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EXPERIMENTAL ARTICLES

Isolation and Characterization of Polyester-Based Plastics-Degrading Bacteria from Compost Soils[1]

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Abstract—Four potential polyester-degrading bacterial strains were isolated from compost soils in Thailand. These bacteria exhibited strong degradation activity for polyester biodegradable plastics, such as polylactic acid (PLA), polycaprolactone (PCL), poly-(butylene succinate) (PBS) and polybutylene succinate-*co*-adipate (PBSA) as substrates. The strains, classified according to phenotypic characteristics and 16S rDNA sequence, belonging to the genera *Actinomadura*, *Streptomyces* and *Laceyella*, demonstrated the best polyester-degrading activities. All strains utilized polyesters as a carbon source, and yeast extract with ammonium sulphate was utilized as a nitrogen source for enzyme production. Optimization for polyester-degrading enzyme production by *Actinomadura* sp. S14, *Actinomadura* sp. TF1, *Streptomyces* sp. APL3 and *Laceyella* sp. TP4 revealed the highest polyester-degrading activity in culture broth when 1% (w/v) PCL (18 U/mL), 0.5% (w/v) PLA (22.3 U/mL), 1% (w/v) PBS (19.4 U/mL) and 0.5% (w/v) PBSA (6.3 U/mL) were used as carbon sources, respectively. All strains exhibited the highest depolymerase activities between pH 6.0–8.0 and temperature 40–60°C. Partial nucleotides of the polyester depolymerase gene from strain S14, TF1 and APL3 were studied. We determined the amino acids making up the depolymerase enzymes had a highly conserved pentapeptide catalytic triad (Gly-His-Ser-Met-Gly), which has been shown to be part of the esterase-lipase superfamily (serine hydrolase).

Keywords: Actinomyces, depolymerase, degradation, polyester, thermophilic bacteria

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As the global population has increased, so too has the accumulation of non-biodegradable waste. Much of this waste comes from plastics used for daily living. Plastic accumulation in the environment leads to both environmental and economic problems (Shimao, 2001). Much research has been dedicated to finding new products that are biodegradable and will not accumulate in the environment (Luengo et al., 2003). In recent years, many researchers have studied biodegradable plastic production for use in packaging applications. Biodegradable plastics do not deleteriously effect the environment and are naturally broken down by enzymes endemic to local microorganisms, producing water, carbon dioxide, methane and biomass as by-product of degradation (Mueller, 2006).

Some types of biodegradable plastics, such as polylactic acid (PLA), poly-L-lactide (PLLA), polycaprolactone (PCL), poly-(butylene succinate) (PBS) and polybutylene succinate-*co*-adipate (PBSA), are particularly promising materials that are commercially available synthetic polyesters. Microorganisms can act on these substances by secreting polyester-degrading enzymes (Shinozaki et al., 2013). Many countries have initiated production of polyester plastics, particularly PBS and PBSA, because PBS is aliphatic polyester that is degraded similarly to and shares properties with polyethylene terephthalate (PET).

Many bacteria in the genera *Bacillus* and *Actinomyces* have the ability to degrade emulsified or powdered plastics or thin films of polyester-based plastics (Hu et al., 2008). Some information on microorganisms that degrade polymers has been reported, but bioplastic-degrading enzymes have not been extensively studied. Previous studies have been reported that depolymerase enzymes such as lipase, esterase and protease, have the ability to degrade polyester-based plastics (Akutsu-Shigeno et al., 2003). Thus, in this study, we have isolated thermophilic bacteria from compost and landfill soils in Thailand and characterized the bioplastic-degrading enzymes found therein.

[1] The article is published in the original.

MATERIALS AND METHODS

Substrates and chemicals. PLA (M_n 5.4 × 10^3), PCL (M_n 1.0 × 10^5), PBS and *p*-nitrophenyl (pNP)-substrates were obtained from Sigma-Aldrich Chemical (USA). PLLA (M_n 1.69 × 10^5) and PBSA (M_n 1.0 × 10^5) were supplied from Prof. Dr. Fusako Kawai at Kyoto Institute of Technology (KIT), Kyoto, Japan. All other chemicals used were of the highest grade available.

Screening of polyester-degrading microorganisms. Seventeen soil samples were collected from composts and landfill soils in Thailand. The depth range of all soil samples was approximately 5–10 cm. All samples were collected in sterile 50 mL tubes and kept at 4°C until used to screen for polyester (PCL, PLA, PLLA, PBS and PBSA)-degrading thermophilic bacteria. Soil samples (1 g each) were suspended in 9 mL sterile distilled water and then serially diluted. 0.1 mL aliquots of 10^0–10^{-4} diluted suspensions were spread on emulsified polyester agar plates and incubated at 50°C for 10 days. Plates were prepared as follows: 1 g of each polyester pellet was dissolved in 20 mL dichloromethane. The solution was emulsified with an ultrasonicator into 1 L of basal medium composed of 200 mg yeast extract, 1 g $(NH_4)_2SO_4$, 200 mg $MgSO_4 \cdot 7H_2O$, 100 mg NaCl, 20 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeSO_4 \cdot 7H_2O$, 0.5 mg $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg $Na_2WO_4 \cdot 2H_2O$, 0.5 mg $MnSO_4$, 1.6 g K_2HPO_4, 200 mg KH_2PO_4 and 20 g agar at pH 7, modified from the method described by Nishida and Tokiwa (Nishida and Tokiwa, 1993). Dichloromethane was removed by incubation at 50°C for 30 min. The colonies that formed clear zones on the plates were selected as polyester-based plastic degrading strains.

