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International Biodeterioration & Biodegradation 49 (2002) 245–252

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Biodegradation of polyurethane: a review

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Abstract

Lack of degradability and the closing of landfill sites as well as growing water and land pollution problems have led to concern about plastics. Increasingly, raw materials such as crude oil are in short supply for the synthesis of plastics, and the recycling of waste plastics is becoming more important. As the importance of recycling increases, so do studies on elucidation of the biodegradability of polyurethanes. Polyurethanes are an important and versatile class of man-made polymers used in a wide variety of products in the medical, automotive and industrial fields. Polyurethane is a general term used for a class of polymers derived from the condensation of polyisocyanates and polyalcohols. Despite its xenobiotic origins, polyurethane has been found to be susceptible to biodegradation by naturally occurring microorganisms. Microbial degradation of polyurethanes is dependent on the many properties of the polymer such as molecular orientation, crystallinity, cross-linking and chemical groups present in the molecular chains which determine the accessibility to degrading-enzyme systems. Esterase activity (both membrane-bound and extracellular) has been noted in microbes which allow them to utilize polyurethane. Microbial degradation of polyester polyurethane is hypothosized to be mainly due to the hydrolysis of ester bonds by these esterase enzymes.

Keywords: Polyurethane; Degradation; Polyurethanase; Esterase

1. Introduction

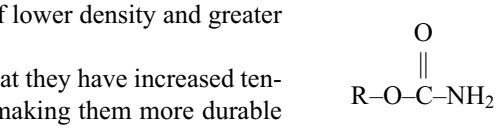
Polyurethanes (PU) are present in many aspects of modern life. They represent a class of polymers that have found a widespread use in the medical, automotive and industrial fields. Polyurethanes can be found in products such as furniture, coatings, adhesives, constructional materials, fibers, paddings, paints, elastomers and synthetic skins.

Polyurethanes are replacing older polymers for various reasons. The United States government is phasing out chlorinated rubber in marine and aircraft and coatings because they contain environmentally hazardous volatile organic compounds (Hegedus et al., 1989; Reisch, 1990). Auto manufacturers are replacing latex rubber in car seats and interior padding with PU foam because of lower density and greater flexibility (Ulrich, 1983).

Other advantages of PUs are that they have increased tensile strength and melting points making them more durable (Bayer, 1947). Their resistance to degradation by water, oils, and solvents make them excellent for the replacement of plastics (Saunders and Frisch, 1964). As coatings, they exhibit excellent adhesion to many substances, abrasion resistance, electrical properties and weather resistance for industrial purposes (Saunders and Frisch, 1964; Urbanski et al., 1977; Fried, 1995).

2. Physical and chemical properties of polyurethanes

Polyurethanes were first produced and investigated by Dr. Otto Bayer in 1937. Polyurethane is a polymer in which the repeating unit contains a urethane moiety. Urethanes are derivatives of carbamic acids which exist only in the form of their esters (Dombrow, 1957). This structure can be represented by the following, generalized amide-ester of carbonic acid:



Variations in the *R* group and substitutions of the amide hydrogen produce multiple urethanes. Although all PUs contain repeating urethane groups, other moieties such as urea, ester, ether and aromatic may be included (Saunders and Frisch, 1964). The addition of these functional groups may result in fewer urethane moieties in the polymer than functional groups.

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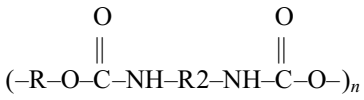
0964-8305/02/$ - see front matter
PII: S0964-8305(02)00051-3

The urethane linkage results most readily through the reaction of an isocyanate, –N=C=O, with an alcohol, –OH (Dombrow, 1957; Kaplan et al., 1968). The hydrogen atom of the hydroxyl group is transferred to the nitrogen atom of the isocynanate (Bayer, 1947). The major advantage of PU is that the chain is not composed exclusively of carbon atoms but rather of heteroatoms, oxygen, carbon and nitrogen (Bayer, 1947). The simplest formula for PU is linear and represented by



where n is the number of repetitions and R2 is a hydrocarbon chain. R represents a hydrocarbon containing the OH group. Diisocyanates are employed in PU production reactions because they will react with any compound containing an active hydrogen (Dombrow, 1957).

