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Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*

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ABSTRACT

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Aim: To select a polyethylene-degrading micro-organism and to study the factors affecting its biodegrading activity.

Methods and Results: A thermophilic bacterium *Brevibaccillus borstelensis* strain 707 (isolated from soil) utilized branched low-density polyethylene as the sole carbon source and degraded it. Incubation of polyethylene with *B. borstelensis* (30 days, 50°C) reduced its gravimetric and molecular weights by 11 and 30% respectively. *Brevibaccillus borstelensis* also degraded polyethylene in the presence of mannitol. Biodegradation of u.v. photo-oxidized polyethylene increased with increasing irradiation time. Fourier Transform Infra-Red (FTIR) analysis of photo-oxidized polyethylene revealed a reduction in carbonyl groups after incubation with the bacteria.

Conclusions: This study demonstrates that polyethylene – considered to be inert – can be biodegraded if the right microbial strain is isolated. Enrichment culture methods were effective for isolating a thermophilic bacterium capable of utilizing polyethylene as the sole carbon and energy source. Maximal biodegradation was obtained in combination with photo-oxidation, which showed that carbonyl residues formed by photo-oxidation play a role in biodegradation. *Brevibaccillus borstelensis* also degraded the CH_2 backbone of nonirradiated polyethylene.

Significance and Impact of the Study: Biodegradation of polyethylene by a single bacterial strain contributes to our understanding of the process and the factors affecting polyethylene biodegradation.

Keywords: biodegradation, *Brevibacillus borstelensis*, polyethylene, thermophilic bacteria, u.v. photo-oxidation.

INTRODUCTION

Nondegradable plastic materials are widely used in industry and agriculture. Because of their high durability, they accumulate in the environment at a rate of *ca* 25 million tons per year (Orhan and Buyukgungor 2000). Polyethylene appears to be one of the most inert plastic materials. Indeed, in a long-term study on the biodegradation of ^{14}C-labelled polyethylene, Albertsson and Karlsson (1990) found that after 10 years of incubation in soil, <0·5% carbon (as CO_2) by weight was evolved from an u.v.-irradiated polyethylene sheet. Nonirradiated polyethylene emitted <0·2% carbon dioxide during the same time. Furthermore, no signs of deterioration could be observed in a polyethylene sheet that had been incubated in moist soil for 12 years (Potts 1978) and only partial degradation was observed in a polyethylene film buried in soil for 32 years (Otake *et al.* 1995).

From a chemical perspective, we would expect polyethylene to be biodegradable, as linear alkenes are usually subject to biodegradation. However, for polyethylene there is an inverse relationship between molecular weight and biodegradability. Linear hydrocarbon oligomers with molecular weights lower than 620 support microbial growth, while those having higher molecular weights are not utilized (Haines and Alexander 1974; Potts 1978). It is widely accepted that the resistance of polyethylene to biodegradation stems from its high molecular weight, its

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three-dimensional structure and its hydrophobic nature, all of which interfere with its availability to micro-organisms. Nevertheless, several studies have demonstrated partial biodegradation of polyethylene after u.v. irradiation (Cornell *et al.* 1984), thermal treatment (Albertsson *et al.* 1998; Volke-Sepulveda *et al.* 2002) or oxidation with nitric acid (Brown *et al.* 1974). Furthermore, a synergistic effect has been found between photo-oxidation and biodegradation of polyethylene (Albertsson *et al.* 1987). Apparently, the biodegradation of polyethylene is enhanced by oxidation pretreatment, which increases surface hydrophilicity by the formation of carbonyl groups that can be utilized by micro-organisms (Albertsson 1978, 1980; Cornell *et al.* 1984).