Identification of polyester-degrading strains. The polyester-based plastic degrading strains were grown in low salt Luria-Bertani (LB_{low}, 10 g/L peptone, 5 g/L NaCl and 5 g/L yeast extraction) broth. All liquid cultures were aerated at 37°C for 3–4 days and harvested by centrifugation. Bacteria genomic DNA was extracted using a modified method by Kieser (Kieser et al., 2000). The 16S rDNA sequence of each isolate was amplified using universal oligonucleotide primers, 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GGTTACCTTGTTACGACTT-3' described by Lane (Lane, 1991). Preliminary sequences obtained in this study were manually compared with 16S rDNA sequences from GenBank using the Blast database on the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple alignments of the sequences obtained were carried out with the program CLUSTAL X version 1.81 (Thompson et al., 1997). A phylogenetic tree was constructed according to the neighbour-joining method of Saitou and Nei (Saitou and Nei, 1987) using the MEGA 5.1 program (Tamura et al., 2011).

Morphological and physiological characteristics of polyester-degrading actinomycetes. Actinomycetes were characterized based on the colour of colony produced, as well as the degree of growth and pigmentation, after growth at 37°C for 21 days using yeast-malt extract agar (ISP-2), oatmeal agar (ISP-3), inorganic salt-starch agar (ISP-4) and glycerol-asparagine agar (ISP-5) as the media of the International *Streptomyces* Project (ISP) described by Shirling and Gottlieb (Shirling and Gottlieb, 1966). Morphological structures were studied between 10–14 days of incubation using scanning electron microscopy on ISP-3 growth medium. Physiological characterization tests, such as growth temperatures and salt tolerace (NaCl final concentration from 2 to 10% w/v), were assessed using ISP-2 medium. Carbon utilization medium (ISP-9), modified from Pridham and Gottlieb medium (Pridham and Gottlieb, 1948) was used to investigate the ability of the strains to use different carbon sources. Each carbon source was added to ISP-9 medium at 1% (w/v) final concentration. The isomers of diaminopimelic acid (DAP) in the cell wall were determined as described by Staneck and Roberts (Staneck and Roberts, 1994). The strains were examined for the decomposition of 1% (w/v) of xylan, avicel, carboxymethylcellulose (CMC), tributyrin and skim milk using 1 L actinomyces agar containing 1.5 g KH_2PO_4, 2 g K_2HPO_4, 1.4 g $(NH_4)_2SO_4$, 2 g yeast extract, 1 g peptone, 2 mL Tween 80, 20 g agar and 1 mL of trace element solution composed of 140 mg/L of $ZnSO_4 \cdot 7H_2O$, 160 mg/L of $MnSO_4 \cdot 5H_2O$, 500 mg/L of $FeSO_4 \cdot 7H_2O$ and 200 mg/L of $CoCl_2 \cdot 6H_2O$ in distilled water. The pH was adjusted to 6.0 and supplemented with substrates as described by Techapun et al. (Techapun et al., 2002).

Optimization for polyester depolymerase production. Different culture conditions for maximum production of depolymerase enzymes were tested using various temperature, pH, carbon sources, nitrogen sources and polyester concentration in basal medium. Each polyester emulsion basal medium with 0.1% (w/v) final concentration was used as enzyme production medium. All experiments were performed in a 250 mL flask containing 20 mL of basal medium. The inoculated medium was incubated with rotary shaking at 150 rpm for 5 days. The culture medium was centrifuged at 11,000 *g* for 10 min to obtain the crude enzyme extract and used for analyzing enzyme activity. Protein concentrations were determined using the Bradford method (Bradford, 1976).

Effects of pH on enzyme activity in the crude enzyme were assayed using pH ranges of 6.0–8.0 in 100 mM Tris–HCl buffer. Effects of temperature were carried out at 40, 50, 60 and 70°C in 100 mM Tris–HCl buffer at pH 8.0. Effects of carbon source on enzyme production were measured using various carbon sources at 0.1% (w/v): sucrose, glucose, fructose, xylose, PCL, PLA, PBS and PBSA. Effects of nitro-

gen source were measured using yeast extract, ammonium sulphate, casein, gelatin and yeast extract with ammonium sulphate. To measure effects of inducer substrate concentration, flasks containing basal medium and different concentrations of polyesters (0.1, 0.5, 1, 1.5, and 2% (w/v)) were prepared at pH 8.0 and inoculated with crude enzyme extract followed by incubation at 50°C for 60 min.

Depolymerase enzyme assay. Enzyme activity was measured by a slightly modified method from Oda et al. (Oda et al., 1997). A reaction mixture composed of 0.5 mL of crude enzyme supernatant plus 2.5 mL of 0.1% (w/v) polyesters emulsion (PCL, PLA, PBS and PBSA) in 100 mM Tris-HCl buffer (pH 8) using an ultrasonic processor was used as substrate and then incubated at 50°C for 60 min. The turbidity derived from insoluble plastics was measured at 650 nm after 60 min. One unit (U) of polyester-degrading activity was defined as a 0.1 turbidity decrease in optical density at 650 nm under the assay conditions described.

Partial characterization of polyester-degrading genes. PCR was used to isolate partial gene fragments of polyester-degrading genes from each bacterial strain. The PCR products were amplified by degenerate primers specifically designed for bioplastic-degrading actinomyces and lipase/esterase proteins, including *Streptomyces flavogriseus* ATCC 33331 (accession no. YP_004925694), *Amycolatopsis orientalis* (accession no. YP_008014213), *Thermobifida alba* strain AHK119 (accession no. BAK48590) (Hu et al., 2010), *Streptomyces albus* (accession no. U03114.2), *Streptomyces coelicolor* (accession no. AF009336.1), *Streptomyces exfoliates* (accession no. AAB51445) and *Acidovorax delafieldii* strain BS-3 (accession no. BAB86909) (Uchida et al., 2000). The degenerate primers used in this experiment were LPAF1 (5'-GGCTWCSGSGSSGGCACCRTSTAC-TAC-3') and LPAR1 (5'-SSWGCCGCCGCCGC-CCATSSWSYRGCC-3') that were designed to amplify an approximately 250 bp portion of the open reading frame encoding the consensus sequence (G-X-S-X-G) of esterase/lipase families.