For industrial applications, a polyhydroxyl compound can be used. Similarly, polyfunctional nitrogen compounds can be used at the amide linkages. By changing and varying the polyhydroxyl and polyfunctional nitrogen compounds, different PUs can be synthesized (Dombrow, 1957). Polyester or polyether resins containing hydroxyl groups are used to produce polyester- or polyether-PU, respectively (Urbanski et al., 1977).

Variations in the number of substitutions and the spacing between and within branch chains produce PUs ranging from linear to branched and flexible to rigid. Linear PUs are used for the manufacture of fibers and molding (Urbanski et al., 1977). Flexible PUs are used in the production of binding agents and coatings (Saunders and Frisch, 1964). Flexible and rigid foamed plastics, which make up the majority of PUs produced, can be found in various forms in industry (Fried, 1995). Using low molecular mass prepolymers, various block copolymers can be produced. The terminal hydroxyl group allows for alternating blocks, called segments, to be inserted into the PU chain. Variation in these segments results in varying degrees of tensile strength and elasticity. Blocks providing rigid crystalline phase and containing the chain extender are referred to as hard segments (Fried, 1995). Those yielding an amorphous rubbery phase and containing the polyester/polyether are called soft segments. Commercially, these block polymers are known as segmented PUs (Young and Lovell, 1994).

3. Polyurethane degradation

After years of production of PUs, manufacturers found them susceptible to degradation. Variations in the degradation patterns of different samples of PUs were attributed to the many properties of PUs such as topology and chemical composition (Pathirana and Seal, 1983). The regularity in synthetic polymers allows the polymer chain to pack easily, resulting in the formation of crystalline regions. This limits accessibility of the polymer chains to degradative agents.

4. Decreasing polyurethane degradation

Research was initiated to elucidate whether additives to the chemical structure of PUs could decrease biodegradation. Kanavel et al. (1966) observed that sulfur-cured polyester and polyether PUs had some fungal inertness. However, they noted that even with fungicides added to the sulfur- and peroxide-cured PUs, fungal growth still occurred on the polyester PUs and most fungicides had adverse effects on the formulations. Kanavel et al. (1966) also recognized the need for physical testing of the PUs after extended exposure to the activity of fungi.

Santerre et al. (1994) varied the amount of degradation products released by varying the physical makeup of the polyester PUs, as coatings on glass tubes or as films. This implied that while urethane and urea groups are susceptible to hydrolysis, they are not always accessible to the enzyme and degradation may never proceed past the polymer surface. Although the polyether PUs showed no significant degradation, they consistently showed higher radiolabel products release from soft-segment-labeled, enzyme-incubated samples than controls. The authors attributed these results to the shielding of ester sites by secondary structures and hydrogen bonding within the hard segment.

Santerre and Labrow (1997) tested the effect of hard segment size on the stability of PUs against cleavage. Analysis was performed with polyether PUs and their susceptibility to cholesterol esterase. Three polyether PUs were synthesized with varying molar ratios of [^{14}C]-diisocyanate to chain extender and constant polyether makeup. A ten-fold increase in enzyme concentration of cholesterol esterase previously used (Santerre et al., 1994) was utilized to approach plateau values for polyether PU hydrolysis. Upon treatment with cholesterol esterase, Santerre and Labrow (1997) observed that radiolabel release was significantly dependent on the amount of hard segment contained within the polymer. In the polymer with the lowest concentration of hard segment, higher numbers of carbonyl groups are exposed to the surface. With increased hard segment size, a greater number of carbonyl groups are integrated into secondary hard segment structures through hydrogen bonding. The investigators also concluded that an increase in hard segment size does lead to restrictions in polymer chain mobility.

In the medical field PUs show resistance to macromolecular oxidation, hydrolysis and calcification (Marchant, 1992). Polyurethane elastomers are being used in place of other elastomers due to higher elasticity and toughness, and resistance to tear, oxidation and humidity (Dombrow, 1957; Saunders and Frisch, 1964; Ulrich, 1983). In addition, polyether derivatives are inexpensive to produce as prepolymers, which can lower the overall cost of polymer production.

