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ORIGINAL PAPER

# Screening of tropical fungi producing polyethylene terephthalate-hydrolyzing enzyme for fabric modification

**Thidarat Nimchua · Douglas E. Eveleigh · Usa Sangwatanaroj · Hunsa Punnapayak**

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**Abstract** Microfungi were selectively isolated for production of polyethylene terephthalate (PET) fiber-degrading enzymes potentially to be used to modify the surface of polyester fabric. A range of fungi were isolated from plant surfaces and soil samples using a polycaprolactone (PCL) plate-clearing assay technique, and screened for cutinolytic esterase (cutinase) activity. Twenty-two of 115 isolates showed clearing indicating the production of cutinase. The ability of the fungi to produce cutinase in mineral medium (MM) using either potato suberin or PET (1 cm of untreated pre-washed PET fiber) fiber as substrates was assessed based on the hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB). All isolates exhibited activity towards *p*-NPB, isolate PBURU-B5 giving the highest activity with PET fiber as an inducer. PBURU-B5 was identified as *Fusarium solani* based on its conidial morphology and also nucleotide sequencing from internal transcribed spacer region of the ribosomal RNA gene (rDNA-ITS). Enzymatic modification of PET cloth material properties using crude enzyme from strain PBURU-B5 showed hydrolysis of ester bonds of the PET fiber. The modification of the PET fabric resulted in increase of water and moisture absorption, and general enhancement of hydrophilicity of the fabric, properties that could facilitate processing of fabric ranging from easier dyeing while also yielding a softer feeling fabric for the user.

**Keywords** Cutinase · Cutinolytic esterase · *Fusarium* · Polyethylene terephthalate

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## Introduction

Polyethylene terephthalate (PET) is the most widely used polymer for the production of synthetic textile fibers over the past 50 years [14, 17, 21, 28, 33]. Its wide use is a result of its strength, combined with its resistance to chemicals and abrasion, stretching, shrinking, and wrinkling [33]. However, the great disadvantage of the PET fiber is its low hydrophilicity and inactive surface. These affect processing of the fibers such as coloring and also coziness of the fabric to the wearer. The conventional modification of PET fiber properties is through strong alkaline treatment under high processing temperature which concomitantly gives both weight loss and excessively hydrophilic fibers. The alkaline wastes and the high processing temperature invoke environmental concern [14, 29]. An interesting alternative in the surface modification of PET fibers is the application of hydrolytic enzymes which can be accomplished under mild conditions. Hydrolases, esterases, and lipases that act on biopolyesters such as cutin and suberin could have potential in surface modification of PET fiber [5, 6, 13, 29]. Although PET-fabrics were initially considered quite inert,

there has been accumulating evidence for enzymatic modification of PET fibers. Sato [25] found changes in the physical properties of PET fibers including their tensile strength, viscoelasticity, and extension behavior following incubation with an enzyme preparation from *Cladosporium cladosporioides*. Treatments of the polyester fabrics with several lipases improved their water wettability and absorbent properties indicating an increase in hydrophilicity due to enzymatic hydrolysis [11, 12]. Polyester-hydrolyzing bacterial and fungal esterases reduce the pilling properties of PET fabrics [2]. Yoon et al. [33] showed the modification of surface of PET fibers by a serine esterase resulted in enhanced the hydrophilicity, wettability, and cationic dye binding ability of the fibers. The treated PET fabrics showed less pilling and improved oil stain removal properties. The application of *Arthrobacter* and *Trichosporon* enzymes for the modification of PET fibers has also been described [23]. Cutinases (EC 3.1.1.74) from the phytopathogenic fungus, *Fusarium solani* f. sp. *pisi* and a thermophilic actinomycete, *Thermomonospora fusca*, have been successfully used for the modification of PET [21, 29] including their ability to improve the hydrophilic properties and activation of the surface of PET fibers [22].

This range of studies illustrates the proof of concept for the modification of PET fibers, yet new and more efficient enzymes need to be considered for industrial development. Esterase activity per se apparently is not the absolute criterion in this regard and the need for correlation to PET fiber modification is necessary. In this study, we address the isolation further fungal esterases including assessment of their efficiency with regard to PET surface modification.