RESULTS AND DISCUSSION

Seventeen soil samples collected from compost and landfill soils in Thailand, were screened for polyester-degrading bacteria using the clear zone method at 50°C for 3–10 days. Our results show that 80 isolates were polyester degrading, 20 isolates were PCL-degrading (25%), 6 isolates were PLA-degrading (7.5%), 3 isolates were PLLA-degrading (3.75%), 15 isolates were PBS-degrading (18.75%) and 10 isolates were PBSA-degrading (12.5%). PLA-degrading bacteria are rarely isolated, and only a few PLA-degrading strains have been isolated and identified. A previous study reported that 39 bacterial strains of the classes *Firmicutes* and *Proteobacteria* can degrade other polyesters, but not PLA (Pranamuda et al., 1997). The population of aliphatic polymer-degrading microorganisms in different ecosystems was found to exist in the following order: PHB = PCL > PBS > PLA (Pranamuda et al., 1997; Suyama et al., 1998). In this study, potential polyester-degrading thermophilic bacteria designated S14, TF1, APL3 and TP4 had the highest clear-zone forming activities (Table 1). Colony formation appeared on the turbid polyester-agar plates, and more than two types of polyester were able to be degraded by these strain. These were then used for further study. Thermophilic microorganisms that can break down biodegradable plastics were essential to the development of high-temperature composting technology. Composting exposed to microbial attack under high temperature is one of the key technologies for biodegradable plastic recycling (Tokiwa et al., 2004). The formation of the clear zones around the colonies indicates that the polymer could be hydrolysed into water-soluble products (Nishida and Tokiwa, 1993).

Table 1. Clear zone formation of thermophilic bacteria on agar plates emulsified with biodegradable plastics[a]

Strain	Clarity of clear zone[b] on plate containing with				
	PLA	PLLA	PCL	PBS	PBSA
Actinomadura sp. S14	–	–	+++	+	–
Actinomadura sp. TF1	+++	+	+	+++	++
Streptomyces sp. APL3	++	++	++	+++	–
Laceyella sp. TP4	–	–	+	+	++

[a] Polylactic acid (PLA), poly-L-lactide (PLLA), polycaprolactone (PCL), poly-(butylene succinate) (PBS) and polybutylene succinate-*co*-adipate (PBSA). [b] Clarity of clear zone: +++ >5 mm diameter, ++ 3–5 mm diameter, + >3 mm diameter. The plates were incubated at 50°C for 5–7 days.

Phylogenetic study of 4 potent polyester-degrading strains was based on the 16S rDNA sequence shown in Fig. 1. Strains S14 (accession no. GQ340794) and TF1 (accession no. KC529344) showed 100% and 99.8% identity with *Actinomadura keratinilytica* and *Actinomadura miaoliensis*, respectively (family *Thermomonosporaceae*). Molecular phylogenetic identification of *Actinomadura* sp. S14 using 16S rDNA sequence have been reported previously (Sriyapai et al., 2011). Strain APL3 (accession no. JN387598) showed 99.5% identity with *Streptomyces antibioticus* (family *Streptomycetaceae*) that has been isolated and used in our laboratory (unpublished). Strain TP4 (accession no. JX402751) showed 100% identity with *Laceyella* sp. P43 (family *Thermoactinomycetaceae*). Strain TF1 could degrade all types of polyesters on the agar plates. Although many thermophilic bacteria and fungi that can degrade polyesters have been reported, nearly all of these bacteria were unable to degrade