Huang and Roby (1986) tested the biodegradability of polyamide-urethanes for medical purposes. They synthesized PUs with long repeating units and alternating amide and urethane groups from 2-aminoethanol. The resulting partial crystalline fibers were observed to undergo hydrolysis by subtilisin less readily than polyamideesters with degradation proceeding in a selective manner. The amorphous regions on the PU were being degraded prior to the crystalline regions. These fibers showed promise as absorbable sutures and implants where in vivo degradation is needed. The investigators also noted that PUs with long repeating units and hydrophilic groups would less likely to pack into high crystalline regions as normal PUs, and these polymers were more accessible to biodegradation.

Tang et al. (1997) added surface-modifying macromolecules (SMM) containing fluorinated end groups to the base PU to reduce the material's susceptibility to hydrolysis by lysosomal enzymes. Synthesized polyester urea-urethanes were radiolabled with [^{14}C] and coated onto small hollow tubes. Biodegradation experiments were carried out using methods previously established by Santerre et al. (1994). Results indicated that degradation was inhibited by the SMM surface. Different SMM formulations provided varying degrees of enzyme resistance. It was noted that some SMM formulations were incompatible with the PU and led to increased biodeterioration. The mechanism of inhibition was not deduced and will be the subject of further study.

In an attempt to increase biocompatibility and reduce bacterial adhesion on PU surfaces, Baumgartner et al. (1997) synthesized phosphonated PUs. They used glycerophosphorylcholine (GPC) as the chain extender, which incorporated phosphorylcholine head groups into the PU backbone. This gave the PU surface some characteristics of a red blood cell surface. Physical tests on the PU showed a small decrease in tensile strength and transition temperature with increasing GPC concentration. Water absorption by the PU was increased with increased GPC content. To test bacterial adhesion to the PU, Baumgartner et al. (1997) used a radial flow chamber. They passed a culture of *Staphylococcus aureus* across phosphonated and unphosphonated PU at a rate of 8 ml min^{-1}. The phosphonated PU showed a decrease in bacterial adhesion with increased GPC content.

5. Increasing polyurethane degradation

Lack of degradability and increasing depletion of landfill sites as well as growing water and land problems have led to concern about plastics (Kawai, 1995). As more and more raw materials (e.g. crude oil) become in short supply for the synthesis of plastics, recycling of waste plastics is becoming important (Schnabel, 1981). Degradability problems promoted researchers to investigate modification or productions that led to either chemically degradable or biodegradable PUs.

Huang et al. (1981) derived polyester PUs from polycaprolactonediols in an effort to produce biodegradable PUs for use in the medical field. Several different PUs were made containing polyester subunits of various lengths. The polymers were subjected to degradation by the enzyme axion and two species of fungi. The enzyme and fungi degraded each PU. In addition, it was also noted that there was an increase in the biodegradability of the polyester PUs with increase in the chain length of the polyesters.

In a later study, Phua et al. (1987) observed that two proteolytic enzymes, papain and urease degraded a medical polyester PU. The PU they tested was Biomer®, segmented, cross-linked polyester PU. Although cross linking was previously described as a way of inhibiting degradation (Kaplan et al., 1968), papain (molecular weight 20.7 kDa) had little difficulty in diffusing into the film and causing breaks in the structural integrity. Urease activity, because of its size (molecular weight 473 kDa), was limited to the PU surface and therefore was not significant. Phua et al. (1987) also proposed that papain degraded the polymer by hydrolyzing the urethane and urea linkages producing free amine and hydroxyl groups. The effect of papain on polyether PU was assessed by Marchant et al. (1987). Comparison of papain activity to aqueous hydrolysis resulted in both releasing degradation products. Ether linkages were non-enzymatically hydrolyzed by water while degradation of the urethane groups was dependent on the presence of the proteolytic enzyme.

Labrow et al. (1996) treated polyester PU and polyether PU with human neutrophil elastase and porcine pancreatic elastase. The polyester PU was readily degraded by porcine pancreatic elastase at a rate 10 times higher than by human neutrophil elastase. The rate of polyester PU degradation by porcine pancreatic elastase was also 10 times higher than its activity against the polyether PU. Human neutrophil elastase had no significant activity against the polyether PU. These results indicate a distinct similarity to the degradation of PUs by cholesterol esterase (Santerre et al., 1993, 1994; Santerre and Labrow, 1997). Inhibition of porcine pancreatic elastase was achieved with the elastase specific inhibitor NMSAAPVCMK.