## Materials and methods

### Screening for PET-hydrolyzing enzymes

Fungi were screened for PET-hydrolyzing enzymes. The sources of fungi were from the surfaces of plants including leaves, flowers, underground storage organs, and fruits, plus soil nearby the roots of these plants. A range of ecologic habitats from habitats in nine Thai provinces were sampled (Table 1). The surface of leaves, flowers, underground storage organs, and fruits were aseptically cut into small pieces (0.5 × 0.5 cm) and placed on selective agar of mineral medium (MM) supplemented with a suspension of polycaprolactone (PCL) 0.5 g/l (MM-PCL) [19]. This cutin analog, PCL (mol. wt. 14,000), was purchased from Sigma-Aldrich Inc. St Louis, MO, USA. For isolation of soil fungi, samples were diluted and spreaded onto MM-PCL plates. All cultures were incubated at 30 °C for 7 days and examined daily for growth and capability to clear the MM-PCL agar plates. The fungi producing clearing zones were isolated and transferred to fresh medium. The active isolates were cultivated in liquid mineral medium (LMM) [19] containing either potato skin suberin 2 g/l, isolated from *Solanum tuberosum* Linn. [30] or PET yarns 2 g/l (1 cm, untreated prewashed 100% PET filament fibers, the kind gift of Dr. Th. Böhme KG, Geretsried, Germany). Inocula were prepared in LMM containing glucose 0.1% (w/v) and incubated at 30 °C for 4 days. These inocula were transferred to the LMM containing suberin or PET fiber, and the cultures incubated at the same temperature up to 21 days. The culture supernatants were assessed for esterase activity daily using *p*-nitrophenyl butyrate (*p*-NPB) as substrate [26]. An enzyme unit (1 U) was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenol per min at pH 7.0 and 25 °C.

### Fungal identification

#### *Morphological observation*

Fungal cultures were grown on potato dextrose agar (PDA) at 30 °C for several days and the colony morphology (color and texture) were examined. Slide cultures were prepared using banana leaf agar (BLA) [27] and stained with lactophenol-cotton blue, and examined by bright-field microscopy (mycelium, mycelium color, septa, and conidia). Isolates were identified to genus level [4] and Fusaria by use of Seifert [27].

#### *Nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing of isolate PBURU-B5*

This isolate produced the most promising enzyme and was focused on. Fresh mycelia of PBURU-B5 grown in potato dextrose broth (PDB) for 5 days (pH 7.0, 30 °C, 150 rpm) were collected by filtration on paper filter under suction and then washed with Tris–HCl buffer (50 mM, pH 7.5). The mycelium was disrupted by grinding in liquid nitrogen in mortar, and genomic DNA was extracted using the CTAB method [24]. The ITS region of the ribosomal DNA (rDNA) was amplified using primers ITS5 (GGAAGTAAAAGTCG TAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) [32]. Amplification reactions were performed using Peltier PTC-100$^{TM}$ Thermal Cycler (MJ ResearchTM Inc. MA, USA) in a total volume of 100 µl containing 10 mM Tris–HCl buffer, 50 mM KCl, 2.5 mM $MgCl_2$, 12.5 pmol each dNTP, 50 pmol each primer, 2 U Taq polymerase, 4 µl of undiluted genomic DNA, and 55.5 µl of sterile distilled water. The amplification conditions included a hot start for 6 min at 80 °C and a denaturation step for 2 min at 95 °C, followed by primer annealing for 30 s at 56 °C and primer extension for 1 min at 72 °C. After 35 cycles, an extension step was followed at 72 °C for 10 min. Successful PCR prod-

**Table 1** Fungal isolates attacking polycaprolactone (PCL): sources and collection sites

| Province | Description of collection site | | | | Source | Fungal strain |
|---|---|---|---|---|---|---|
| | Mean temp. (°C) | Elevation (m) | Collection date | Area | | |
| Bangkok | 32.0 | 11 | March 2005 | Shady lowland | Banana (*Musa sapientum* Linn.) leaf | *Fusarium* sp. PBURU-B1 |
| | | | | | Potato (*Solanum tuberosum* Linn.) peel | *Fusarium* sp. PBURU-B2 |
| | | | | | Tomato (*Lycopersicon esculentum*) peel | *Fusarium* sp. PBURU-B19 |
| | | | | | Soil near by root of banana (*Musa sapientum* Linn.) | *Penicillium* sp. PBURU-B13 |
| | | | | | Soil near by root of tomato (*Lycopersicon esculentum*) | *Fusarium* sp. PBURU-B7, *Fusarium* sp. PBURU-B8 |
| Chiangmai | 20.2 | 1,100 | June 2005 | Open highland | Soil near by root of fairy rose (*Rosa chinensis*) | *Aspergillus* sp. PBURU-B10 |
| | | | | | Water Lily (*Nymphaea stellata* Wild.) leaf | *Fusarium solani* PBURU-B5 |
| | | | | | Soil near by root of lychee tree (*Litchi chinensis* Sonn.) | *Aspergillus* sp. PBURU-B3 |
| Lampang | 28.0 | 262 | September 2005 | Shady lowland | Soil near by root of mango tree (*Mangifera indica* Linn.) | *Fusarium* sp. PBURU-B16, *Fusarium* sp. PBURU-B22 |
| Pathumthani | 29.7 | 11 | August 2005 | Shady lowland | Burma Padauk (*Pterocarpus indicus*) bark | *Fusarium* sp. PBURU-B11 |
| | | | | | Bodhi tree (*Ficus religiosa* Linn.) bark | *Fusarium* sp. PBURU-B15 |
| | | | | | Soil near by root of dwarf date palm (*Phoenix loureri* Kunth.) | *Fusarium* sp. PBURU-B20 |
| Prachinburi | 30.7 | 17 | June 2005 | Shady lowland | Soil near by root of jack fruit (*Artocarpus heterophyllus* Lam.) | *Aspergillus* sp. PBURU-B9 |
| Rayong | 29.3 | 66 | August 2005 | Shady lowland | Soil near by root of fan palm tree (*Licuala grandis* H. Wendl.) | *Aspergillus* sp. *PBURU-B17, Fusarium* sp. PBURU-B4, *Fusarium* sp. PBURU-B12 |
| | | | | | Soil near by root of mango tree (*Mangifera indica* Linn.) | *Fusarium* sp. PBURU-B18, *Penicillium* sp. PBURU-B6 |
| | | | | | Soil near by root of lychee tree (*Litchi chinensis* Sonn.) | *Fusarium* sp. PBURU-B21 |
| Samutsakorn | 27.0 | 0.5 | August 2005 | Muddy shore | Mangrove (*Rhizophora apiculata* Blume) leaf | *Fusarium* sp. PBURU-B14 |