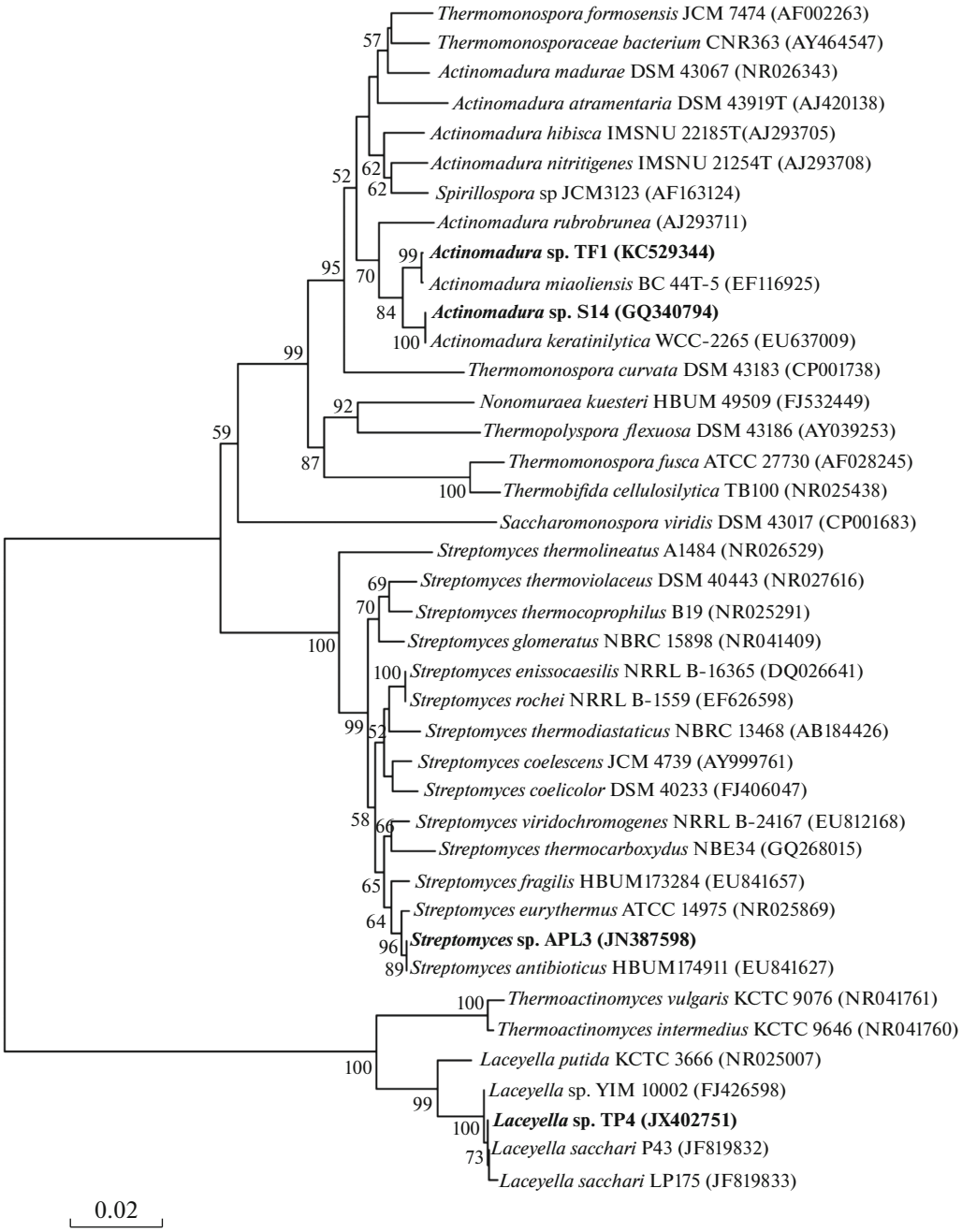

Fig. 1. Phylogenetic position of 4 polyester-degrading thermophilic bacteria among the neighboring species. Potential polyester degrading thermophilic bacterial is shown in bold. The phylogenetic tree was constructed by the method of Neighbor Joining (NJ). Each name at the termini represents the species from which the 16S rDNA originated. Segments corresponding to an evolutionary of 0.02 are shown. Bootstrap values are expressed as percentages of 1,000 replications.

polyesters such as PCL, PLA, PLLA, PBS and PBSA. Only a few PLA-degrading bacteria have been reported, nearly all of which belong to the actinomyces family, particularly the genera *Amycolatosis* (Matsuda et al., 2005; Li et al., 2008; Penkhrue et al., 2015) and *Actinomadura* (Sukkhum et al., 2011). Thermophilic composting is one of the most promising technologies for recycling biodegradable plastics (Tokiwa et al., 1992). In addition, a few studies have been published regarding a role for the genera *Actinomadura*, *Streptomyces* and *Laceyella* in high-temperature degradation of polyesters (Sukkhum et al., 2011; Chua et al., 2013;Hanphakphoom et al., 2014). This study is the first report for the optimization of polyester depolymerase by themophillic *Actinomadura*, *Streptomyces* and *Laceyella*.

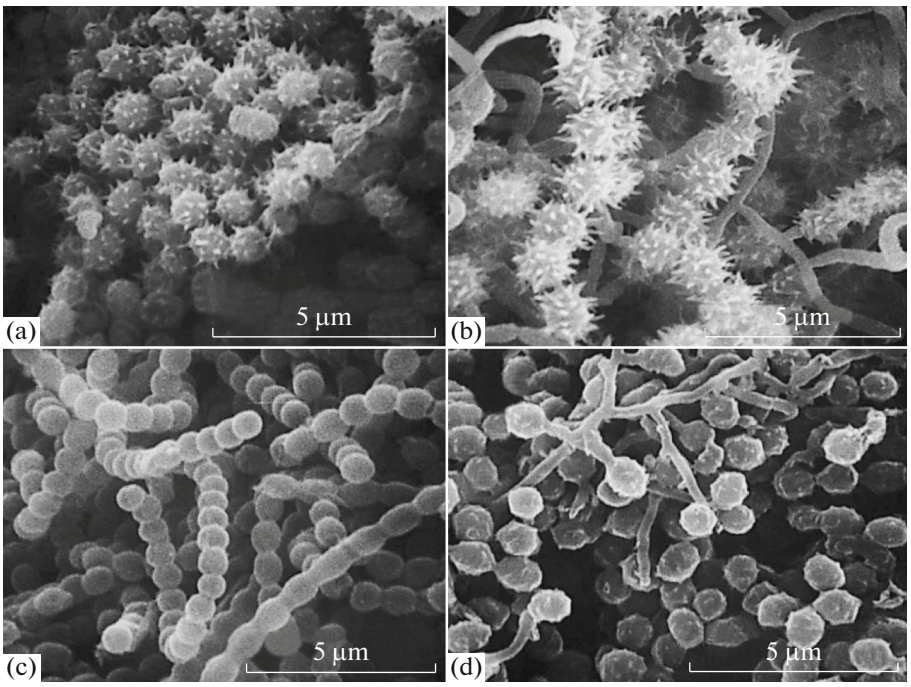

Fig. 2. SEM of spore of 4 polyester-degrading thermophilic bacteria grown on ISP agar for 7–14 day at 50°C. Strain: *Actinomadura* sp. S14 (a); *Actinomadura* sp. TF1 (b); *Streptomyces* sp. APL3 (c); *Laceyella* sp. TP4 (d). Bar, 5 μm.