6. Fungal biodegradation

After years of production of PUs, manufacturer's found them susceptible to degradation. Variations in the degradation patterns of different samples of PUs were attributed to the many properties of PUs such as molecular orientation, crystallinity, cross-linking, and chemical groups presented in the molecular chains which determine the accessibility to degrading-enzyme systems (Pathirana and Seal, 1983). The regularity in synthetic polymers allows the polymer chains to pack easily, resulting in the formation of crystalline regions. This limits accessibility of the polymer chains to degradation whereas, amorphous regions on the PU can

degrade more readily. Huang and Roby (1986) observed PU degradation proceeded in a selective manner, with the amorphous regions being degraded prior to the crystalline regions. Also, it was observed that PUs with long repeating units and hydrolytic groups would be less likely to pack into high crystalline regions as normal polyurethanes, and these polymers were more accessible to biodegradation. Several investigators have suggested microbial attack on PUs could be through enzymatic action of hydrolases such as ureases, proteases and esterases (Evans and Levisohn, 1968; Hole, 1972; Flilip, 1978; Griffin, 1980).

Several reports have appeared in the literature on the susceptibility of PUs to fungal attack (Darby and Kaplan, 1968; Kaplan et al., 1968; Ossefort and Testroet, 1966). These studies revealed that polyester-type PUs are more susceptible to fungal attack than other forms. In addition, polyether PUs were noted to be moderately too highly resistant. Boubendir (1993) isolated enzymes with esterase and urethane hydrolase activities from the fungi *Chaetomium globosum* and *Aspergillus terreus*. These organisms did not grow solely on PU and the enzymes had to be induced. Induction of the enzymes was accomplished by addition of liquid polyester PU to the growth media. Activity of the enzymes was determined by assays based on ethyl carbamate (urethane) as artificial substrate.

Four species of fungi, *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans* and *Cladosporium* sp. were isolated based on their ability to utilize a colloidal polyester PU (Impranil DLN^{TM}) as the sole carbon and energy source (Crabbe et al., 1994). *Curvularia senegalensis* was observed to have a higher PU-degrading activity and therefore subsequent analysis of this fungal isolate was carried out. An extracellular polyurethanase (PUase) displaying esterase activity was purified from this organism. The protein has a molecular mass of 28 kDa, is heat stable at 100°C for 10 min and inhibited by phenylmethylsulphonylfluoride (PMSF).

Wales and Sagar (1988) proposed a mechanism for the degradation of polyester PUs by extracellular esterases. Polyurethane degradation is the result of synergistic activity between endopolyurethanases and exopolyurethanases. Endoenzymes hydrolyze the PU molecule at random locations throughout the polymer chain leading to loss of tensile strength. Exoenzymes remove successive monomer units from the chain ends however, show little loss of tensile strength.

7. Bacterial biodegradation

In a large-scale test of bacterial activity against PUs, Kay et al. (1991) investigated the ability of 16 bacterial isolates to degrade polyester-PU. Seven of the isolates tested degraded PU when the media was supplemented with yeast extract. Two isolates, *Corynebacterium* sp. and *Pseudomonas aeruginosa*, could degrade PU in the presence of basal media. However, none of the isolates grew on PU alone. Physical tests of the degraded polyester PU revealed different but significant decreases in tensile strength and elongation for each isolate. In a further study, (Kay et al., 1993), tested the chemical and physical changes in degraded polyester PU. Polyurethanes taken from *Corynebacterium* sp. cultures had significant reductions in both tensile strength and elongation after three days of incubation. Infra-red spectrophotometer analysis revealed the ester segment of the polymer to be the main site of attack. The investigators noted that supplementing the media with glucose inhibited esterase production. However, addition of PU did not increase esterase activity.

