Biodegradation (2007) 18:333–342
DOI 10.1007/s10532-006-9067-5

ORIGINAL PAPER

Degradation of polyethylene succinate (PES) by a new thermophilic *Microbispora* strain

Kim-Chi Hoang · Min Tseng · Wei-Jye Shu

Received: 21 March 2006 / Accepted: 14 June 2006 / Published online: 16 November 2006

Abstract Thermophilic actinomycetes were isolated from sediment of the Chingshuei hot spring in north Taiwan, and the strain HS 45-1 was selected from colonies which formed distinct clear zones on agar plate with emulsified polyethylene succinate (PES). The film of PES disappeared within 6 days in liquid cultures at 50°C. The strain HS 45-1 was also able to degrade poly (ϵ-carpolactone) (PCL) and poly (3-hydroxybutyrate) (PHB) films completely within 6 days in liquid cultures. Basing on the results of phynotypic characteristics, phylogenetic studies and DNA-DNA hybridization, strain HS 45-1 should be assigned to *Micorbispora rosea* subsp. *taiwanensis.*

Keywords Degradation · Polyethylene succinate · Thermophile · *Micorbispora rosea* subsp. *taiwanensis*

K.-C. Hoang · W.-J. Shu (✉)
Department of Chemical and Material Engineering, Ta-Hwa Institute of Technology, 1, Ta-Hwa Road, Chiung-Lin, Hsinchu 30743, Taiwan, ROC
e-mail: shu@thit.edu.tw

M. Tseng
Bioresource Collection and Research Center, Food Industry Research and Development Institute, 331, Shih-Pin Road, Hsinchu 300, Taiwan, ROC

Abbreviations
PES polyethylene succinate
PCL poly (ϵ-carpolactone)
PHB poly (3-hydroxybutyrate)

Introduction

Up to year 2000, about 140 million tones of synthetic polymers were produced worldwide every year. Moreover, production of those polymers would gradually increase to about 200 million tones per year. Environmental pollution from synthetic polymers has been recognized as a large problem. So, bio-degradable plastics have been the subject of many research papers. Several kinds have been produced, such as PHB, PCL, polylactic acid (PLA), PES and so on. Among these polymers, PES is a thermoplastic polyester prepared either by ring-opening copolymerization of succinic anhydride with ethylene oxide or by poly-condensation of succinic acid and ethylene glycol. It can be used for most application in which polyethylene is currently used and its yield strength is higher than that of polyethylene.

The clear zone technique, a powerful method for ecological investigation of plastic degradation, was suggested by Nishida and Tokiwa (1993). The thermophilic composting was one of the promising

technologies in recycling biodegradable plastics (Tokiwa et al. 1992). And, thermophilic microorganisms play an important role in the composting process. Intensive study on this newfound strain as a useful microorganism for industrial application is necessary. Furthermore, actinomycetes also play an important role in polyester degradation (Tokiwa and Pranamuda 2001). Most of studies about high temperature of PES-degradation were focused on bacteria. A thermophilic bacterium, for example, is able to degrade PES at 50°C (Tansengco and Tokiwa 1998). Pranamuda et al. (1997) had isolated a PLA-degradable actinomycete, *Amycolatopsis* strain HT-32, and Jarerat and Tokiwa (2003) had isolated the *Saccharothrix waywayanrdensis* and *Kibdelosporangiumaridum.* Sanchez et al. (2000) had found the PCL-degradable fungi, *Aspergillus sp.* However, the studies about the PES degradation by actinomycetes on high temperature are still rare in the literature.

In this research, we have studied the degradation of PES at 50°C with thermophilic actinomycete isolated from culture collections. The best one of PES-degrading strain was selected to illustrate the degradation of PES and other commercially available biodegradation polyester.

Materials and methods

Materials

PES ($\overline{Mn}$ = 1.0 × 10^4), PCL ($\overline{Mn}$=6.8×10^5), and PHB ($\overline{Mn}$ = 5.4 ×10^5) were obtained from Aldrich Chemical Co. All the polymers mentioned above were directly used as received and their chemical structures were given in (Fig. 1.)

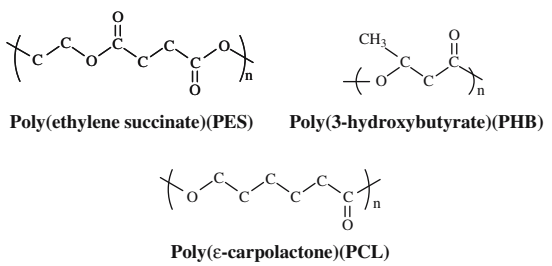

Fig. 1 The chemical structures of PES, PHB, and PCL

Samples collection

Sediment samples were collected from the Chingshuei hot spring in north Taiwan with a depth range about 5 cm. The highest temperature of hot spring was 83.6 °C, and pH was 8.16. All samples were collected in sterile 50-ml tubes and kept refrigerated until they were processed.