ucts were cleaned of primers and salts, using the QIAquick PCR Purification Kit (QIAGEN Inc. Valencia, CA, USA). ABI PRISM® 3100 BigDye Terminators v3.0 Cycle Sequencing reactions (Applied Biosystems, Foster City, CA, USA) were prepared according to the manufacturer's protocol, using primers ITS5 and ITS4 and the PCR product as templates [32]. Sequences were analyzed on an ABI PRIAM® 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

### Preparation of crude enzyme from PBURU-B5

Crude extracellular PET-hydrolyzing enzyme was prepared by culturing isolate PBURU-B5 in production medium (mineral medium containing 0.2% (w/v) suberin, pH 7.0) in two litre shake flasks (500 ml working volume, 10 flasks) with incubation on rotary shaker at 150 rpm (30 °C). After 21 days, culture supernatant was recovered following filtration through filter paper under suction. The residual materials, suberin, and mycelia (352 g wet weight), were also extracted by stirring in 0.1% Triton X-100 in one liter of 50 mM phosphate buffer (pH 7.0) at 4 °C overnight [8]. The insoluble residues were again removed by filtration. These culture supernatant (4,640 ml) and Triton X-100 wash (974 ml) were pooled and then lyophilized. This crude enzyme was dissolved in 200 ml 50 mM phosphate buffer (pH 7.0) and concentrated by ammonium sulfate precipitation (50–80% saturation), followed by removal of the salt by dialysis. This crude enzyme was used for further study. The enzyme activity was determined using *p*-NPB as described above. Protein concentration was determined using the Bradford assay [3].

### Determination of enzymatic effects towards the PET materials

#### *Measurement of PET-hydrolyzing activity*

The terephthalic acids (TPA), PET monomers, released from PET fibers were measured following incubation of PET yarn (0.2 g, in 100 ml 50 mM phosphate buffer, pH 7.0) with the enzyme preparation (20, 40, or 80 U). Reaction mixtures were incubated at 30 °C and sampled periodically up to 168 h, followed by analysis via absorbancy at 240 nm [33]. Controls included mixtures lacking enzyme and also inactivated autoclaved enzyme. All assays were minimally performed in duplicate.

#### *Measurement of water absorption of the enzyme treated PET fabrics*

The effect of the enzyme on modification in the water adsorption ability of PET fabrics was determined using the rising height test following a standardized method (DIN 53924). The PET fabrics (weave, warp, and weft: 167 dtex, 192 g/m$^2$) were a kind gift of Suthep Permpornsakul from Asia Fiber Public Company, Bangkok, Thailand. The swatches (25 × 3 cm) were incubated with enzyme preparations (20, 40, and 80 U) in 100 ml of 50 mM phosphate buffer, pH 7.0 at 30 °C on a rotary shaker at 125 rpm for up to 168 h. Controls were performed without enzymes and treated with the same buffers. After the treatment, residual proteins on the fabric surface were removed by rinsing with sodium carbonate solution 2 g/L, pH 7.0 at 70 °C on a rotary shaker at 125 rpm for 30 min [22]. The fabrics were washed several times with distilled water and dried at room temperature. The rising height of water in treated PET fabrics was measured.

#### *Measurement of moisture content of enzyme treated PET fabrics*

Enhanced hydrophilicity of treated PET fabrics was demonstrated by measuring the changes of moisture content of fabrics before and after treatment with the enzyme. The swatches (10 × 10 cm) were pre-treated with enzyme preparations (20, 40, and 80 U) as described above. The moisture content of enzyme treated-fabrics was determined using an AD 4715 infrared moisture determination balance (A&D weight, Bradford, MA, USA) for 5 min. All analyzes were performed five times.