Halim El-Sayed et al. (1996) tested the growth of several species of bacteria on PU military aircraft paint. The investigators isolated *Acinetobacter calcoaceticus*, two *Pseudomonas* sp., *Pseudomonas cepacia*, and *Arthrobacter globiformis*. In addition, the U.S. Navy supplied two strains of *A. calcoaceticus*, *Pseudomonas aeruginosa* and *Pseudomonas putida*. All species were capable of utilizing the polyurethane paint as a sole carbon and energy source with the exception of *P. cepacia*. Using fluorescein diacetate as an esterase substrate, the remaining species showed esterase activity in the absence of PU. This data indicated that the PUases were constitutively expressed.

In an additional study, *Comamonas acidovorans* strain TB-35 was isolated from soil samples by its ability to degrade polyester PU (Nakajima-Kambe et al., 1995). Solid cubes of polyester PU were synthesized with various polyester segments. The cubes were completely degraded after 7 days incubation when they were supplied as the sole carbon source and degraded 48% when they were the sole carbon and nitrogen source. Analysis of the breakdown products of the PU revealed that the main metabolites were from the polyester segment of the polymer. Further analysis of strain TB-35 revealed that the degradation products from the polyester PU were produced by an esterase activity (Nakajima-Kambe et al., 1997). Strain TB-35 possess two esterase enzymes, a soluble, extracellular and one membrane-bound. The membrane-bound enzyme was found to catalyze the majority of the polyester PU degradation. The membrane-bound PUase enzyme was purified and characterized (Akutsu et al., 1998). The protein has a molecular mass of 62 kDa, heat stable up to 65°C and inhibited by PMSF. The structural gene, *pudA*, for the PU esterase was cloned in *Escherichia coli*. Upon nucleotide sequencing of the open reading frame (ORF), the predicted amino acid sequence contained a Gly-X-Ser-X-Gly motif characteristic of serine hydrolases. The highest degree of homology was detected with the *Torpedo californica* acetylcholinesterase (T AchE), possessing the Ser–His–Glu catalytic triad, with the glutamate residue replacing the usual aspartate residue. Similarity in the number and positions of cysteine and salt bonds was very apparent between PudA and T AchE, as were also identities of sequences and their positions in the α-helix and β-strand regions between

Table 1
Characteristics of purified PUase isolated by our laboratory

Bacterial isolate	Molecular mass (kDa)	Enzyme specificity	Inhibition	Heat stable[a]
P. fluorescens	29	Protease	PMSF	+
P. fluorescens	48[bc]	Esterase	PMSF	−
P. chlororaphis	63[bc]	Esterase/protease	PMSF	+
P. chlororaphis	31	Esterase	PMSF	+
C. acidovorans	42	Esterase/protease	PMSF/TI	+

[a]Enzyme activity (100%) after 10 min at 100°C.
[b]Enzyme has been cloned and expressed in *E. coli.*
[c]Gene has been sequenced.

the two. In the neighborhood of the glutamate residue of the Ser^{199}–His^{433}– Glu^{324} catalytic domain of PudA, there were three hydrophobic domains, one of which constituted the surface-binding domain, which occurred in the C-terminus of most bacterial poly(hydroxyalkanoate)(PHA) depolymerases.

Previous reports in the literature on the degradation of PU have focused on fungal attack with few studies addressing bacterial degradation of these polymers. Blake and Howard (1998) reported bacterial degradation of a polyester PU (Impranil DLN) by a species of *Bacillus*. The pattern of degradation involved the binding of cells to the polymer with subsequent floc formation, and the degradation of substrate. Several members of the genus *Pseudomonas* have been isolated for their ability to utilize a polyester PU as the sole carbon and energy source. Our laboratory is currently studying the PUase enzymes and genes that encode them from three species of *Pseudomonas* (Table 1). Interestingly, the three species of bacteria produce different PUase activities but are inhibited by serine hydrolase inhibitors. These data suggest that either esterase and/or protease activities are involved in the degradation of Impranil.