Biodegradation resulting from the utilization of polyethylene as a nutrient (i.e. a carbon source) may be more efficient if the degrading micro-organism forms a biofilm on the polyethylene surface. However, the hydrophobicity of the polyethylene interferes with the formation of a microbial biofilm. Attempts to facilitate colonization of polyethylene by adding nonionic surfactants to the culture medium promoted the biodegradation of polyethylene (Albertsson *et al.* 1993; Ehara *et al.* 2000). Presumably, the surfactant increased the hydrophilicity of the polyethylene surface and thus facilitated the adhesion of bacteria to the polymer.

Recently, we isolated a strain of *Rhodococcus ruber* that was found to colonize and degrade polyethylene. The ability of this bacterium to form a biofilm on polyethylene was attributed to the hydrophobicity of its cell surface (Gilan *et al.* 2004). Addition of a small amount (0·05%) of mineral oil to the culture medium increased both biofilm formation and the subsequent biodegradation of the polyethylene, presumably by increasing the hydrophobic interactions between the bacterial biofilm and the polymer (Gilan *et al.* 2004).

In the present study, we describe a newly isolated bacterial thermophilic strain, *B. borstelensis*, which was more effective in degrading branched low-density polyethylene than the *Rhodococcus* strain. We studied the effect of u.v. photo-oxidation on the extent of biodegradation and analysed the activity of the bacterial biofilm in the course of colonization of the polyethylene.

MATERIALS AND METHODS

Bacterial strain and culture conditions

Nutrient broth (NB) or nutrient agar (NA) media (Difco) were used to maintain the bacterial cultures. Unless otherwise specified, liquid cultures (100 ml) were incubated in flasks (250 ml) on a rotary shaker (150 rev min^{-1}) at 50°C.

Bacterial strains assayed for their ability to utilize polyethylene as the sole source of carbon and energy were grown in two different minimal media: (i) synthetic medium (SM) medium containing (g l^{-1} of distilled water): NH_4NO_3, 1·0; $MgSO_4 \cdot 7H_2O$, 0·2; K_2HPO_4, 1·0; $CaCl_2 \cdot 2H_2O$, 0·1; KCl, 0·15; and yeast extract (Difco), 0·1; and 1·0 mg l^{-1} of each of the following micro-elements: $FeSO_4 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$ and $MnSO_4$; (ii) VB medium (Vogel and Bonner 1956) modified as follows (g l^{-1}): KNO_3, 2·0; KH_2PO_4, 5·8; K_2HPO_4, 3·7; $MgSO_4 \cdot 7H_2O$, 0·25; yeast extract (Difco), 0·1; and 1 ml of trace-elements stock solution containing (g l^{-1} in 1 mol HCl l^{-1} solution): $FeSO_4 \cdot 7H_2O$, 2·78; $MnCl_2 \cdot 4H_2O$, 1·98; $CoSO_4 \cdot 7H_2O$, 2·81; $CaCl_2 \cdot 2H_2O$, 1·67; $CuCl_2 \cdot 2H_2O$, 0·16; $ZnSO_4 \cdot 7H_2O$, 0·29. pH 7·8. The first medium was used as general medium for isolation of strains capable of growing on polyethylene as the sole carbon source, and the second was found to be more suitable for strain 707 (see below). In some experiments, mineral oil [light-white oil; $d = 0{\cdot}84$ g l^{-1} (Sigma) or a nonionic surfactant, a polysorbate [Tween 60, 80 or 85 (Sigma)], was added to the minimal medium at a concentration of 0·01–0·5% (v/v) to test the effect of these substances on the colonization and biodegradation of polyethylene.

Biodegradation tests were performed with polyethylene films that had been dried overnight at 60°C, weighed, disinfested (30 min in 70% ethanol) and added to flasks, each containing 100 ml of mineral medium (*ca* 300 mg of film per flask). Each test consisted of three flasks (replicates).

Polyethylene

Branched low-density (0·92 g cm^{-3}) polyethylene (LDPE) with an average molecular weight of 191 000 (Ipiten®111) (Carmel Olefins, Haifa, Israel) produced in film form by Plastophil Hazorea (Kibbutz Hazorea, Israel) was used. The type designated LDPE did not contain any additives, whereas the type designated LDPE-L0235 contained a u.v. photosensitizer (an undisclosed compound designated L0235). Both types of film were kindly provided by Mr R. Harpaz of Plastopil Hazorea.