Screening of the aliphatic polyester-degrading actinomycetes

One gram of aliphatic polyester powder was dissolved in 50 ml of methylene chloride. The solution was emulsified into basal medium containing (per liter): yeast extract, 0.1 g; $FeSO_4{\cdot}7H_2O$, 10 mg; $MgSO_4{\cdot}7H_2O$, 0.2 g; $(NH_4)_2SO_4$, 1 g; $CaCl_2{\cdot}2H_2O$, 20 mg; NaCl, 0.1 g; $Na_2MoO_4{\cdot}2H_2O$, 0.5 mg; $NaWO_4{\cdot}2H_2O$, 0.5 mg; $MnSO_4{\cdot}H_2O$, 0.6 mg; and detergent (Poas, Nice Co., Taiwan), 50 mg. Methylene chloride was evaporated using a proctor laboratory hood. Agar (18 g) was added to the emulsified medium with a pH of 7.2, which was then sterilized in an autoclave at 121 °C for 15 min and poured into Petri dishes. The purified isolates of actinomycetes were streaked out on emulsified PES, PCL, and/or PHB/agar plate, and incubated at 30 °C for 7 days. The degradation ability of the isolates was determined by the formation of a clear zone around the colonies.

Analysis of biodegradability

The isolates were grown in 250 ml Erlenmeyer flasks with 100 ml of basal medium and 100 mg aliphatic polyester (PES, PHB and PCL) films. The aliphatic polyester films were prepared by heat pressed method. The films with a thickness about 188 μm to 220 μm (Fig. 2) was sterilized with 75% (wt./vol.) alcohol and radiated for 10 minutes. The flasks were incubated on a rotary shaker (180 rpm) at 50 °C. Culturing was done in triplicate and analyzed daily for the water-soluble total organic carbon (TOC), pH and cell weight. The TOC was measured by using a Shimadzu TOC-1010 analyzer.

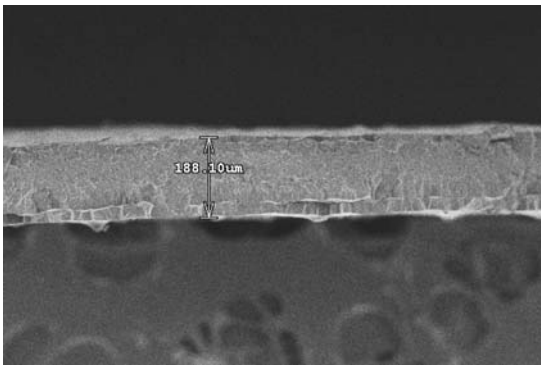

Fig. 2 Scanning electron micrographs of PES film thickness

Scanning electron microscope (SEM) observation

The aliphatic polyester films were directly retrieved from the culture broths and air dried overnight, and then, the degrading microorganism was observed with a scanning electron microscope (Hitachi-S-3000N) at various stages of cultivation.

Microorganisms and cultural condition

The thermophilic microorganisms were isolated using the dilution plating method, from a sediment sample of hot spring in Yilan county, Taiwan. The medium used for isolation was a modified HV agar (Hayakawa and Nonomura 1987) (1.0 g of humic acid, 1.0 g yeast extract, 1.7 g KCl, 0.5 g Na_2HPO_4, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.02 g $CaCO_3$, 20 mg nalidixic acid, 50 mg cyclohexamide, 0.25 mg biotin, 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, p-aminobenzoic acid, and agar 18.0 g, distilled water 1.0L, pH 7.2) and incubated at 50 °C for 1 week, then observed in long working distance objective lens. The powdery colonies with branched hyphae were picked and streaked on oatmeal agar plate then maintained as glycerol suspensions (20%, v/v) at -80 °C. *M. rosea* subsp. *taiwanensis* BCRC 16808 was used for the comparative studies. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (125 rpm/min) of YG broth (yeast extract 1%, glucose 1%, pH 7.0) at 50 °C for 1 week.

Identification of actinomycetes

Morphological characteristics of the strain were observed by scanning electron microscope (S-420, Hitachi, Tokyo) following incubation on oatmeal agar for 7 days at 50°C and fixation by 4% osmium tetraoxide solution, then dehydration by serial ethanol, acetone and critical point drying. Cultural characteristics were tested by using 7 days cultures grown at 50 °C on various agar media (Table 1). The *ISCC-NBS Color-Name Charts* (Kelly 1966) were used for determining color designations of substrate and aerial mycelia. Media and procedures used for physiological and biochemical features and carbon source utilization were those described by (Shirling and Gottlieb 1966).

