000) and *P. funiculosum* IFO 6345 (Miyazaki et al. 2000). The enzymatic degradation of PHB by fungal PHB depolymerases usually results in the formation of 3-hydroxybutyrate and its dimers as the final hydrolysis products (Oda et al. 1995; Scherer 1996; Iyer et al. 2000; Kim et al. 2002b).

Characterization of fungal PMA hydrolase

The extracellular PMA hydrolase from the slime mold *Physarum polycephalum* was recently isolated and characterized (Korherr et al. 1995; Karl and Holler 1998). However, it was assumed that this enzyme is excreted from the plasmodia of *P. polycephalum* under unfavorable environmental conditions (Korherr et al. 1995). The PMA depolymerase does not degrade PHB, which is the reduced form of PMA. It is a glycoprotein with a M_r of 97,000 and exists in multiple forms. In particular, in contrast to the general properties of PHB depolymerases, it is neither a metallo- nor a serine-hydrolase and is most active at pH 3.5. Although the extracellular PMA hydrolase from *Comamonas acidovorans* was recently purified (Gödde et al. 1999), any extracellular PMA depolymerase from non-PMA-producing eukaryotic organisms has not yet been isolated.

Biodegradation of synthetic polyesters

Poly(ε-caprolactone)

PCL, a synthetic linear polyester with an almost 50% crystallinity, is biologically degradable and consists of 6-hydroxyhexanoates. Generally, the environmental degradation of PCL appears to occur by the action of bacteria that are widely distributed in the ecosystem (Nishida and Tokiwa 1993; Mergaert and Swings 1996; Suyama et al. 1998). However, several studies have shown that some filamentous fungi and yeasts also hydrolyze PCL to water-soluble products, as listed in Table 1, although little regarding their PCL depolymerases is known. Fields et al. (1974) reported that *Aureobasidium pullulans* (formerly *Pulllularia pullulans*) can efficiently degrade a lower molecular weight (Mw) PCL film (Mw=1,250), but the extent of decomposition of a PCL film with a Mw above 15,000 by the fungus was negligible. In contrast, *Penicillium* sp. strain 14-3 (Tokiwa et al. 1976) and a yeast, *Cryptococcus laurentii* (Benedict et al. 1983b), degrade PCLs with relatively higher Mws (25,000 and 35,000, respectively). Another study showed that *Aspergillus fischeri*, *A. flavus*, *Penicillium funiculosum*, *Chaetomium*

globosum, and a *Fusarium* sp. could metabolize PCLs with Mws in the range 7,130–35,000 (Benedict et al. 1983a). However, in that case, increasing the Mw of the polyester reduced its degradability. Recently, Sanchez et al. (2000) reported the breakdown of a high Mw PCL (Mw=67,300) at 50 °C by a thermotolerant *A. fumigatus* ST-01 and a thermophilic *Thermoascus aurantiacus*. These results suggest that one of the most important factors influencing PCL biodegradation is the PCL-degrading microorganisms that produce different types of PCL hydrolases, even though the biodegradability of a polymer is related to its Mw.

Tokiwa and Suzuki (1977b) showed that various esterases and lipases could hydrolyze PCL. In particular, the lipases of *Rhizopus delemar* (Tokiwa and Suzuki 1977b) and *R. arrhizus* (Tokiwa et al. 1986) could readily hydrolyze synthetic polyesters such as PCL, poly(ethylene dicarboxylic acids), and poly(butylene dicarboxylic acids). However, PCL is not susceptible to bacterial (Jaeger et al. 1995) and fungal PHB depolymerases (Oda et al. 1995; Kim et al. 2002b). Some fungal phytopathogens belonging to *Fusarium* spp can produce cutinase to degrade a cutin polyester that is the structural component of a plant cuticle (Baker and Bateman 1978). There is some evidence suggesting that the *Fusarium* PCL depolymerases are true cutinases. Murphy et al. (1996) reported that a cutinase-negative gene-replacement mutant of *F. solani* did not degrade both PCL and cutin, while the wild-type strains of *Fusarium* spp commonly hydrolyzed both polyesters. The production of PCL depolymerase in the wild-type strains was induced by adding the cutinase inducer, 16-hydroxyhexadecanoic acid, to the culture medium in addition to the PCL hydrolysates. In contrast, no PCL depolymerase was induced in the cutinase-negative mutant by the same inducers. Another study showed that the cutinase-negative mutant of *F. solani* produces a second PCL depolymerase, which can be induced by Tween 80 and tributyrin, but not by PCL or cutin, suggesting that the enzyme is a lipase distinct from cutinase (Murphy et al. 1998). The fungal degradation of the plant polyesters (cutin, suberin) is well documented by Kolattukudy (2001).