## Results and discussion

A diverse variety of plant surfaces and soil from a range of environmental niches yielded 22 fungi out of 115 isolates that were capable of degrading PCL, as indicated by zone of clearing on MM-PCL agar plates (Fig. 1). Such zones could be easily observed after 3–5 days of incubation period. This diversity is not remarkable in that cutin is abundant and is widespread occurring on the surfaces of all aerial parts of plants (stems, petioles, leaves, flower parts, fruits, and some seed coats), but also on internal parts such as juice sacks of citrus [15]. The total amounts are considerable. In higher plant leaves, cutin ranges from 20 to 600 μg/cm$^2$ of the surface area, while in some fruits with a well developed cuticle, the cutin content may reach 1.5 mg/cm$^2$ [15]. Thus with the ubiquity of cutin, it was not surprising that cutin degraders were generally isolated from different aerial parts of plants (fruit peel, leaf, and bark) and also soil nearby roots, and all of which produced cutinolytic esterase (Table 1). In analogous interpretation in that cutin is widely prevalent, it was not remarkable to find PET degrading fungi from low (0.5 m, Samutsakorn Province) and high (1,100 m, Chiangmai

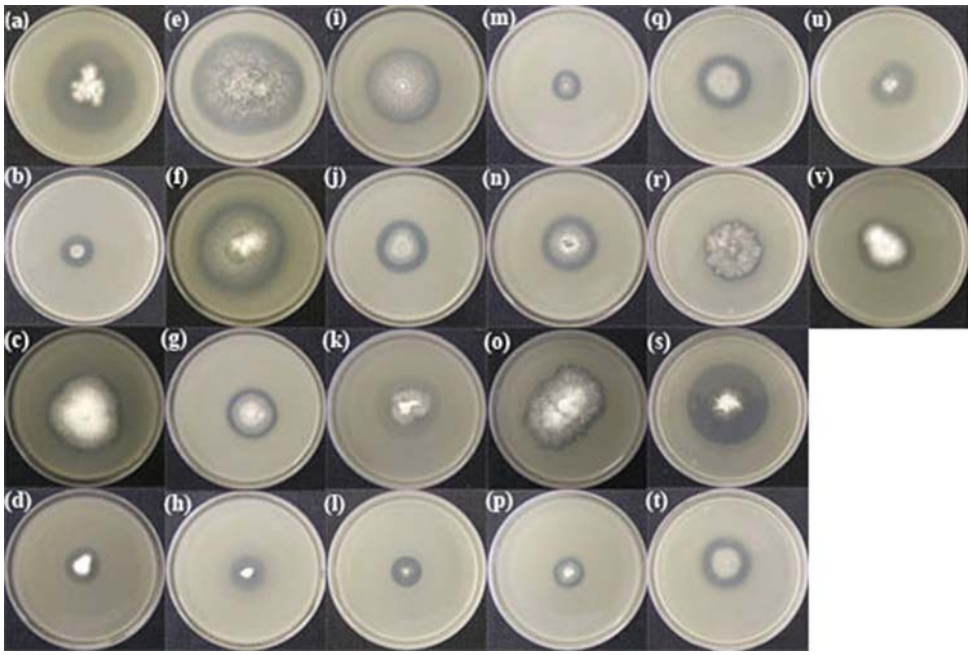


**Fig. 1** The esterase activity of the fungal isolates (All isolates were *Fusarium* spp. apart from the *Aspergillus* spp. PBURU-B3, PBURU-B9, PBURU-B10, and PBURU-B17, and *Penicillium* spp. PBURU-B6 and PBURU-B13. See Table 1 for the origin of the isolates.) towards PCL as evidenced by clearing zones on polycaprolactone plates. **a** PBURU-B1, **b** PBURU-B2, **c** PBRU-B3, **d** PBURU-B4, **e** PBURU-B5, **f** PBURU-B6, **g** PBURU-B7, **h** PBURU-B8, **i** PBURU-B9, **j** PBURU-B10, **k** PBURU-B11, **l** PBURU-B12, **m** PBURU-B13, **n** PBURU-B14, **o** PBURU-B15, **p** PBURU-B16, **q** PBURU-B17, **r** PBURU-B18, **s** PBURU-B19, **t** PBURU-B20, **u** PBURU-B21, and **v** PBURU-B22

Province) altitudes, at ranges from 20.2 (Chaingmai) to 32.0 °C (Bangkok), and from seven provinces. The sampling collections included the rainy season (June, July, August and September) and the dry season (March). Noting that this is a limited survey, what was apparently remarkable was the dominance of *Fusarium* cultures, 16 of 22 isolates, perhaps reflecting the common phyllosphere status of Fusaria and the selective isolation technique. A few of the more generally occurring *Aspergillus* and *Penicillium* species were isolated from soil and found to attack PCL.