Isomers of diaminopimelic acid (A_2pm) and sugars in whole-cell hydorlysates were determined by the method of (Hasegawa et al. 1983) and the N-acyl types of muramic acid were determined by the method of (Kawamoto et al. 1981). Presence of mycolic acids was examined by TLC following (Minnikin et al. 1975), and phospholipids were extracted and identified following the method of. (Minnikin et al. 1984). Menaquinones were extracted and purified by the method of (Collins et al. 1997), then analyzed by HPLC (Model 600, Waters) on a chromatograph equipped with a Nova-Pak C18 column. Cellular fatty acid compositions of strain HS 45-1 were determined using the Sherlock Microbial Identification System (MIDI). Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer and analyzed with a 5890 gas chromatograph equipped with a flame ionization detector and an automatic injector with a G1512A controller (Hewlett Packard). Identification of the peaks was made by comparing the results with the built-in TSBA 40 database (MIDI).

Total DNA was extracted from 7-day cultured cells by using the QIAGEN® Genimic DNA Kit. The G+C content of the DNA was determined by the HPLC method of (Tamaoka and Komagata 1984). The total DNA was prepared by using the same method as above. The 16S rRNA gene was PCR-amplified by using methods of Nakajima (Nakajima et al. 1999) and was sequenced directly

Table 1 Cultural characteristics of strain HS 45-1 on various media

Medium	Growth	Substrate mycelium	Aerial mycelium	Sporulation
Yeast extract-malt extract agar (ISP 2)	Good	Strong yellowish* brown	White	Poor
Oatmeal agar (ISP 3)	**Good**	Strong yellow	None	None
Glycerol asparagines agar (ISP 5)	Moderate	Yellowish white	White	Moderate
Glucose-asparagine agar +1% Yeast extraact	Good	Deep yellow	None	None
Oatmeal agar + Yeast extract	Good	Strong yellow	None	None
Inorganic salt-starch + 1% Yeast extract	Good	Dark orange yellow	None	None
Modified Bennett's agar	Good	Deep orange yellow	None	None
Yeast extract-starch agar	Good	Moderate orange yellow	None	None
1/5 Yeast extract-starch agar	Moderate	Pale yellow	Yellowish white	Good
1/20 V-8 juice agar	Moderate	Yellowish white	White	Good

Note: colors were taken from ISCC-NBS COLOR CHARTS (Kelly, 1964).

on an ABI model 3730 automatic DNA sequencer by using BigDye Terminator V3.1 Kit (Applied Biosystems). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein 1993) and MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar et al. 2001) after multiple alignment of data by CLUSTALX (Thompson et al. 1997). Distances (distance options according to the Kimura two-parameter mode) (Kimura 1980, 1983) and clustering were calculated with the neighbor-joining method (Saitou and Nei 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data by performing 1000 resamplings (Felsenstein 1985).

The 16S rRNA gene sequence determined in this study has been deposited in GenBank under the accession number DQ 246622. The accession numbers of the reference strains, which are closely related to strain HS 45-1, are indicated in (Fig. 3).

DNA-D NA hybridization was carried out according to the method of (Ezaki et al. 1989).

Results and discussion

Isolation and identification of microorganism

The clear-zone method was used to screen the PES-degrading microorganisms. It is the simplest method for investigating microbial degradation of aliphatic polyester since the formation of a clear-zone around the colony indicates solubilization of the polymer by enzyme(s) secreted from the microbes (Nishida and Tokiwa 1993; Pranamuda et al 1995). The ''clear- zone'' formed when strains secrete extra cellular enzymes in order to break down compounds into soluble materials (Fields et al. 1974). Out of ten strains were isolated from the sediment of hot spring, an active PES-degrading actinomycete strain HS 45-1 had been isolated successfully (Fig. 4).

Strain HS 45-1 produced branched and unfragmented substrate mycelia and monopodially branching aerial mycelia. The aerial hyphae bored non-motile spores on short sporophores in characteristics longitudinal pairs. Spores were oval with a smooth surface (Fig. 5). The cultural characteristics of strain HS 45-1 are shown in (Table 1). The growth was good on most of media and the substrate mycelia on most media tested were yellowish white to strong yellow. Aerial mycelia were produced on some of media. Sporulations were poor on most of media, and no soluble pigment was produced in all of media. The results of physiological and biochemical test of strain HS 45-1 and reference strain were indicated in (Table 2). Strain HS 45-1 could utilize glucose, sucrose, raffinose, cellulose, mannitol and fructose. It was positive for gelatin liquefaction, casein, L-tyrosin , xanthine, urea and aesculin hydrolysis.