Poly(L-lactide)

PLA is a biocompatible thermoplastic with a melting temperature of 175 °C and a glass transition temperature of 60 °C and is synthesized by the polymerization of L-lactic acid. Originally, Williams (1981) reported the biodegradability of PLA by the lipase from *R. delemar* and the proteinase K from *Tritirachium album*. PLA can also be hydrolyzed by the polyester polyurethane depolymerase from *Comamonas acidovorans* (Akutsu et al. 1998). However, it is currently believed that PLA is more resistant to microbial attack in the environment than other microbial and synthetic polyesters (Suyama et al. 1998). In fact, reports on the environmental degradation of PLA by microorganisms are relatively rare and most PLA-degraders isolated to date are restricted to actinomycetes belonging to the genus *Amycolatopsis* (Pranamuda et al. 1997; Ikura and Kudo 1999; Pranamuda and Tokiwa 1999; Nakamura et al. 2001), although the breakdown of the polymer by *Bacillus brevis* (Tomita et al. 1999) and some unidentified fungal strains (Torres et al. 1996) has been reported.

The attempt to screen PLA-degrading fungi was first conducted by Torres et al. (1996). They demonstrated that, of the 14 fungal strains tested, only two strains of *F. moniliforme* and *P. roqueforti* could assimilate DL-lactic acid and the partially soluble racemic oligomers (Mw=1,000), which are PLA derivatives. In particular, they observed that one strain of *F. moniliforme* was able to grow on a poly(lactic acid-*co*-glycolic acid) copolyester (Mw=150,000) film, but its ability to mineralize the polymer was relatively poor. Apparently, *Tritirachium album* ATCC 22563 is the only PLA-degrading fungus that has been identified to date (Jarerat and Tokiwa 2001a). In that case, the PLA degradation rate by *T. album* was greatly increased by adding 0.1% gelatin to the culture medium, implying that gelatin induces the production of a protease capable of giving rise to chain scission of the PLA. In fact, the culture supernatant of *T. album* exhibited hydrolytic activity towards silk fibroin and elastin in addition to PLA, but not towards PHB, PCL, and polybutylene succinate (PBS). From these results, it was concluded that the hydrolase from *T. album* might be a protease rather than a lipase. Similarly, Oda et al. (2000) reported that various commercial prokaryotic and eukaryotic proteases exhibited hydrolytic activity against PLA.

Aliphatic polyalkylene dicarboxylic acids

A variety of aliphatic polyesters consisting of dicarboxylic acid units, such as PEA, PES, polypropylene adipate (PPA), PBA, PBS, and polyhexylene carbonate, have been widely produced commercially. Several studies have proven the biodegradability of these synthetic polyesters, either by measuring microbial growth on an agar plate containing the respective emulsified polymer substrate (Pranamuda et al. 1995; Suyama et al. 1998; Tansengco and Tokiwa 1998; Jarerat and Tokiwa 2001a, 2001b), or by enzymatic degradation tests using fungal hydrolases, such as lipase (Tokiwa and Suzuki 1977b; Tokiwa et al. 1986; Walter et al. 1995), PEA depolymerase (Tokiwa and Suzuki 1977a), and PHB depolymerase (Scherer et al. 1999).

From the fungal degradation studies of low Mw PEA, PPA, and PBA, Darby and Kaplan (1968) reported that several filamentous fungi, such as *Aspergillus flavus*, *A. niger*, *A. versicolor*, *Aureobasidium pullulans*, *P. funiculosum*, and *Chaetomium globosum*, can hydrolyze the polyesters. However, increasing the Mw and hydrocarbon content in the polymers consisting of dicarboxylic acid units reduces their biodegradability. In an early study, the PEA-degrading enzyme from *Penicillium* sp. strain 14-3