Ultraviolet irradiation of polyethylene

To simulate partial photolysis during the natural weathering of polyethylene exposed to the sun (e.g. polyethylene used for soil mulching or greenhouse cover), polyethylene samples were subjected to partial photolysis in a QUV Accelerated Weathering Tester (Q-Panel, Cleveland, OH, USA). The polyethylene was subjected to a programme of alternating exposure to u.v. (312 nm) and humidity – five cycles per day – (four of 4 h each and one of 3 h at 70°C) separated by 1-h intervals (at 50°C), during which water condensed on the polyethylene surface. Unless otherwise specified, the overall cumulative u.v. irradiation time to which the polyethylene samples were exposed was 60 h. Prior to transfer to liquid culture media, the polyethylene

films were cut into pieces (*ca* 3 cm × 3 cm each), weighed, disinfected in 70% ethanol and air-dried for 15 min in a laminar-flow hood.

Isolation of polyethylene-degrading bacteria

From a group of bacterial isolates that had been initially screened for utilization of a mixture of liquid waxes, a number of bacterial isolates were screened for their ability to grow on polyethylene as the sole source of carbon and energy. Soil samples taken from a polyethylene-waste disposal site at the polyethylene production plant of Carmel Olefins were plated on SM medium supplemented with 10 ml of a mixture containing intermediate-size polyethylene oligomers in the form of liquid waxes. To isolate mesophilic and thermophilic bacteria separately, plates were incubated for 2 weeks at either 30 or 50°C. Wax-degrading bacteria were identified by the production of clear zones around the colonies growing in the opaque wax-containing medium. These colonies of wax-degrading bacteria were further tested for their ability to grow in SM medium containing polyethylene as the sole carbon source.

Determination of dry weight of residual polyethylene

To facilitate accurate measurement of the weight of the residual polyethylene, the bacterial biofilm was washed off the polyethylene surface with a 2% (v/v) aqueous sodium dodecyl sulphate solution for 4 h and then with distilled water. The washed polyethylene was placed on a filter paper and dried overnight at 60°C before weighing.

Determination of molecular weight of polyethylene

The correlation between the intrinsic viscosity of the polyethylene and its molecular weight was used to estimate the reduction in the number average molecular weight (M_n) of biodegraded polyethylene. The intrinsic viscosity was determined by dissolving the polyethylene samples in 20 ml of *p*-xylene in screw-cap test tubes immersed in a 20-l glass water tank heated to 81 ± 0·05°C. The viscosity of a range of concentrations of dissolved polyethylene was measured with a capillary viscometer immersed in the heated glass water tank. The intrinsic viscosity and M_n were calculated using the 'Mark–Houwink' equation, according to Harris (1952).

Evaluation of bacterial hydrophobicity

Bacterial cell-surface hydrophobicity was estimated by the bacterial adhesion to hydrocarbon (BATH) test (Rosenberg *et al.* 1980), which is based on the affinity of bacterial cells for an organic hydrocarbon such as hexadecane. The more hydrophobic the bacterial cells, the greater their affinity for the hydrocarbon, resulting in transfer of cells from the aqueous suspension to the organic phase and a consequent reduction in the turbidity of the culture. For the BATH test, bacteria were cultured in NB medium until the mid-exponential phase, centrifuged and washed twice with phosphate–urea–magnesium (PUM) buffer containing (g l^{-1}): K_2HPO_4, 17; KH_2PO_4, 7·26; urea, 1·8 and $MgSO_4 \cdot 7H_2O$, 0·2. The washed cells were resuspended in PUM buffer to an O.D. 400 nm value of 1·0–1·2. Aliquots (1·2 ml each) of this suspension were transferred to a set of test tubes, to which increasing volumes (ranging: 0–0·2 ml) of hexadecane were added. The test tubes were shaken for 10 min and then allowed to stand for 2 min to facilitate phase separation. The turbidity of the aqueous suspensions was measured at O.D. 400 nm. Cell-free buffer served as the blank.