Growth of *Comamonas acidovorans* on PU resulted in obtaining growth parameters for K_S and μ_{max} of 0.3 mg ml^{-1} and 0.7 doublings h^{-1}, respectively (Allen et al., 1999). Our laboratory has purified and characterized a 42 kDa PUase enzyme displaying esterase/protease activity (Allen et al., 1999). Nakajima-Kambe et al. (1995, 1997) reported a strain of *Comamonas acidovorans* that could utilize solid polyester PU as the sole carbon and nitrogen source. These authors indicated the role of an extracellular membrane bound esterase activity in PU degradation. Purification of the membrane bound esterase revealed a thermally labile protein having a 62 kDa molecular mass (Akutsu et al., 1998).

Growth of *Pseudomonas chlororaphis* on polyurethane resulted in values of 0.9 mg ml^{-1} and 1.3 doublings h^{-1} for K_S and μ_{max}, respectively (Ruiz et al., 1999a). Two PUase enzymes have been purified and characterized, a 65 kDa esterase/protease and a 31 kDa esterase (Ruiz et al., 1999b). A third PUase enzyme, 60 kDa esterase, has been partially purified and characterized (Ruiz et al., 1999a). In addition to the enzymology, a PUase gene encoding a 63 kDa protein has been cloned in *E. coli*. The gene encoding a 65 kDa PUase, *pueA*, has been expressed and sequenced (Genebank, Accession AF069748). An ORF, 1854 nucleotides in length, was observed in the cloned insert by codon analysis, starting with an AUG codon in nucleotide 175 and stopping with an UGA codon in nucleotide 2029. Identity searching in database with the BLAST program indicated a strong similarity of this ORF to several lipase genes. Therefore, the sequenced gene was called *pueA* (polyurethanase-esterase A). The initiating AUG codon was preceded 7 nucleotides by a plausible ribosomal binding site with an AAGAGG. The deduced protein PueA has 617 amino acid residues and a molecular mass of 65 kDa. The encoded amino acid sequence of PueA was aligned with six other extracellular lipases from *Pseudomonas fluorescens* and *Serratia marcescens*. The PueA amino acid sequence showed a high identity with these lipases (ranging from 58% to 75%).

Two highly conserved regions were observed for PueA and the extracellular lipases (Fig. 1). A putative catalytic domain for serine hydrolases, amino acids 203–211, was observed. The sequence motif, G-H-S-L-G, as well as the location were conserved among all seven polypeptides. In addition, a second conserved region was observed for PueA and the six lipases. A common motif among secreted lipases was observed at amino acids 364–400. The secretion sequence motif, G-G-X-G-X-D-X-X-E was conserved as well as the location. Interestingly, the location of the secretion motif is consistently 163 amino acid residues downstream from the putative catalytic motif for each of the seven polypeptides. The gene encoding the 31 kDa protein has not been isolated, however the *P. chlororaphis* library generated is currently being screened for its presence. In addition, a second PUase gene has been cloned and sequenced. PueB encodes a 60 kDa PUase and also contains a putative catalytic domain for serine hydrolases as well as the secretion sequence motif, G-G-X-G-X-D-X-X-E. Interestingly, *pueA* and *pueB* are contained within an operon similar to that of the ABC protein operon found in *Pseudomonas fluorescens* (Fig. 2). The proposed PUase operon containing PueA and PueB in *P. chlororaphis* appears to be a re-arrangement or an insertional event between the two ABC protein operons observed in *P. fluorescens*.

Growth of *Pseudomonas fluorescens* on PU resulted in values of 0.9 mg ml^{-1} and 1.6 doublings h^{-1} for K_S and μ_{max}, respectively (Howard and Blake, 1999). Two PUase enzymes have been purified and characterized from this bacterial isolate, a 29 kDa protease (Howard and Blake, 1999) and a 48 kDa esterase (Vega et al., 1999). In addition, to the enzymology of the PUases the gene encoding a 48 kDa protein has been cloned and expressed in *E. coli* (Vega et al., 1999). The gene encoding PulA has been sequenced (Genebank, Accession AF144089). The deduced amino acid sequence has 461 amino acid residues and a molecular mass of 49 kDa. The PulA amino acid sequence showed high identity with the six lipases (58–75%) and PueA from