Morphological, physiological and chemotaxonomic analyses indicated that strains S14 and TF1 belong to the genus *Actinomadura*, strain APL3 belongs to the genus *Streptomyces* and strain TP4 belongs to the genus *Laceyella* shown in Table 2. These strains grew well on all of media tested and colonies could be observed after 24 h. The spore chain formations in these strains grew on ISP3 for 7–14 day at 50°C and were viewed using a scanning electron microscope (Fig. 2). Strain S14 and TF1 contained *meso*-diaminopimelic acid. The substrate hyphae of strain S14 colonies were cream on ISP2, ISP3 and ISP4. However, aerial mycelia of strain S14 were white on ISP2. Strain TF1 formed cream substrate mycelium on ISP2 and grey-white on ISP3 and ISP4. The colour of aerial mycelium is blue-grey on ISP3 and ISP4. Both strain showed temperature ranges for growth on ISP3 between 40–60°C, with optimal growth at 50°C, but only strain TF4 grew on medium containing NaCl at 2–6%. Strain S14 utilized arabinose, fructose, glucose, inositol, mannitol, rhamnose and xylose, but did not degrade CMC and avicel. Although strain S14 was identified by 16S rDNA sequence as *A. keratinilytica* similar to strains such as *A. keratinilytica* T16-1 (Sukkhum et al., 2011) and *A. keratinilytica* WCC-2265^T (Puhl et al., 2009), some phenotypic characterizations such as colony colour, % NaCl tolerance and growth on sugars were different. Strain TF1 utilized all sugars as sole carbon sources, and degraded CMC, avicel and xylan. Phenotypic characterizations, genetic data and degradation properties of strain TF1 as shown in Table 2 and Fig. 1 were different from type strain of *A. miaoliensis* BC44T-5^T (Tseng et al., 2009); hence, it is proposed that the strain should be classified as representative of a novel species of polyester-degrading *Actinomadura*. Strain APL3 contained *LL*-isomer diaminopimelic acid. The substrate mycelia were cream on ISP2 and brown on ISP3 and ISP4. The aerial mycelia were white on ISP2, brown on ISP3 and grey on ISP4. This strain grew at 30–50°C, optimally at 45°C and tolerated NaCl at 2–6%. It could utilize all sugars and was positive for CMC, avicel and xylan degradation. Morphological, physiological and genetic characteristics of strain APL3 were not similar to previously published strains of polyester-degrading *Streptomyces* (Calabia and Tokiwa, 2006). Strain TP4 was closely related to *L. sacchari* LP175 (Hanphakphoom et al., 2014), with a 16S rDNA sequence similarity of 99% and the morphological and physiological characteristics of strain TP4 were partially similar to *L. sacchari* LP175. The substrate mycelia of strain TP4 were yellow-brown on ISP2 and brown on ISP3 and ISP4. The aerial mycelia were yellow on ISP2, white on ISP3 and brown on ISP4. This strain grew at 40–50°C, with optimum growth at 45°C and tolerated NaCl at 2–6%. It could utilize glucose, mannitol, raffinose and sucrose and degraded CMC, avicel and xylan.

The depolymerase activities from 4 polyester degrading strains in basal medium containing inducer substrates at pH values between 6.0–8.0 (Fig. 3) and temperatures between 40–70°C were evaluated

Table 2. Morphological and physiological characteristics of polyester-degrading thermophilic bacteria. Strain 1, *Actinomadura* sp. S14; 2, *Actinomadura* sp. TF1; 3, *Streptomyces* sp. APL3; 4, *Laceyella* sp. TP4

Characteristic	1	2	3	4
DAP type	Meso	Meso	LL-isomer	–
Conidia				
chain arrangement	Flexous	Flexous	Flexous/straight	Unbranched/bifurcated
number in chain	10–15	10–15	>15	1
Shape/ornamentation	Globose/spiny	Globose/spiny	Globose/smooth	Globose
Color of colony				
ISP2	Cream	Cream	Cream	Yellow-brown
ISP3	Cream	Gray-white	Brown	Yellow-brown
ISP4	Cream	Gray-white	Brown	Brown
Aerial hyphae				
ISP2	White	–	White	Yellow-brown
ISP3	–	Blue-gray	Brown	Brown
ISP4	–	Blue-gray	Gray	Gray
Growth at/in				
30°C	–	–	+	–
50°C	+	+	+	+
2–6% NaCl	–	+	+	+
Utilization of				
arabinose	+	+	+	–
fructose	+	+	+	–
glucose	+	+	+	+
inositol	+	+	+	–
mannitol	+	+	+	+
raffinose	–	+	+	+
rhamnose	+	+	+	–
sucrose	–	+	+	+
xylose	+	+	+	–
Degradation				
CMC	–	+	+	+
avicel	–	+	+	+
xylan	+	+	+	+
tributyrin	+	+	+	+
skim milk	+	+	+	+