Estimation of bacterial biomass colonizing the polyethylene

As the bacterial cells were strongly attached to the polyethylene surface, it was impossible to estimate the population density by standard techniques, such as direct cell counting or plating. Therefore, the population density of the biofilm on the polyethylene surface was estimated by determining the concentration of extractable protein. Colonized polyethylene samples taken from the bacterial liquid culture were washed briefly with water and then boiled for 30 min in 5 ml of 0·5 mol l^{-1} NaOH. The suspension was centrifuged, the supernatant was kept aside and the pellet was subjected to the same procedure once again. The two supernatants were combined and the protein concentration was determined according to Sedmak and Grossberg (1977).

Estimation of bacterial biofilm viability

The activity and viability of the bacterial biofilm was determined by two indirect methods: (i) by measuring the hydrolysis of fluoroscein diacetate (FDA) which yields fluoroscein according to Schnurer and Rosswall (1982); and (ii) by determining cell viability with a Live/dead® *Bac*Light™ Bacterial Viability Kit (Molecular Probes Eugen, OR, USA) according to the manufacturer's instructions. For the first method, a piece of polyethylene film was transferred from SM cultures to a flask containing 60 ml of sodium phosphate buffer, 60 mmol l^{-1}, pH 7·6. A 0·3-ml aliquot of FDA solution in acetone was added to give a final concentration of 10 μg ml^{-1}. The flasks were shaken at 140 rev min^{-1} at 30°C, and 1-ml aliquots were withdrawn at various times during the incubation. The samples were centrifuged at 13 400 *g* and read in a spectrophotometer at 494 nm. Samples without FDA served as the blanks and a

sample of polyethylene from a sterile SM medium served as the control.

FTIR of polyethylene

Changes in the polyethylene structure following u.v. irradiation and subsequent incubation with bacteria were analysed by ATR-FTIR spectroscopy (Irscope 2, Ettlingen, Germany; Equinox 55, Bruker, USA). Three types of polyethylene samples were analysed: (i) untreated; (ii) u.v. irradiated (as described above); and (iii) u.v. irradiated and then incubated with strain 707.

Identification of strain 707

Total genomic DNA for 16S rDNA amplification was isolated from cells of strain 707 grown to the late exponential phase by means of a standard protocol (Ausubel *et al.* 1992). Amplification of the 5′ end of the 16S rDNA gene was performed with universal primers (*Escherichia coli* numbering): forward primer (8-F) 5′-AGAGTTTGA-TYMTGGCTCAG-3′ and reverse primer: (1942R) 5′-GGTTACCTTGTTACGACTT-3′ (Lane 1991). The similarity of the sequence obtained against known deposited 16S rDNA sequences from closely related bacteria was tested with BLASTN 2.2.1 (Altschul *et al.* 1997).

RESULTS

Isolation of strain 707

In the screening for bacteria capable of growing on a carbon-free SM supplemented with liquid wax, a few bacterial colonies were shown to utilize and degrade the wax, as was evident from the clear zones around these colonies. One such bacterium – a thermophilic, gram-positive, spore-forming rod with a growth optimum at 50°C – was designated as strain 707. It could grow in SM or VB medium containing polyethylene films as the sole carbon source. Based on 16S rRNA sequence, with 99·7% similarity with *B. borstelensis*, strain 707 was identified as *B. borstelensis* (GenBank accession number AY764129).