Examination by bright field light microscopy of the morphology of PBURU-B5 grown on BLA for 5 days showed typical Fusaria characteristics [4, 27]. It produced both macro and microconidia (Fig. 2). Macroconidia are hyaline and canoe-shaped in side view. Microconidia were ellipsoidal, produced from long monophialides in aerial mycelium. Chlamydospore usually occurred singly or in pairs. The colony grew to 5 cm diameter in 4 days and was whitish (Fig. 2). It was apparently *F. solani.*

The sequence of the ITS region of rDNA of PBURU-B5 yielded a sequence to *F. solani* sequences in Genbank,

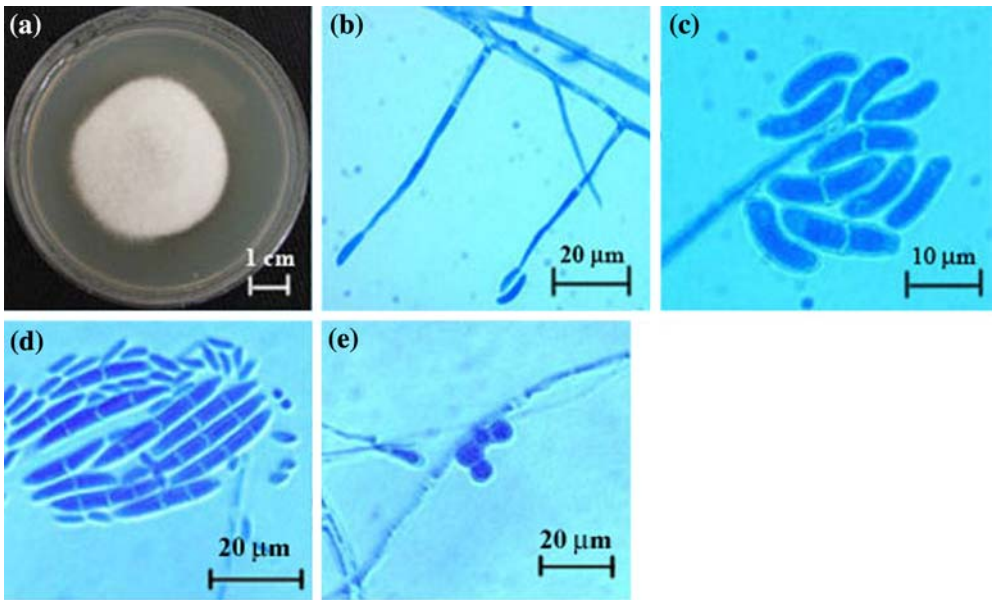


**Fig. 2** Colony and morphology of *Fusarium solani* PBURU-B5. **a** Colony (5 cm in diameter) grown on PDA for 4 days at 30 °C. **b** Septate hyphae and conidia. **c** Ellipsoidal-shaped microconidia. **d** Canoe-shaped macroconidia. **e** Chlamydospores

**Table 2** Maximum esterase yields from the polycaprolactone attacking isolates

| Isolate | Maximum esterase activity (mU/ml)[a] | |
|---|---|---|
| | Suberin powder | PET fiber |
| *Fusarium* sp. PBURU-B1 | 76 | 3 |
| *Fusarium* sp. PBURU-B2 | 36 | 6 |
| *Aspergillus* sp. PBURU-B3 | 20 | 5 |
| *Fusarium* sp. PBURU-B4 | 20 | 7 |
| *Fusarium* sp. PBURU-B5 | 308 | 61 |
| *Penicillium* sp. PBURU-B6 | 21 | 8 |
| *Fusarium* sp. PBURU-B7 | 23 | 8 |
| *Fusarium* sp. PBURU-B8 | 494 | 6 |
| *Aspergillus* sp. PBURU-B9 | 99 | 4 |
| *Aspergillus* sp. PBURU-B10 | 132 | 5 |
| *Fusarium* sp. PBURU-B11 | 552 | 5 |
| *Fusarium* sp. PBURU-B12 | 29 | 9 |
| *Penicillium* sp. PBURU-B13 | 84 | 5 |
| *Fusarium* sp. PBURU-B14 | 11 | 11 |
| *Fusarium* sp. PBURU-B15 | 527 | 3 |
| *Fusarium* sp. PBURU-B16 | 353 | 3 |
| *Aspergillus* sp. PBURU-B17 | 25 | 7 |
| *Fusarium* sp. PBURU-B18 | 135 | 4 |
| *Fusarium* sp. PBURU-B19 | 1,126 | 4 |
| *Fusarium* sp. PBURU-B20 | 777 | 5 |
| *Fusarium* sp. PBURU-B21 | 20 | 5 |
| *Fusarium* sp. PBURU-B22 | 1,053 | 3 |

Enzymes from culture supernatants of isolates grown in liquid mineral medium containing either suberin powder or PET fiber as sole carbon source (see text)

[a] Determined with *p*-nitrophenyl butyrate as substrate—the extinction coefficient of *p*-nitrophenol = $1.84 \times 10^4\ M^{-1}\ cm^{-1}$

including the following: NRRL 22354 (AF178402), NRRL 32817 (DQ094583), NRRL 232756 (DQ094535), FRC# s1592 (DQ094746), FRC#s669 (DQ094765), FRC#s1223 (DQ094767), FRC#s752 (DQ094725) and FRC#s617 (DQ094694). On this basis isolate PBURU-B5 is identified as *F. solani* and its ITS sequence was submitted to GenBank—accession number EU008940. In an earlier initial study, identification based on its morphology alone, suggested it to be *F. oxysporum* [22]. The current study is in greater detail both as regards identification and also total enzymology.