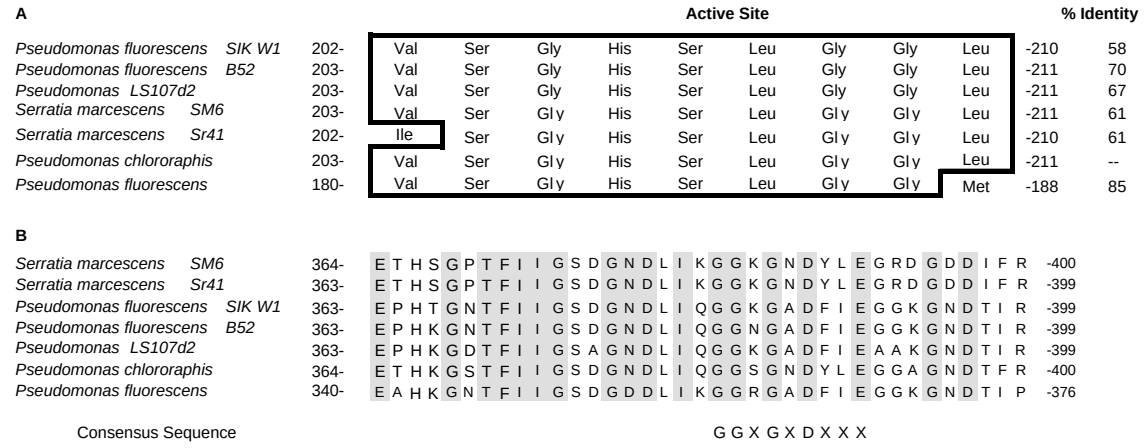


Fig. 1. Comparison of putative active sites and secretion sites for the PueA from *P. chlororaphis* and six extracellular lipases. The numbers indicate the position of the amino acid residues within the protein sequence. Percent identity of complete gene when compared to PueA is indicated. Panel A, Active site region with identical residues boxed. Panel B, Secretion signal region with identical residues shaded.

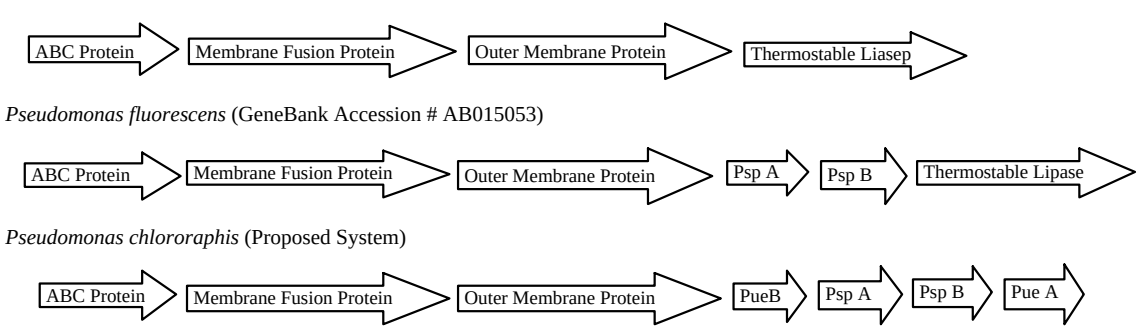


Fig. 2. Comparison of the ABC reporter operons from *Pseudomonas fluorescens* and the PUase operon from *Pseudomonas chlororaphis*. The ABC reporter protein, membrane fusion protein and outer membrane protein are involved in translocation of the extracellular protein. The PspA and PspB proteins are serine protease homologues.

P. chlororaphis (85%). The size of the *pulA* mRNA was determined by Northern blot analysis. A 1500 nucleotide transcript was detected from *P. fluorescens* grown in various carbon sources. The transcript analysis indicates that the *pulA* gene is constitutively expressed. The gene encoding for the 29 kDa protease has not been isolated, however the *P. fluorescens* library generated is currently being screened for its presence.