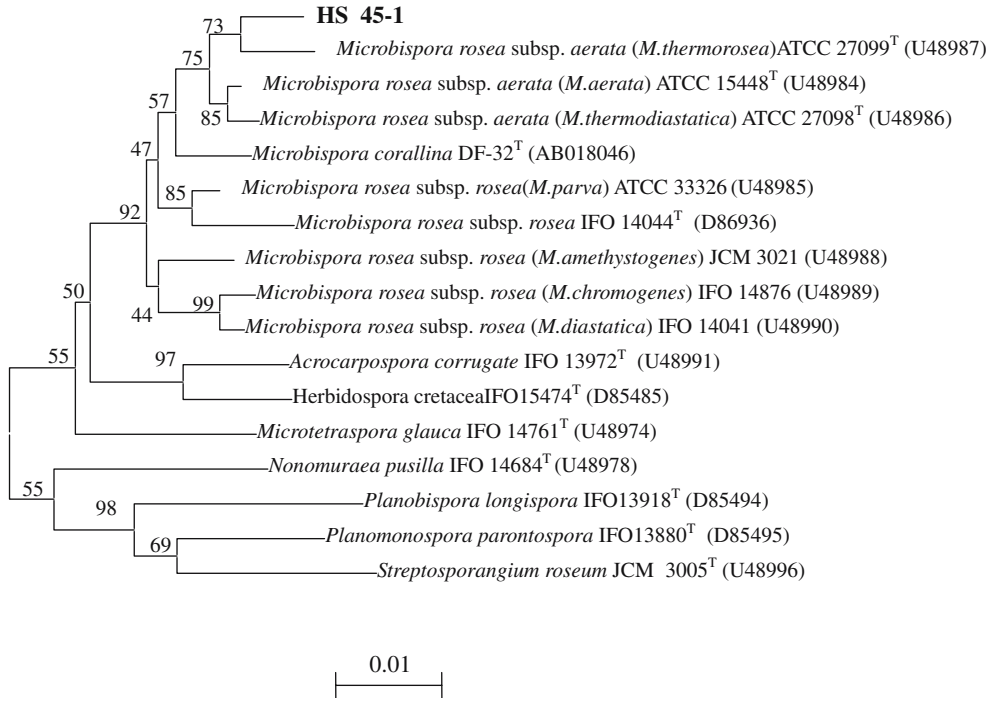

Fig. 3 The phylogenetic tree based on the 16S rRNA gene sequences of the isolate HS 45-1

Strain HS 45-1 contained galactose, glucose, mannose, ribose and a trace amount of madurose in the whole-cell hydrolysates and the cell-wall peptidoglycan contained *meso*- and 3-hydroxy-A_2pm. Predominant menaquinone were MK-9(H_2) and MK-9(H_4). Mycolic acids were not detected. The fatty acid methyl ester profiles were: iso-$C_{16:0}$ (56.8%), iso$C_{15:0}$ (7.02%), anteiso-$C_{17:0}$

Fig.4 The colonies and clear-zone formation by strain HS 45-1 on agar plate with emulsified PES-agar plate at 50 °C

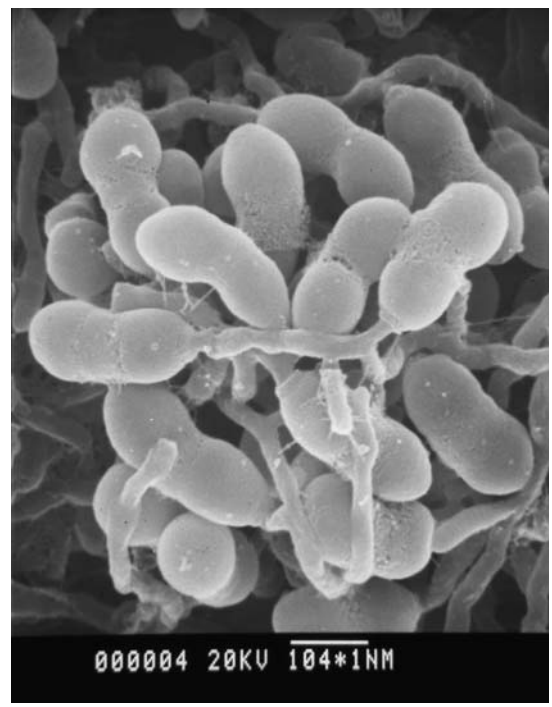

Fig. 5 Scanning electron micrograph of strain HS 45-1 on oatmeal agar at 50 °C for 7 days