There was no obvious correlation between esterase activities produced by the fungi and sizes of clearing zones (Fig. 1 and Table 2). However, the size of the clearing zone includes the amount of enzyme, its release, and also the rate of growth of the fungus. Such a correlation between enzyme yield and clearing zone size is further obscured in that some enzyme apparently remains mycelial bound (Table 3). Further enzyme was released following Triton X-100 treatment.

The use of PCL as a selective isolation substrate merits comment. Cutinase, a serine esterase, secreted by many phytopathogenic fungi including *F. solani* f. sp. *pisi* [19], can have low substrate specificity. Certain cutinases hydrolyze cutin and are also capable of degrading PET [18, 29]. Cutinase is induced by cutin or suberin [19], and can be repressed by glucose [18]. On these general bases, PCL hydrolysis was used as an initial screen to find fungal isolates producing enzyme that may be active on aromatic synthetic polyesters such as PET.

All isolates when cultivated on suberin produced esterases with maximum activities ranging from 11 (PBURU-B14) to 1,126 mU/ml (PBURU-B19) (Table 2). However, it is noteworthy that only one fungus (*F. solani* PBURU-B5) was able to grow to any reasonable extent on PET fibers with activity (61 mU/ml) after 21 days growth (Table 2).

The apparent yields released to the extracellular fluid appear only half of total esterase activity (Table 3) in part reflecting that the enzyme can bind to the cell and/or the residual suberin in the medium. Such bound enzyme was

**Table 3** Purification of *Fusarium solani* PBURU-B5 cutinolytic esterase

| Preparation step | Volume (ml) | Esterase activity (mU/ml) | Total activity (U) | Protein concentration (μg/ml) | Total protein (μg) | Specific activity (U/mg) | Yield (%) |
|---|---|---|---|---|---|---|---|
| Extracellular fluid | 4,640 | 160 | 742 | 108 | 501 | 1.48 | 50 |
| Triton X-100 wash | 974 | 758 | 738 | 276 | 269 | 2.75 | 50 |
| Crude extract[a] (extracellular fluid and Triton X-100 washed solution) | 5,614 | 265 | 1,488 | 134 | 752 | 1.98 | 100 |
| Freeze dried and dissolved in buffer | 200 | 6,543 | 1,309 | 320 | 64 | 20.45 | 88 |
| Ammonium sulfate precipitation (50-80%) | 51 | 17,489 | 892 | 515 | 26 | 33.96 | 60 |

[a] The extracellular fluid (4,640 ml) contained secreted cutinase which was about 50% of total esterase. The enzyme that was adsorbed to mycelium and suberin was recovered from Triton X-100 washing step was also about 50%. Extracellular fluid and Triton X-100 extract were pooled, the enzyme recovered by ammonium sulfate precipitation