was purified and characterized (Tokiwa and Suzuki 1977a). The enzyme, with a M_r of 25,000, exhibited its highest activity at pH 4.5 and 45 °C and was remarkably activated by divalent cations, such as Ca^{2+} and Cd^{2+}. It was assumed that this fungal PEA depolymerase is a lipase exhibiting a broad substrate specificity capable of acting upon various bio- and synthetic polymers. This means it can degrade not only the substrates for lipase, such as plant oils, triglycerides, and the methyl esters of fatty acids, but also the aliphatic polyesters composed of dicarboxylic acids. However, it does not degrade aromatic polyesters, such as polybutylene terephthalate and polyethylene tetrachlorophthalate. In addition, the non-specific hydrolysis of microbial and synthetic polyesters was observed in the case of the hydrolase from *Aspergillus fumigatus* M2A (Scherer et al. 1999). Even though the fungal enzyme was referred as a PHB depolymerase, it showed its highest hydrolytic activity for PEA and degraded PES and PBA more efficiently than PHB.

Aromatic polyalkylene dicarboxylic acids

Compared with most aliphatic polyesters, aromatic polyesters, such as poly(ethylene terephthalate) and poly(butylene terephthalate), have excellent material properties. However, it is now considered that their susceptibility to microbial attack is negligible (Müller et al. 2001), although some growth of *A. niger* on the surfaces of some aromatic polyesters has been observed (Huang and Byrne 1980). Therefore, to increase the biodegradability of aromatic polyesters, recent studies focused on the synthesis of aliphatic–aromatic copolyesters (Müller et al. 2001; Ki and Park 2001). In fact, it was reported that the incorporation of aliphatic dicarboxylic acids or polyethylene glycol in polyester chains greatly enhances the degradation rate by the lipase from *R. delemar* (Nagata et al. 1997). To date, some bacterial species capable of decomposing aliphatic–aromatic polyalkylene dicarboxylic acid copolyesters with different compositions have been isolated from composts (Kleeberg et al. 1998). However, little is known about the fungal strains that can cause the environmental degradation of these polymers.

Microbiological and environmental aspects of the fungal degradation of polyesters

In soil systems, the fungal biomass generally exceeds the bacterial biomass (Killham 1994). Therefore, the fungal contribution to the biological recycling of organic substances in the environment is expected to be much greater than the bacterial contribution. Actually, it was reported that the decomposition of organic matter in acidic soils is predominantly achieved by fungal strains exhibiting a greater tolerance to acid conditions, compared with bacterial heterotrophs (Killham 1994). In a study on the relative contributions of fungi and bacteria to cellulase activities in soil, Rhee et al. (1987) indicated that fungi are the dominant contributors to the degradation of cellulosic materials in a soil ecosystem. Recently, Sang et al. (2002) estimated the relative contributions made by fungi, actinomycetes, and bacteria to the in situ biodegradation of a microbial polyester film in soil. They compared the degradation abilities and population of poly(3HB-*co*-3HV)-degrading fungi with those of bacteria and actinomycetes and found not only that the population of fungi dramatically increased during poly(3HB-*co*-3HV) film degradation but also that the degradation rate of the fungi was much higher than that of either the actinomycetes or the bacteria. On the basis of these descriptions, it is believed that many fungal strains may play a considerable role in decomposing various polymeric materials in soil systems.

Composting is a microbial process that is regarded to be one of the most promising technologies for the biological management of solid wastes. As the temperature rises during the active composting stage, thermophilic heterotrophs replace mesophilic forms. Mineralization of the various types of aliphatic polyesters by thermotolerant or thermophilic bacteria in the composting process has been extensively investigated (Mergaert et al. 1994; Tansengco and Tokiwa 1998; Jarerat and Tokiwa 2001b; Sakai et al. 2001). In contrast, the ecological importance of fungi participating in the thermophilic biodegradation of polyesters is not well understood. This is despite the fact that a few polyester-degrading thermotolerant or thermophilic fungi have been isolated from soil and compost (Mergaert et al. 1994; Sanchez et al. 2000).

Currently, the annual worldwide use of plastic materials is gradually increasing and, for this reason, the importance of both developing and improving a technology for the eco-friendly management of waste plastic materials is greatly emphasized. From this point of view, it is expected that a more intensive study on the fungal degradation of polyesters will not only emphasize the ecological significance of fungi, but also contribute to the development of a more efficient strategy for the biological treatment of wastes. In addition, further advances in biochemistry and molecular genetics concerning fungal enzymes catalyzing the degradation of the polyesters will open up new fields in environmental biotechnology and bioengineering for fine chemicals.

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