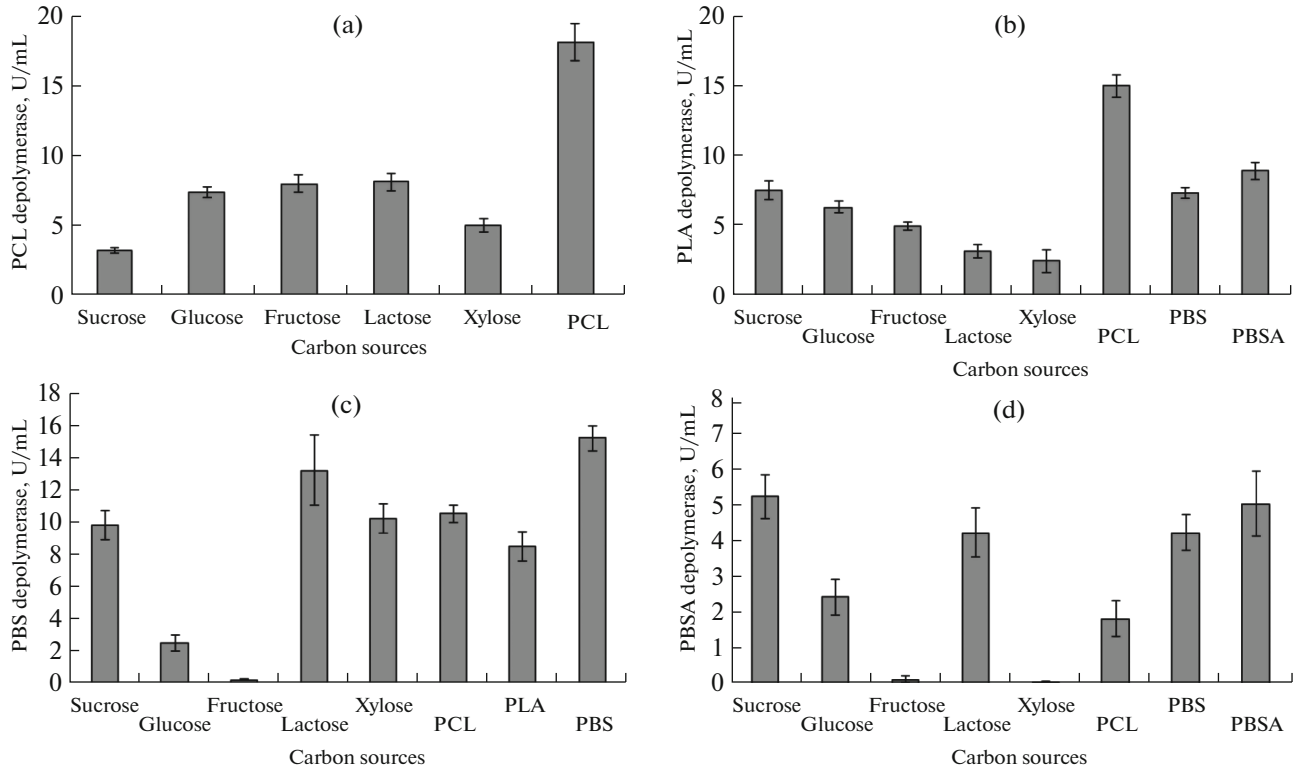

Fig. 3. Effect of carbon sources on the polyester-hydrolyzing activity of polyester-degrading thermophilic bacteria: *Actinomadura* sp. S14 (a); *Actinomadura* sp. TF1 (b); *Streptomyces* sp. APL3 (c); *Laceyella* sp. TP4 (d). The strain showed slightly activity in basal medium without carbon source. The concentration of each inducer was 0.1%. The inoculated medium was incubated with rotary shaking at 50°C for 3–5 days.

(Fig. 4). The effects of various inducers on depolymerase activities is summarized in Figs. 5 and 6. In this study found that the growth of polyester degrading bacteria must be optimized by controlling of temperature, pH, nitrogen source and carbon source to help in the production of large amount of enzyme. Yeast extract combined with $(NH_4)_2SO_4$ as a nitrogen source was a typical inducer of depolymerase activity in all polyester-degrading thermophilic bacteria. Strain S14 produced the highest PCL depolymerase at 50°C and pH 8.0 for 5 days with final concentration of 1% (w/v) PCL. PCL was the best carbon source and degradation activity of the PCL emulsion by culture supernatant was 18.3 U/mL. Strain TF1 produced the highest PLA depolymerase at 50°C and pH 8.0 for 5 days with final concentration of 0.5% (w/v) PLA. The highest activity on PLA emulsion by culture supernatant was 22.3 U/mL, and PLA was the best carbon source compared with other sugars. Strain APL3 produced the highest PBS depolymerase at 45°C and pH 7.0 for 5 days with final concentration of 1% PBS. PBS was the best carbon source and the degradation of PBS emulsion by culture supernatant was 19.4 U/mL. Strain TP4 produced the highest PBSA depolymerase at 50°C and pH 7.0 for 5 days using 0.5% (w/v) PBSA and the activity of approximately 6.3 U/mL. The depolymerase enzyme production of 4 strains exhibited activity over all temperature evaluated (Fig. 4). Strain S14, TF1 and TP4 produced the highest PCL PLA and PBSA depolymerase at 50°C, respectively, while, strain APL3 had an optimum temperature at 40°C for production of these enzymes. A recent study on the production of PLA-degrading enzyme by *A. keratinilytica* T16-1 demonstrated production of PLA-degrading enzyme at neutral pH (6.8) and 46°C. Under these conditions, the model predicted a PLA-degrading activity of 254 U/mL (Sukkhum et al., 2012). Previously, Hanphakphoom et al. reported that *L. sacchari* LP175 could produce PLLA-degrading activity of 5.07 U/mL, when the culture was grown at 50°C for 4 days (Hanphakphoom et al., 2014). Several thermal stable polyester depolymerases have been characterized from thermophilic *Streptomyces* (Calabia and Tokiwa, 2006). In this study, all strains grew and produced degradation enzymes with polyesters (PCL, PLA, PBS and PBSA) as the sole carbon source. These results strongly suggest that the degradation of PCL, PLA, PBS and PBSA emulsions by strains TF1, APL3 and TP4 is a result of co-degradation by co-inducers.

As a result of partial esterase/lipase gene amplification, PCR products with a molecular size of approximately 250 bp formed for all of the actinomyces strains

