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Polyester-degrading thermophilic actinomycetes isolated from different environment in Taiwan

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Abstract Thermophilic actinomycetes strains were isolated from various environment in Taiwan and screened for degradation of poly(ethylene succinate) (PES), poly(ε-caprolactone) (PCL) and/or poly(β-hydroxybutyrate) (PHB) by the clear-zone method. Out of 341 strains of thermophilic actinomycetes, 105 isolates were PHB-degraders (30.8%), 198 isolates were PCL-decomposers (58.1%), and 99 isolates could degrade PES (29.0%). Furthermore, 77 isolates could degrade both PHB and PCL (22.6%), 35 isolates could degrade both PHB and PES (10.3%), 81 isolates could degrade both PES and PCL (23.8%) and 31 isolates could degrade the three polyesters used in this study (9.1%). Base on the morphological and chemical characteristics, these 31 isolates belonging to *Actinomadura* (12.9%), *Microbispora* (25.8%), *Streptomyces* (48.4%), *Thermoactinomyces* (9.7%) and *Saccharomonospora* genus (3.22%).

Keywords Thermophile actinomycetes · Microbial degradation · Polyester · Environment

Abbreviations

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

Introduction

To reduce the environmental impact of plastics, developed nations are beginning to impose strict control or mandating recovery, while industry is proactively developing biodegradable plastics as an alternative. Several biodegradable plastics, such as poly(hydroxyalkanoic acids) (PHA), poly (β-hydroxybutyrate) (PHB), poly(ε-caprolactone) (PCL), poly(ethylene succinate) (PES), poly(lactic acid) (PLA) and poly(tetramethylene succinate) (PTMS), with properties comparable to conventional plastics have been developed (Pranamuda and Tokiwa 1999; Jarerat and Tokiwa 2001).

Thermophilic composting was one of the promising technologies in recycling biodegradable plastics (Tokiwa et al. 1992) and thermophilic microorgan-

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isms play an important role in the composting process, hence, thermophilic microorganisms that are able to degrade polyesters at high temperatures are especially required. However, most studies on high-temperature polyester degradation have been focused on bacteria and fungi (Takeda et al. 1998; Tansengco and Tokiwa 1998; Sanchez et al. 2000). Actinomycetes are antibiotic-producing microorganisms, and a vast amount of reports on the enzyme-producing and degradation abilities in vitro. Actinomycetes are usually considered to be most active in the later stages of decomposition of plant and other materials, playing an important role in polyester degradation (Tokiwa and Pranamuda 2001). Recently, some information on actinomycetes that degrade polymer at high temperature has been reported (Kleeberg et al. 1998; Jarerat and Tokiwa 2001; Calabia and Tokiwa 2004; Tokiwa and Calabia 2004). Therefore, we tried to isolate the thermophilic actinomycetes from different environments and screen the polymer-degrading ability to investigate the distribution and population of polyesters-degrading microorganisms at a temperature of 50°C using the clear zone methods.

Materials and methods

Materials

The aliphatic polyester samples used were: poly(ethylene succinate) (PES) with a number-average molecular weight ($\overline{Mn}$) of 1.0×10^4, poly(ε-caprolactone) (PCL, $\overline{Mn} = 6.8 \times 10^5$), and poly($\beta$-hydroxybutyrate) (PHB, $\overline{Mn} = 5.4 \times 10^5$). These polyesters were obtained from Aldrich Chemical Co. (Steinheim, Germany).

Samples collection

There are 274 samples from ground soil, garden soil, compost, sediment of rivers or lakes and hot spring soil were collected in Taiwan between 2000 and 2005. At each of these locations, the depth range of all the samples was about 5 cm. All samples were collected in sterile 50-ml tubes, air dried for 7 days and kept at room temperature until they were processed.

Thermophilic actinomycetes isolation

Thermophilic actinomycetes were isolated by culture on modified HV agar (Hoang et al. 2007a) at 45–50°C for 7–10 days, then observed the plates on light microscope with long working distance objective lens. The powdery colonies with branched hyphae were picked and streaked on oatmeal agar (20.0 g of oatmeal, 1.0 mg each of $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, agar 18.0 g, in 1.0 l of distilled water, pH 7.2) plate then maintained as glycerol suspensions (20%, v/v) at −80°C.

Screening of aliphatic polyester-degrading thermophilic 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. 1.8% (w/v) Difco Bacto-agar 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 45–50°C for 7 days. The degradation ability of the isolates was determined by the formation of a clear zone around the colonies.

Identification of actinomycetes could degrade three polyesters

The 31 strains that could degrade three polyesters were grown at 50°C on oatmeal agar for 7 days. The morphological characteristics of these strains were observed on light microscope with long working distance objective lens.