Since anaerobic degradation of solid waste such as polyurethane is an attractive option both for disposal of waste and bioconversion to potentially useful end-products a range of anaerobic bacterial isolates was selected from our culture collection based on documented proteolytic activity. *Anaerovibrio lipolytica* was also included and was the only isolate known to have lipolytic activity in our culture collection. The isolates tested included *Clostridium perfringens* 3624A and 3624BWT, *Butyrivibrio fibrisolvens* H17c, *Prevotella ruminicola* GA33, *Selenomonas ruminantium* GA192 and *Anaerovibrio lipolytica* 7553. Cultures were grown in a modified rumen fluid containing medium with added carbohydrate and used to inoculate Impranil DLN polyester polyurethane agar plates CRF-M + PU). *Anaerovibrio lipolytica* consistently produced large zones of clearing adjacent to colonies or streaks which were easily visible without staining to the naked eye. The clearings were marked and obvious when viewed on a light box and after Coomassie Blue staining (Howard and Hilliard, 1999). It is highly likely that a range of other lipolytic anaerobic bacteria can be isolated with polyurethane degrading activity.

8. Degradation of polyurethane by polyurethanase enzymes

Enzyme molecules can easily come in contact with water-soluble substrates thus allowing the enzymatic reaction to proceed rapidly. However, the enzyme molecules are thought to have an extremely inefficient contract with insoluble substrates (e.g. PU). In order to overcome this

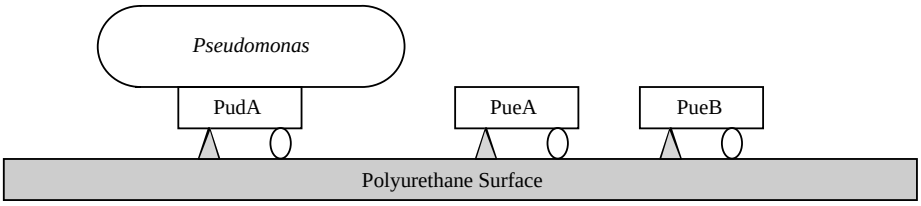


Fig. 3. Putative model of roles for PUase enzymes in degradation of polyurethane. PudA is a cell associated protein. PueA and PueB are extracellular proteins. ▲ represents catalytic domain of PUase, represents substrate binding domain of PUase.

obstacle, enzymes that degrade insoluble substrates possess some characteristic that allow them to adhere onto the surface of the insoluble substrate (van Tilbeurgh et al., 1986; Fukui et al., 1988; Hansen, 1992).

The observations made by Akutsu et al. (1998) for the polyurethanase PudA indicate that this enzyme degrades PU in a two-step reaction: hydrophobic adsorption onto the PU surface followed by the hydrolysis of the ester bonds of PU. The PU esterase was considered to have a hydrophobic-PU-surface binding domain (SBD) and a catalytic domain. The SBD was show to be essential for PU degradation. This structure observed in PudA has also been reported in poly(hydroxyalkanoate) (PHA) depolymerase, which degrades PHA. PHA is an insoluble polyester synthesized as a food reserve in bacteria. In PHA depolymerase enzymes, the hydrophobic SBD has been determined by amino acid sequence analysis and its various physicochemical and biological properties (Fukui et al., 1988; Shinomiya et al., 1997). Another class of enzymes that contain a SBD is cellulases. Several cellulase enzymes have been observed to contain three main structural elements: the hydrolytic domain, a flexible hinge region, and a C-terminus tail region involved in substrate binding (Knowles et al., 1987; Bayer et al., 1985; Langsford et al., 1987).

Thus far, only two types of PUase enzymes have been isolated and characterized: a cell associated, membrane bound PU-esterase (Akutsu et al., 1998) and soluble, extracellular PU-esterases (Ruiz et al., 1999b; Allen et al., 1999; Vega et al., 1999). The two types of PUases seem to have separate roles in PU degradation. The membrane bound PU-esterase would allow cell-mediated contact with the insoluble PU substrate while, the cell-free extracellular PU-esterases would bind to the surface of the PU substrate and subsequent hydrolysis (Fig. 3). Both enzyme actions would be advantageous for the PU-degrading bacteria. The adherence of the bacteria cell to the PU substrate via the PUase would allow for the hydrolysis of the substrate to soluble metabolites which would then be metabolism by the cell. This mechanism of PU degradation would decrease competition between the PU-degrading cell with other cells and also allow for more adequate access to the metabolites. The soluble, extracellular PU-esterase would in turn hydrolyze the polymer into smaller units allowing for metabolism of soluble products and easier access for enzymes to the partially degraded polymer.

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