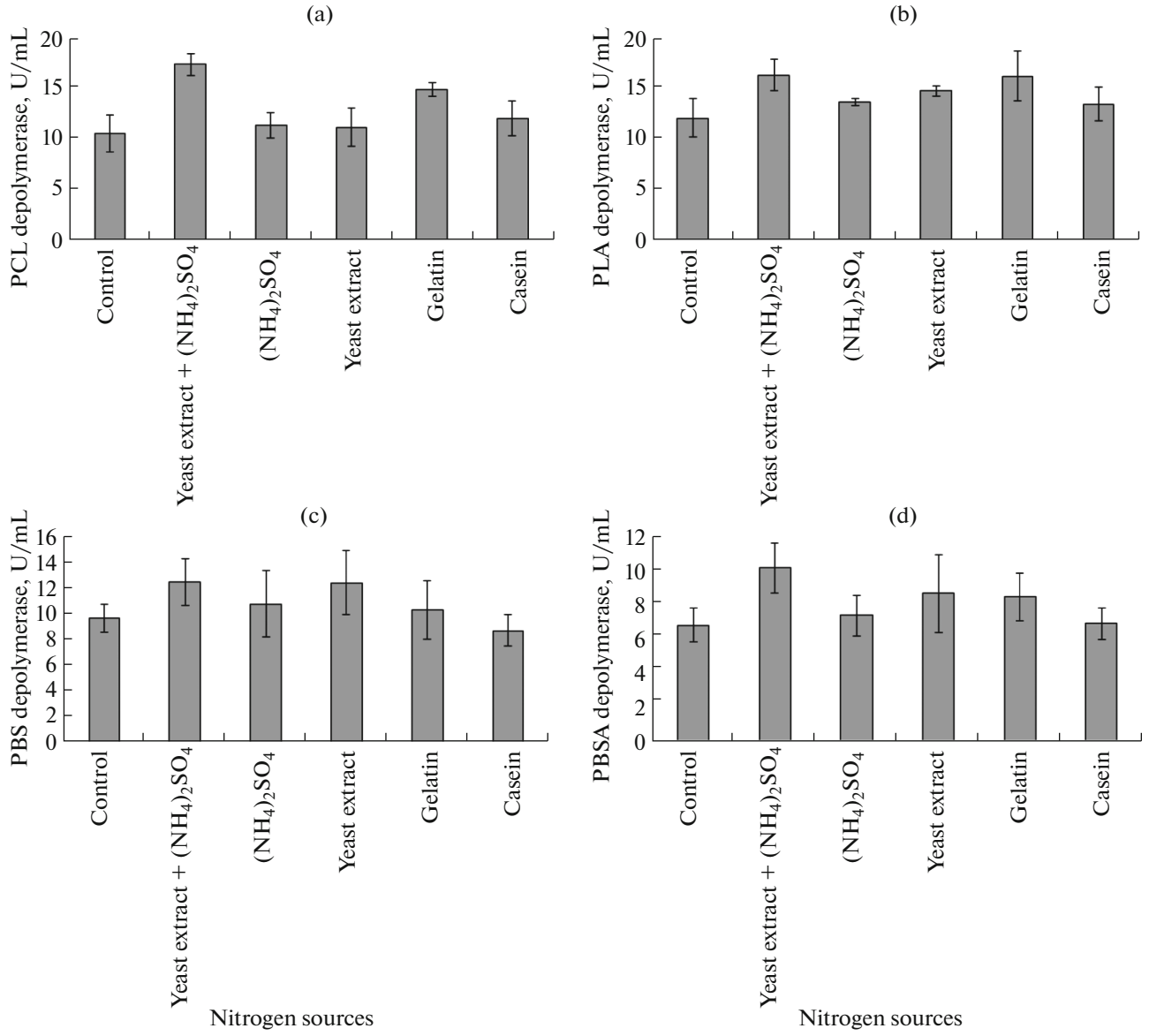

Fig. 4. Effect of nitrogen sources on the polyester-hydrolyzing activity of polyester-degrading thermophilic bacteria: *Actinomadura* sp. S14 (a); *Actinomadura* sp. TF1 (b); *Streptomyces* sp. APL3 (c); *Laceyella* sp. TP4 (d). Basal medium supplement with each the best carbon source inducer (PCL, PLA, PBS and PBSA) was used as control experiments. The strain showed slightly activity in basal medium without carbon source. The concentration of each nitrogen source was 0.1%. The inoculated medium was incubated with rotary shaking at 50°C for 3–5 days.

tested (Fig. 6). The amino acid identities of LipS14, LipTF1 and LipAPL3 were most closely related to the esterase/lipase superfamily. The sequences displayed the typical catalytic triad (GHSMG). Sequence analysis revealed that these enzymes were members of the serine hydrolase superfamily containing the GxSxG motif (Fig. 7). Esterases and lipases belong to the hydrolase super-family. They share the same consensus motif, have multifunctional properties and make up a subgroup of the serine hydrolase family. All translated sequences of LipS14, LipTF1 and LipAPL3 shared identity with a lipase/esterase from *Streptomyces flavogriseus* ATCC 33331 (YP_004925694), *Amycolatopsis orientalis* (YP_008014213) and *Thermobifida alba* strain AHK119 (BAK48590). Hu et al. reported that recombinant Est119 enzymes from *T. alba* strain AHK119 exhibited a conserved lipase box and degraded on aliphatic-aromatic copolyester (Hu et al., 2010). However, the primer did not show a band in strain TP4. Further studies on the mecha-