Wall composition (*meso*- and LL-diaminopimelic acid isomer, A_2pm) and whole-cell sugars were determined by the method of Hasegawa et al. (1983).

Results and discussion

Thermophilic actinomycetes isolation

To isolate thermophilic actinomycetes, 274 samples collected from compost, sediment of lakes or rivers, and soils at different locations in Taiwan were collected. A total of 341 isolates were picked. Results shown in (Table 1) indicated that 168 (49.3%) isolates from ground soil, 55 (16.1%) isolates from garden soil as well as 38 (11.1%) from hot spring soil were identified as thermophilic actinomycetes and however, only 27 (7.9%) and 53 (15.5%) isolates from the compost and the sediment samples, respectively, were isolated. These observations indicated that the frequency of the occurrence of thermophilic actinomycetes in soils was much higher than that in the compost or sediment.

Screening of polyester-degrading thermophilic actinomycetes

Among the 341isolates tested, 198 (58.1%) isolates could degrade PCL, 105 (30.8%) isolates were PHB-degraders, and 99 (29.0%) isolates were PES-decomposers (Table 1). It was reported that the population of microbes isolated from soil that could grow on emulsified PCL agar medium at 50°C was 3–49% (Tansengco and Tokiwa 1998). In addition, the presence of PES-degrading microorganisms in natural environments was found to be fairly limited compared to that for other reported aliphatic polyesters and 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 (2001). The state of distribution would decrease in the order of PCL = PHB > PES > PBS > PLA. However, we found the population of PCL-degrading thermophilic actinomycetes was 58.1%, this result indicated that the isolates could degrade PCL were obviously more numerous than those for PHB and PES.

Among the 198 PCL-degrading actinomycetes, 93 (47.0%) and 35 (17.7%) strains were isolated from ground soil or garden soil, 21 (10.6%) strains from hot spring soil, and 30 (15.2%) and 19 (9.6%) strains from the sediment and the compost, respectively (Table 1). The distribution of PCL-degrading actinomycetes indicated that a total of 149 (75.3%) PCL-degraders were isolated from the soils of the ground, garden and hot spring. In addition, within the group of PHB- and PES- degraders, 51 (48.6%), 23 (21.9%), 8 (7.6%) of 105 isolates and 38 (38.4%), 19 (19.2%), 20 (20.2%) of 99 isolates, were isolated from the ground, garden and hot spring soil, respectively. In addition, a total of 82 (78.1%) PHB-degraders and 77(77.8%) PES-degraders were also isolated from the soils and the numbers of PHB-, PCL-, or PES-degrading actinomycetes isolated from sediment (9–30 isolates) and compost (10–19 isolates) is much lower than those from the soils.

The distribution of polyester degraders isolated in different environments was shown in (Table 1). We found the state of distribution decreased in the order of PCL = PES > PHB in hot spring soil, PCL > PHB > PES in ground soil, garden soil and sediment, and PCL > PES > PHB in compost (Table 1). In contrast, the distribution of the mesophilic actinomycetes isolated from the sediment decreased in the

Table 1 Screening of actinomycetes isolates could degrade different polyesters

Strains source	No. of isolates capable of degrading (% of actinomycetes isolated)						
	PCL	PHB	PES	PCL & PHB	PCL & PES	PHB & PES	PCL, PHB & PES
Ground soil	93 (47.0%)	51 (48.6%)	38 (38.4%)	34 (44.2%)	32 (39.5%)	16 (45.7%)	14 (45.2%)
Garden soil	35 (17.7%)	23 (21.9%)	19 (19.2%)	18 (23.4%)	16 (19.8%)	7 (20.0%)	6 (19.4%)
Hot spring soil	21 (10.6%)	8 (7.6%)	20 (20.2 %)	7 (9.1%)	14 (17.3%)	4 (11.4%)	4 (12.9%)
Sediment	30 (15.2%)	13 (12.4%)	9 (9.1%)	9 (11.7%)	8 (9.9%)	2 (5.7%)	2 (6.5%)
Compost	19 (9.6%)	10 (9.5%)	13 (13.1%)	9 (11.7%)	11 (13.6%)	6 (17.1%)	5 (16.1%)
Total	198 (100%)	105 (100%)	99 (100%)	77 (100%)	81 (100%)	35 (100%)	31 (100%)

PHB: Poly(β-hydroxybutyrate), PCL: Poly(ε-caprolactone), PES: Poly(ethylene succinate).

order of PHB > PCL > PES (Hoang et al. 2007b). These results indicated that the number of polyester-degraders was differed greatly depending on differences in natural surroundings.