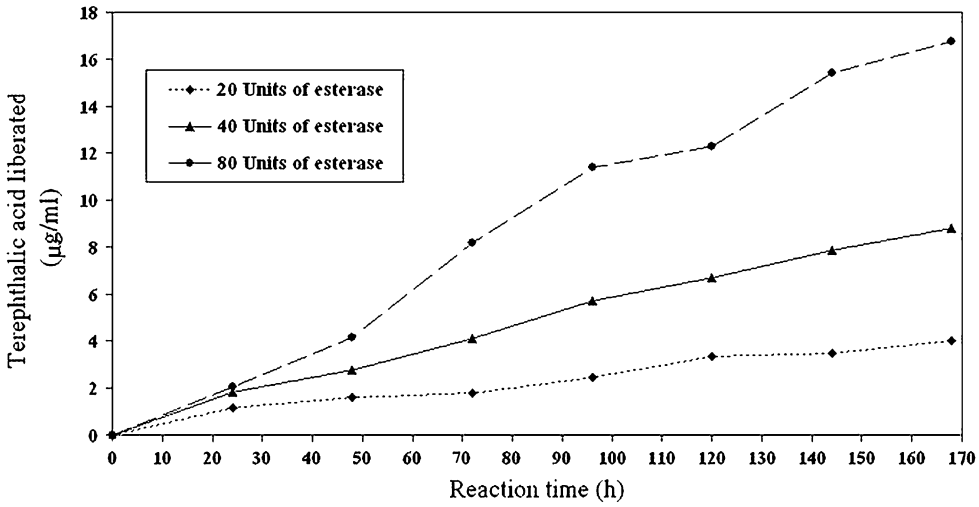


**Fig. 3** *Fusarium solani* PBURU-B5 esterase activity towards PET fibers assessed by terephthalic acid release

readily recovered by treatment with buffer containing Triton X-100 [8]. Recovery enzyme using ammonium sulfate precipitation (50–80% saturation) was yielded almost 60% of total activity and with 17-fold of specific activity from crude extract (Table 2). This crude enzyme was used for further studies. However, esterase activity obtained from culture extract of PBURU-B5 (0.308 U/ml) was average compared to the other isolates, the range being from 0.011 to 1.126 U/ml (Table 2). The yield from PBURU-B5 of 0.3 U/ml is reasonable compared to other microbes. For instance out of 232 gram-positive and gram-negative bacterial isolates, only *Pseudomonas aeruginosa* (10 isolates) produced more than 0.5 U/ml esterase [7]. A *Botrytis cinerea* strain yielded 1.2 U/ml cutinase [10] and that from *Monilinia fructicola* showed 8.92 U/ml of esterase activity [31]. Other fungi give lower yields, for example *Venturia inaequalis* only 0.12 U/ml [16]. The *F. solani* PBURU-B5 yields are reasonable and have yet to be optimized.

The PBURU-B5 enzyme is active towards PET fiber in proportional manner of a result of significance with such insoluble substrates as fabric (Fig. 3). The moisture content of enzyme treated PET fabrics was also increased (Fig. 4). The relative percentage moisture increases were in proportion to the amount of enzyme applied. The enzyme treatment clearly decreases the hydrophobicity of the PET fabric (Table 4, Fig. 4). The increased water and moisture absorption by treated PET fabrics are analogous to those obtained with microbial enzymes from *Aspergillus* sp. strain St 5 [9] and *Thermomonospora fusca* [1].

Aromatic polyesters have previously been considered as very resistant to degradation by hydrolytic enzymes [20]. However, the current results add to the accumulating evidence that esterases and cutinases from various fungi and bacteria can hydrolyze ester bonds in PET [21, 22, 33]. The screening using PCL was useful in selecting a variety of

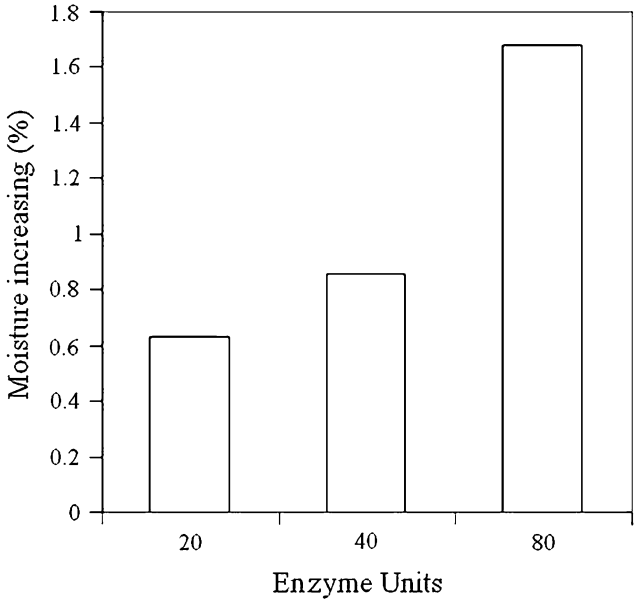


**Fig. 4** Increased hydrophilicity of PET fabric treated with *Fusarium solani* PBURU-B5 esterase (as increased moisture content—details see text)

**Table 4** Increased water absorption of PET fabric in relation to *Fusarium solani* PBURU-B5 esterase treatment

| Treatment | Rising height of water (mm/10 min)[a] |
|---|---|
| Control | 10 |
| Inactivated enzyme | 12 |
| 20 U esterase | 25 |
| 40 U esterase | 37 |
| 80 U esterase | 60 |

[a] Assay—see text

fungi producing esterases. However, in that only one strain produced an enzyme with major activity towards PET fiber, a caution is raised in using this substrate in an absolute manner. That does not detract from the isolation of a truly positive strain of *F. solani*. The overall results support the concept of the enzymatic modification of aromatic synthetic polyesters and potential use in textile finishing processes.