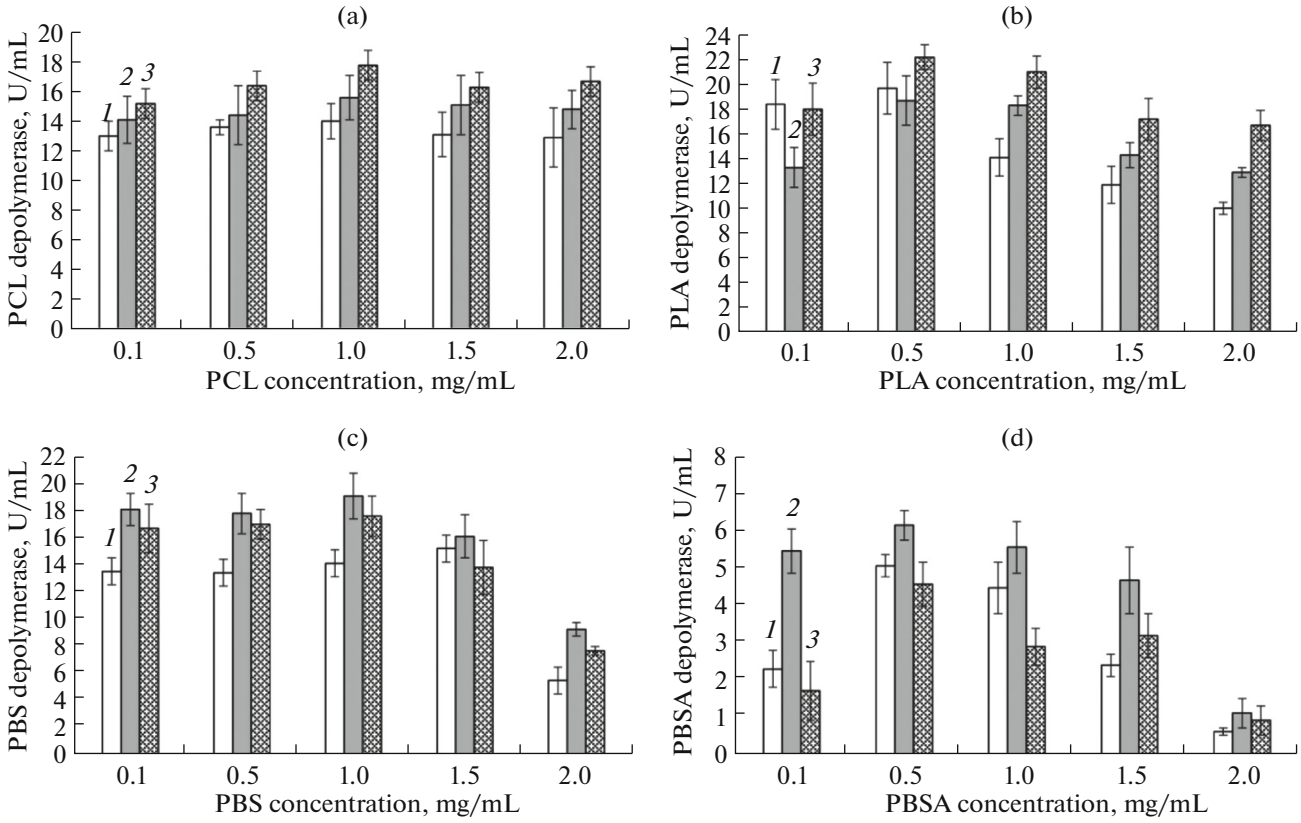

Fig. 5. Effects of pH on each depolymerase activity from polyester-degrading thermophilic bacteria: *Actinomadura* sp. S14 (a); *Actinomadura* sp. TF1 (b); *Streptomyces* sp. APL3 (c); *Laceyella* sp. TP4 (d). The pH values: 6 (*1*); 7 (*2*); 8 (*3*).

nism of polyester degradation and depolymerase genes in bacteria that degrade bioplastics are necessary to fully take advantage of their environmental advantage. Although there have been several reports about polyester-degrading *Actinobacteria* and their polyester-degrading enzymes, little information about their genes has been reported (Matsuda et al., 2005; Hu et al., 2010).

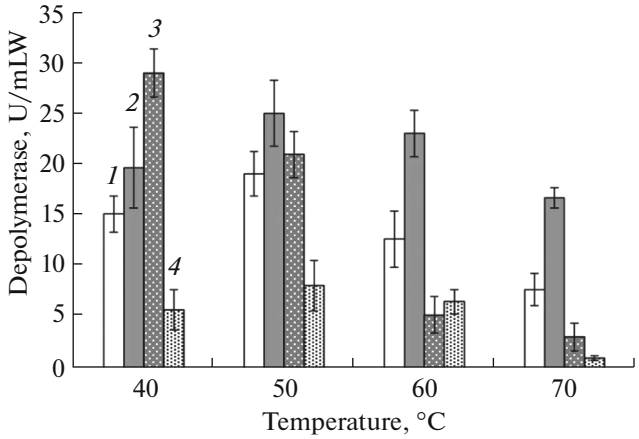

Fig. 6. Effects of temperature on each depolymerase activity from polyester-degrading thermophilic bacteria: *Actinomadura* sp. S14 (PCL depolymerase) (*1*); *Actinomadura* sp. TF1 (PLA depolymerase) (*2*); *Streptomyces* sp. APL3 (PBS depolymerase) (*3*); *Laceyella* sp. TP4 (PBSA depolymerase) (*4*).

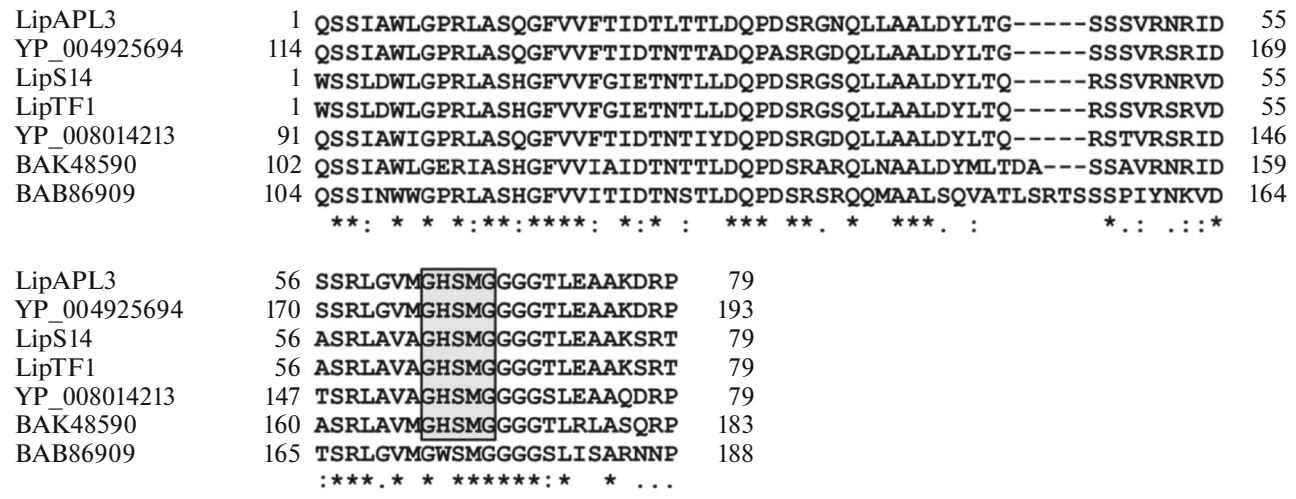

Fig. 7. Alignment of partial depolymerase-like lipase/esterase from *Actinomadura* sp. S14 (LipS14), *Actinomadura* sp. TF1 (LipTF1), *Streptomyces* sp. APL3 (LipAPL3), *Streptomyces flavogriseus* ATCC 33331 (YP_004925694), *Amycolatopsis orientalis* (YP_008014213), *Thermobifida alba* strain AHK119 (BAK48590) and *Acidovorax delafieldii* strain BS-3 (BAB86909). The conserved amino acids of catalytic serine hydrolase superfamily (GX_1SX_2G) is boxed. Conserved and identical amino acids are indicated by asterisks (*), while colon (:) and dot (.) indicate similar amino acids.

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