Table 2 The chemical and physiological characteristics of strain HS 45-1 and reference strains

Characteristics	*M. rosea* subsp. *taiwanensis* T	HS 45-1
Substrate hydrolysis:		
Casein	+*	+
Adenine	–	–
L-tyrosine	+	+
Hypoxanthine	–	–
Xanthine	–	+
Starch	–	–
Urea	+	+
Aesculin	–	+
DNase production	–	–
Nitrate reduction	+	+/–
Melanin production	–	–
Gelatin liquification	+	+
Carbon source utilization:		
Glucose	+	+
Sucrose	+	+
Fructose	+/–	+
Xylose	+	–
Arabinose	+	–
Raffinose	+	+
Rhamnose	–	+/–
Mannitol	+	+
Inositol	–	–
Salicin	–	–
Cellulose	+	+

*+: positive, -: negative, +/–: doubtful

(6.37%), 10-methyl$C_{17:0}$ (5.56%) and other minor components. The G+C content of the DNA was 70.5 mol%.

The almost-complete 16S rRNA gene sequence (1473 nt) of isolate HS 45-1 was determined. Preliminary comparison of the sequence against the GenBank database indicated that members of the genus *Microbispora* were its closest phylogenetic neighbours. The Neighbor-joining tree, as shown in (Fig. 3) (Saitou and Nei 1987), based on almost complete 16S rDNA sequences showing the phylogenetic position of strain HS 45-1 within the radiation of *Microbispora* species. Numbers at nodes indicate percentage of 1000 bootstrap resamplings and only values over 50 % are given. Bar means 0.01 substitutions per nucleotide position. Binary similarity values ranged between 97.1% (*M. rosea* subsp. *rosea* IFO 14876^T), and 99% (*M. rosea* subsp. *taiwanensis* BCRC 16808 and with the members of the *Microbispora* genus.

DNA-DNA hybridization rates determined with the isolate HS 45-1 to its closest type strains of *M. rosea* subsp. *taiwanensis* BCRC 16808 was 90.5 %. Therefore, basing on the results of phenotypic and genotypic data, we propose that the strain HS 45-1 should be classified as *M. rosea* subsp. *taiwanensis.*

The degradation ability of PES film at 50 °C

The strain HS 45-1 was grown in the liquid basal medium with 100 mg PES films. The PES films were degraded completely after 6 days, water-soluble TOC, pH, weight loss and dry cell were then measured as shown in (Fig. 6). During cultivation, the pH dropped and the TOC increased, indicative of the degraded acidic substances in the cultivation broth as water-soluble substances. After 12 days, the TOC decreased due to a quick assimilation by the strain. The cell weight increased on the reason that the strain could assimilate the degradation products of PES.

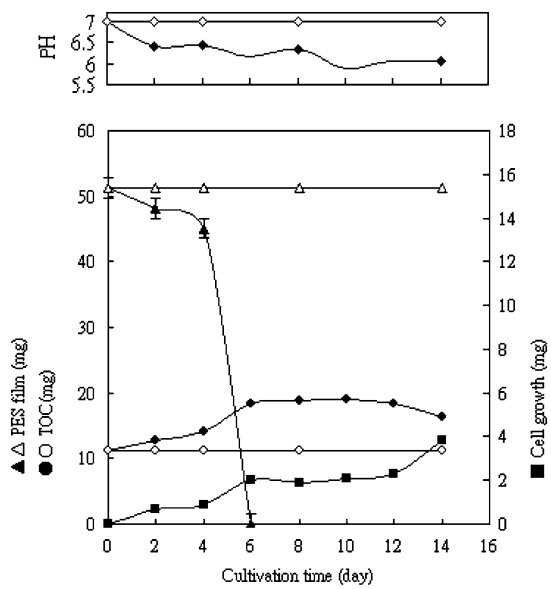

Fig. 6 Time course of PES film degradation by strain (HS 45-1) at 50 °C. Residual polymer(▲, Δ), water-soluble total organic carbon (TOC) (●, ○), dry cell weight(■), and pH(□, □). Solid symbols: inoculated cultures; Open symbols: control without cell inoculation. Bars show standard errors of duplication.

SEM observation of PES film sample

PES films were incubated in liquid cultures as a substrate to examine their degradability. The films would conform to degrade at day 2 and the degradation was quickly and completely degraded after 6 days at 50°C. The surface morphology of the film was observed with SEM. The surfaces of PES films became rough and small cracks were formed in the inoculated culture after 2 days (Fig. 7b). On the contrary, the film surfaces were smooth in the system without cell inoculation cultures (Fig. 7a).