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## References

1. Alisch-Mark M, Herrmann A, Zimmermann W (2006) Increase of the hydrophilicity of the polyethylene terephthalate fibres by hydrolases from *Thermomonospora fusca* and *Fusarium solani* f. sp. *pisi*. Biotechnol Lett 28:681–685
2. Anderson B, Borch K, Abo M, Damgaard B (1999) A method for treating polyester fabrics. PCT Patent WO9901604
3. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254
4. Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi, 4th edn. APS Press, Minnesota, pp 6–56
5. Carvalho CML, Aires-Barros MR, Cabral JMS (1998) Cutinase structure, function and biocatalytic applications. Electron J Biotechnol 1(3):160–173
6. Carvalho CML, Aires-Barros MR, Cabral JMS (1999) Cutinase: from molecular level to bioprocess development. Biotech and Bioeng 66(1):17–34
7. Fett WF, Gerard HC, Moreau RA, Osman SF, Jones LE (1992) Screening of nonfilamentous bacteria for production of cutin-degrading enzymes. Appl Environm Microbiol 58:2123–2130
8. Fernando G, Zimmermann W, Kolattukudy PE (1984) Suberin-grown *Fusarium solani* f. *pisi* generates a cutinase-like esterase which depolymerizes the aliphatic components of suberin. Physiol Plant Pathol 24:143–155
9. Fischer-Colbrie G, Heumann S, Liebminger S, Almansa A, Cavaco-Paulo A, Guebitz G (2004) New enzymes with potential for PET surface modification. Biocatal Biotransformation 22:341–346
10. Gindro K, Pezet R (1999) Purification and characterization of a 40.8-kDa cutinase in ungerminated conidia of *Botrytis cinerea* Pers.: Fr. FEMS Microbiol Lett 171:239–243
11. Hsieh YL, Hartzell MM, Boston MG, Clarkson KA, Collier KD, Graycar TP, Larenas EA (1997) Enzyme treatment to enhance wettability and absorbancy of textiles. PCT Patent WO9733001
12. Hsieh YL, Cram LA (1998) Enzymatic hydrolysis to improve wetting and absorbancy of polyester fabrics. Textile Res J 68:311–319
13. Khoddami A, Morshed M, Tavani H (2001) Effects of enzymatic hydrolysis on drawn polyester filament yarns. Iran Polym J 10(6):363–370
14. Kim DY, Rhee YH (2003) Biodegradation of microbial and synthetic polyesters by fungi. Appl Microbiol Biotechnol 61:300–308
15. Kolattukudy PE (2001) Cutin from plants. Biopolymers 3a. Wiley-VCH, Weinheim, pp 1–40
16. Köller W, Parker DM (1989) Purification and characterization of cutinase from *Venturia inaequalis*. Phytopathology 79:278–283
17. Lewin M, Pearce EM (1998) Handbook of fiber chemistry, 2nd edn. Marcel Dekker, New York, pp 2–13
18. Lin TS, Kolattukudy PE (1978) Induction of biopolyester hydrolase (cutinase) by low levels of cutin monomers in *Fusarium solani* f. sp. *pisi*. J Bacteriol 133:942–951
19. Murphy CA, Cameron JA, Huang SJ, Vinopal RT (1996) *Fusarium* polycaprolactone depolymerase is cutinase. Appl Microbiol Biotechnol 62(2):456–460
20. Müller RJ, Kleeberg I, Deckwer WD (2001) Biodegradation of polyesters containing aromatic constituents. J Biotechnol 86:87–95
21. Müller RJ, Schrader H, Profe J, Dresler K, Deckwer WD (2005) Enzymatic degradation of poly (ethylene terephthalate): rapid hydrolyse using a hydrolase from *T.* fusca. Macromol Rapid Commun 26:1400–1405
22. Nimchua T, Punnapayak H, Zimmermann W (2007) Comparison of the hydrolysis of polyethylene terephthalate fibers by a hydrolase from *Fusarium oxysporum* LCH I and *Fusarium solani* f. sp. *pisi*. Biotechnol J 2:361–364
23. Oda K, Kimura Y (1998) Method for decomposing polyesters containing aromatic moieties, a dernier reduction method of fiber, and microorganisms having activity of decomposing the polyester. Jap Patent JP19980319251
24. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018
25. Sato M (1983) Deterioration of filaments and films of polyethylene terephthalate with enzyme of *Cladosporium cladosporioides*. FERM J–8, Sen. I Gakkaishi 39:67–77
26. Sebastian J, Chandra AK, Kolattukudy PE (1987) Discovery of a cutinase-producing *Pseudomonas* sp. cohabiting with an apparently nitrogen-fixing Corynebacterium sp. in the phylloplane. J Bacteriol 169:131–136
27. Seifert K (1996) Fuskey: *Fusarium* interactive key. Agriculture and Agri-Food Canada
28. Shimado M (2001) Biodegradation of plastics. Curr Opin Biotechnol 12:242–247
29. Vertommen MAME, Nierstrasz VA, VanderVeer M, Varmoeskerken MMCG (2005) Enzymatic surface modification of poly (ethylene terephthalate). J Biotechnol 120:376–386
30. Walton TJ, Kolattukudy PE (1972) Determination of structures of cutin monomers by a novel depolymerization procedure and combined gas chromatography and mass spectroscopy. Biochemistry 11:1885–1897
31. Wang GY, Michailides TJ, Hammock BD, Lee YM, Bostock RM (2000) Affinity purification and characterization of a cutinase from the fungal plant pathogen *Monilinia fructicola* (Wint.) Honey. Arch Biochem Biophys 381:31–38
32. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322
33. Yoon MY, Kellis J, Poulose AJ (2002) Enzymatic modification of polyester. AATCC Rev 2:33–36