Among the largest number of PCL-degrading actinomycetes (198 isolates), 77 (38.9%) isolates were PCL and PHB-degraders, 81 (40.9%) isolates were PCL and PES-degraders, and 31(15.7%) isolates could degrade the three polyesters PCL, PES and PHB (Table. 1). In addition, within the 105 PHB-degraders and 99 PES-degraders, 35 isolates could degrade both PHB and PES. Among the 31 isolates that were capable of degrading PHB, PCL and PES, 14 isolates originated in ground soil (45.2%), four isolates come from hot spring (12.9%), two isolates from sediment (6.5%), five isolates from compost (16.1%) and six isolates from garden soil (19.4%). The sorting of thermophilic actinomycetes capable of degrading different polyesters was shown in (Table 1). From the soils of ground, garden and hot spring, a total of 149 different PCL-degrading isolates, 59 and 62 isolates could also degrade PHB and PES, respectively. A total of 27 isolates were obtained capable of degrading both PHB and PES and 24 isolates formed clear zones on plates containing PCL, PHB and PES. However, less isolates that degraded these three polyesters were obtained from the sediment and compost compared to isolates from the soils. Thus, we divided the polyester-degrading thermophilic actinomycetes into three main groups: (1). The PCL, PHB and PES-degrading isolates are specialized to depolymerize only one polyester. (2). Isolates showed degradation ability toward various combinations of two polyesters among PCL, PHB and PES. (3). Isolates could hydrolyze all three polyesters: PCL, PHB and PES, showed a wide substrate spectrum of polyester degradation.

We found that the clear zones formed by certain isolates on one polyester were not distinct as on others. Obviously, all thermophilic actinomycetes isolates degrade three polyesters with different extent, implying that different enzymes may be involved in the degradation of PCL, PHB and PES or the degrading agents excreted by the isolates demonstrate a wide range of substrate specificity. There are several PHB depolymerase have been isolated and purified from various microorganisms, it shows that extracellular PHB depolymerases are ubiquitous in the environment (Tokiwa and Calabia 2004). Most PHA-degrading bacteria have been analyzed produce only one PHA depolymerase, but *Pseudomonas lemoigne* produces at least five different extracellular PHA-depolymerases (Tokiwa and Calabia 2004).The three of them PHB-depolymerases (PHB-depolymerases A,B and D) are specific for PHB and P(3HB-*co*-3HV). The other two PHB depolymerases (PHB-depolymerases C and poly (D-3-hydroxyvalerate (PHV) depolymerase) also degrade both PHB and PHV (Jendrossek 2001). The isolates *Streptomyces* strain MG, *Aspergillus* ST-01 and *Bacillus* TT96 produced more than one enzyme that could degrade PHB, PCL, PBS and PES. It is likely that the enzymes produced from these strains have a wide range of substrate specificity (Tokiwa and Calabia 2004). Further study is necessary to find out the nature of the enzymes produced by the actinomycetes strains.

Identification of actinomycetes could degrade three polyesters

To characterize, the 31 isolates which have the ability to degrade three polyesters. These 15 (48.4%) isolates were therefore identified as members of the genus *Streptomyces*, 8 isolates (25.8%) were assigned to the genus *Microbispora* and four isolates (1.3%) were identified as the genus *Actinomadura*. The three isolates (0.97%) were considered as member of the genus *Thermoactinomyces.* One blue-color isolate was assigned to the genus *Saccharomonospora*

Some thermophilic actinomycetes could degrade polyesters were reported, including the degradation of terephthalic acid by *Thermobifida fusca* (former name: *Thermomonospora fusca*) (Kleeberg et al. 1998); degrading of poly(tetramethylene succinate, PTMS), PCL, PHB and PLA by *Microbispora rosea* subsp. *aerata* IFO 14046; *Microbispora rosea* subsp. *aerata* IFO 14047, *Excellospora japonica* IFO 144868, *E. viridilutea* JCM 339 in PTMS degradation (Jarerat and Tokiwa 2001); degradation of PHB, PES, poly(ester cargonate, PEC) ,PCL and poly(butylenes succinate, PBS) by *Streptomyces* sp. strain MG (Calabia and Tokiwa 2004).

Conclusions

Three hundrend and fourty-one thermopnilic actinomycetes isolated from various environments were capable of degrading polyesters PCL, PHB and PES. The occurrence of PCL-degrading actinomycetes was more frequent than PHB or PES degraders and the abundance of polyester degraders was distributed mainly on soils from the ground, garden and hot spring. The 31 isolates of PHB-, PCL- and PES-degraders were identified as members of the genus *Actinomadura*, *Microbispora*, *Streptomyces*, *Thermoactinomyces* and *Saccharomonospora*. To our knowledge, this is the first time to survey the distribution of polyester-degrading thermophilic actinomycetes from Taiwan, and also the first report of polyester-degradation in genera *Actinomadura* and *Saccharomonospora*.

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