Degradation of other polyesters

The formation of the clear-zones around the colonies is indicated that the polymer could be hydrolyzed by the enzyme into water-soluble products. (Nishida and Tokiwa 1993) The clear-zone formed not only on PES-emulsified agar plate but also on PHB, PCL via HS45-1 after 2 days (Fig. 8 and Fig. 9). The degradation of

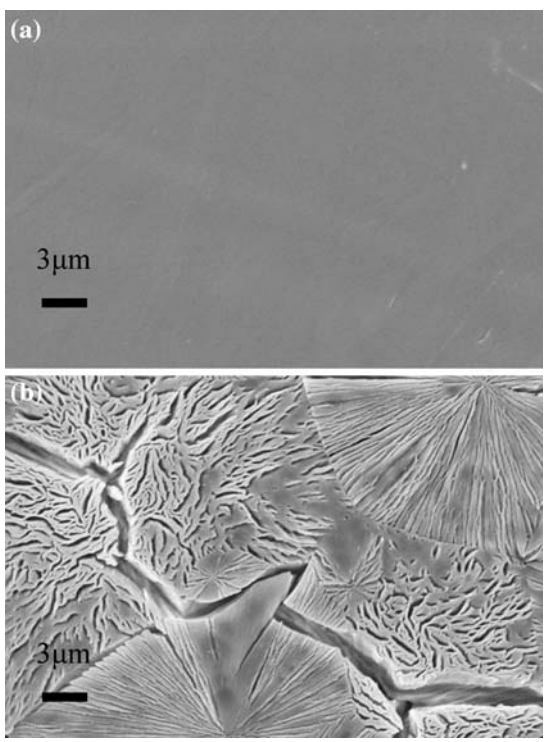

Fig. 7 Surface structures of PES on SEM. (a) Without cell inoculation; (b) With cell inoculation HS-45-1

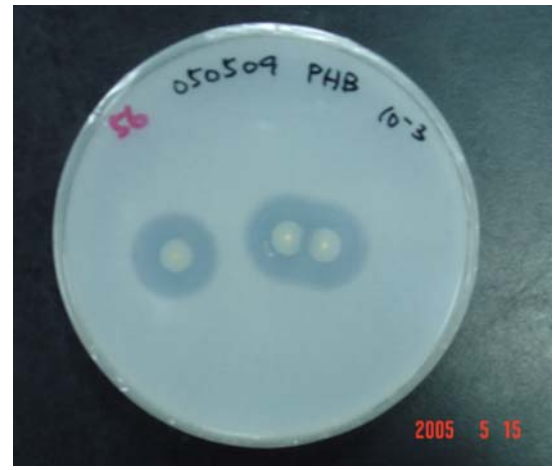

Fig. 8 The colonies and clear-zone formation by strain HS 45-1 on agar plate with emulsified PHB-agar plate at 50 °C

Fig. 9 The colonies and clear-zone formation by strain HS 45-1 on agar plate with emulsified PCL-agar plate at 50 °C

PHB and PCL films were examined by SEM after 4 days of cultivation (Fig. 10a,b and Fig. 11a,b). The PHB and PCL films were degraded more than 99% weight within 6 days at 50°C by the strain HS45-1. The PLA films, however, were not degraded. That indicated the distribution of PLA-degrading microorganism in the environment is limited.

Degradation of other strains

The degradation activity of HS 45-1 was also compared with other strains within the same genus. As shown in (Table 3), all of the strains could not degrade PLA except M. rosea subsp.

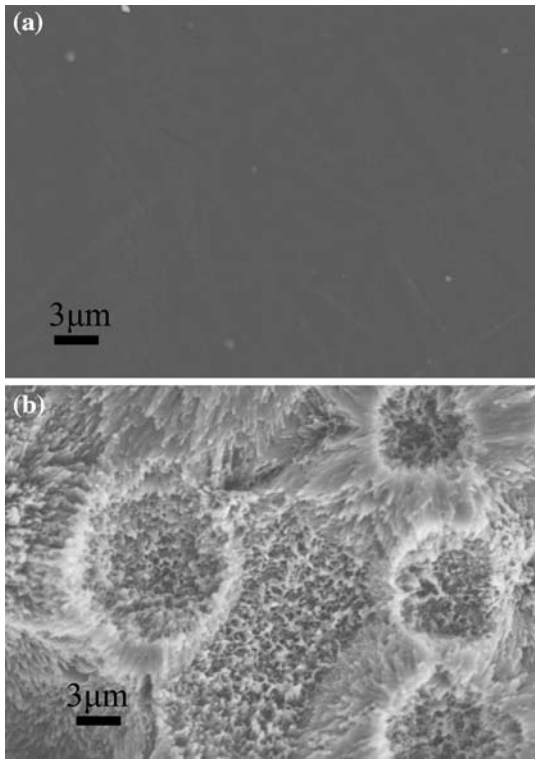

Fig. 10 Surface structures of PHB on SEM. (a) Without cell inoculation (b) With cell inoculation HS 45-1

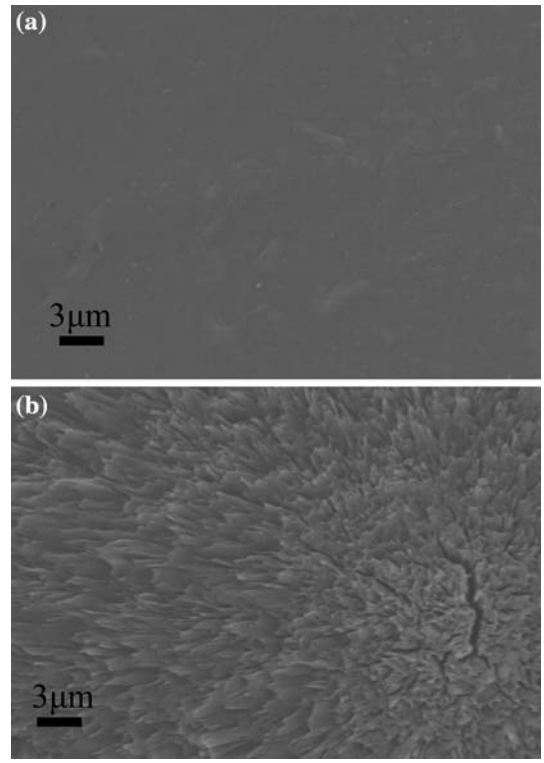

Fig. 11 Surface structures of PCL on SEM. (a) Without cell inoculation (b) With cell inoculation HS 45-1

Table 3 Clear zone forming ability of *Microbispora* spp. obtained from the culture collection on agar plates emulsified with polyester

Strain Number	Optimal temp.	28 °C				37 °C				50 °C			
		PES	PHB	PCL	PLA	PES	PHB	PCL	PLA	PES	PHB	PCL	PLA
HS-45-1	50 °C	–	–	–	–	–	–	–	–	+++	+++	+++	–
M. rosea subsp. *rosea*(BCRC11622)	28 °C	–	+	++	–								
M. mesophila(BCRC12464)	28 °C	–	–	–	–								
M. amethystogenes(BCRC13315)	28 °C	–	–	–	–								
M. rosea subsp. *rosea*(BCRC13321)	28 °C	+	+	++	–								
M. rosea subsp. *rosea*(BCRC13325)	28 °C	–	–	–	+								
M. rosea subsp. *Nonnitritogenes* (BCRC13326)	28 °C	–	–	++	–								
M. rosea subsp. *rosea*(BCRC13358)	28 °C	–	++	+	–								
M. rosea subsp. *rosea*(BCRC13370)	28 °C	–	+	–	–								
M. corallina (BCRC16341)	28 °C	–	+	–	–								
M. rosea subsp. *aerate*(BCRC11655)	37 °C					–	++	–	–				
M. rosea subsp. *aerata*(BCRC12458)	50 °C									–	–	++	–
M. rosea subsp. *aerata*(BCRC16358)	50 °C									–	++	+++	–

1. Polycaprolactone(PCL); polyhydroxybutyrate(PHB); poly(ethylene succinate) (PES); and polylactide(PLA)

2. Clear-zone forming adility: -, clear zone not formed; +, clear zone smaller than 1 mm; ++, Clear zone between 1 and 3 mm; +++, clear zone larger than 3 mm

3. Growth and clear zone formation on agar plates with emulsified polyester

rosea BCRC 13325 and the degradability of PCL was better than PHB and PES at 50 °C or 37 °C or 28 °C. This can be explained that the PLA degraders were limited to members of the family *Pseudonocardiaceae* and related genera (Jarerat et al. 2002). There is more information available about the existence of other PLA-degrading microorganisms in literature, such as Pranamuda et al. 1997, Tansengco and Dogma 1998, Ikura and Kudo 1999, and Kohei et al. 2001. The state of distribution would decrease in the order of PCL=PHB>PES>PBS>PLA. The report of Jarerat and Tokiwa (2003) showed that the strain *M. rosea subsp. rosea* IFO 14046 could degrade poly(tetramethylene succinate) (PTMS) film about 50% after 8 days in liquid culture. But in our study, the strain HS 45-1 could degrade PES film 100% after 6 days in liquid culture.

Conclusions

Thermophilic actinomycetes were successfully isolated and the strain HS 45-1 was selected from colonies which formed distinct clear zones on agar plate with emulsified PES. The strain HS 45-1 was able to degrade PES films completely within 6 days in liquid cultures at 50 °C and was also able to degrade PCL and PHB films more than 99% weight. According to our authentication, strain HS 45-1 should be assigned to *Micorbispora rosea* subsp. *taiwanensis.* The isolation of thermophilic microorganisms should be useful for the development of high temperature composting technology.

Acknowledgments This work is supported by the Ministry of Economic Affairs, R. O. C. (project no. 93-EC-17-A-17-R7-0525). We also thank Mr. Y. K. Lin for assistance in samples collection.

References

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1997) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Felsenstein J (1993) PHYLIP (phylogeny inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA

Fields RD, Rodrigue F, Finn RK (1974) Microbial degradation of polyesters: Polycaprolactone degraded by *P. pullulans*. J Appl Polym Science 18:3571–3576

Hasegawa T, Takizawa M, Tanida S (1983) A rapid analysis for chemical grouping of aerobic actinomycetes. J Gen Appl Microbiol 29:319–322

Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 65:501–509

Ikura Y, Kudo T (1999) Isolation of a microorganism capable of degrading poly-(L-lactide). J Gen Appl Microbiol 45:247–251

Jarerat A, Pranamuda H, Tokiwa Y (2002) Poly (L-lactide)-Degrading Activity in Various Actinomycetes, Macromol. Biosci 2:420–428

Jarerat A, Tokiwa Y (2003) Poly (L-lactide) degradation by *Saccharothrix wayandensis*. Biotechnol Lett 25:401–404

Jarerat A, Tokiwa Y (2003) Poly (L-lactide) degradation by *Kibdelosporangium aridum*. Biotechnol Lett 25:2035–2038

Kawamoto I, Oka T, Nara T (1981) Cell wall composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensi*s, and related organisms. J Bacteriol 146:527–534

Kelly KL (1966) Inter-Society Color Council–National Bureau of Standards Color-Name Charts Illustrated with Centroid Colors. US Government Printing Office, Washington, DC

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. J Mol Evol 16:111–120

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge

Kohei N, Toshio T, Naoki A, Yoshiyuki K (2001) Purification and Characterization of an Extracellular Poly(L-Lactic Acid) Depolymerase from a Soil Isolate, *Amycolatopsis* sp. Strain K104–1. J Gen Appl Microbiol 67:345–353

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics 17:1244–1245

Minnikin DE, Alshamaony L, Goodfellow M (1975) Differentiation of *Mycobacterium, Nocardia*, and related taxa by thin layer chromatographic analysis of whole-cell methanolysates. J Gen Microbiol 88:200–204

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Nakajima Y, Kitpreechavanich V, Suzuki K, Kudo T (1999) *Microbispora corallina* sp. nov. a new species of the genus *Microbispora* isolated from Thai soil. Int J Syst Bacteriol 49:1761–1767

Nishida H, Tokiwa Y (1993) Distribution of poly (3-hydroxy-Butyrate) and poly (ϵ-caprolactone) aerobic degrading microorganism in different environments. J Environ Polym Degrad 1:227–233

Pranamuda H, Tokiwa Y, Tanaka H (1995) Microbial degradation of an aliphatic polyester with a high melting point poly (tetramehtylene succinate). Appl Environ Microbiol 61:1828–1832

Pranamuda H, Tokiwa Y, Tanaka H (1997) Polylactide Degradation by an *Amycolatopsis* sp. J Gen Appl Microbiol 63:1637–1640

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Tansengco ML, Tokiwa Y (1998) Thermophilic microbial degradation of poly(ethylene succinate). World J Microbiol & Biotechnol 14:133–138

Tansengco ML, Dogma IJ Jr (1998) Comparative population study of aliphatic polyesters-degrading microorganisms at 50 °C. Chem Lett 1043–1044

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Tokiwa Y, Iwamoto A, Koyama M, Kataka N, Nishida H (1992) Biological recycling of plastics containing ester bonds. Makromolekulare Chemic-Makromolecular Symposia 57:273–279

Tokiwa Y, Pranamuda H (2001) Microbial degradation of aliphatic polyester. In: Doi Y, Steinbuchel A (eds) Biopolymers, Vol 3, Weinheimi Wiley-VCH pp 85–103

Sanchez JG, Tsuchii A, Tokiwa Y (2000) Degradation of polycaprolactone at 50°C by a thermotolerant *Aspergillus* sp. Biotechnol Lett 22:849–853