Effect of carbon and nitrogen depletion on the biodegradation of polyethylene

It has been suggested that nitrogen limitation may facilitate the biodegradation of complex, slowly biodegradable polymers such as lignin (Iioshi *et al.* 1998). Therefore, we were interested in evaluating whether nitrogen limitation would enhance the biodegradation of polyethylene by *B. borstelensis* strain 707. However, as strain 707 was isolated under conditions of carbon deprivation, we tested not only the effect of nitrogen (KNO_3) but also carbon (mannitol) limitation, alone and in combination, on the biodegradation of polyethylene by *B. borstelensis* strain 707 (Table 1).

Table 1 Effect of mannitol and potassium nitrate concentration on the biodegradation of (u.v. irradiated, for 60 h) polyethylene (LDPE-L0235) by *Brevibacillus borstelensis* strain 707 after 30 days of incubation at 50°C

Nutrient composition	Mannitol* (%)	KNO_3* (%)	Dry weight loss of polyethylene ± S.D. (%)†
Complete medium (control)	100	100	6·2 ± 1·2
Carbon limitation	50	100	5·7 ± 0·1
	20	100	6·8 ± 0·4
	0	100	11·0 ± 1·1
Nitrogen limitation	100	50	8·0 ± 0·6
	100	20	9·7 ± 0·7
	100	0	7·2 ± 0·1
Carbon and nitrogen limitation	50	50	6·9 ± 0·4
	20	20	6·8 ± 0·7
	0	0	7·9 ± 0·7

*Maximal concentrations (100%) of mannitol and potassium nitrate in VB medium were 5 and 2 g l^{-1} respectively.
†Experimentally obtained values minus values for noninoculated control, which were normally <0·2%, are given ($n = 3$).

The data show that the highest degree of polyethylene biodegradation (11·0 ± 1·1% weight loss) after 30 days of incubation was achieved in a mannitol-free medium supplemented with the standard nitrogen concentration. This is not surprising as carbon deprivation promotes utilization of polyethylene as the sole carbon source. Surprisingly, the presence of mannitol (even at a level of 5 g l^{-1}) did not prevent the biodegradation of polyethylene, although, in general, the extent of biodegradation was lower in the mannitol-free media. Regardless of the concentration of mannitol *B. borstelensis* strain 707 was capable of degrading polyethylene under conditions of reduced concentrations of nitrogen. When the polyethylene was incubated in a medium containing 100% mannitol (5 g l^{-1}) and a reduced nitrogen concentration (20%; 0·4 g l^{-1}) it degraded and lost 9·7 ± 0·7% of its weight. This weight loss was higher than the control (100% mannitol and 100% KNO_3; 6·2 ± 1·2%) but was similar to that obtained in a mannitol-free medium supplemented with 2 g l^{-1} of nitrogen (11·0 ± 1·1%).

Ultraviolet irradiation of polyethylene as a photo-oxidative pretreatment to biodegradation

For both types of polyethylene film – standard LDPE and LDPE-L0235 containing a pro-oxidant – u.v. irradiation enhanced the biodegradation by *B. borstelensis* (measured as dry weight loss) compared with nonirradiated polyethylene

Table 2 Effect of 60-h u.v. irradiation on the biodegradation by *Brevibacillus borstelensis* strain 707 of standard (LDPE) and pro-oxidant containing (LDPE-L0235) polyethylene films after 30-days incubation, at 50°C, in a mannitol-free VB medium containing 2 g l^{-1} KNO_3

Polyethylene	u.v. irradiation	Dry weight loss ± S.D. (%)*
LDPE	+	6·2 ± 0·3 b
LDPE	−	2·5 ± 0·3 c
LDPE-L0235	+	7·8 ± 0·8 a
LDPE-L0235	−	5·6 ± 0·5 b

*Experimentally obtained values minus values for noninoculated control, which were normally <0·2% ($n = 3$). Values accompanied by a similar letter are not significantly different according to Duncan's multiple-range test ($P = 0·05$).

samples (Table 2). Ultraviolet irradiation for 60 h before incubation with the bacterium increased the biodegradation of LDPE-L0235 by *ca* 39% and when compared with the standard irradiated LDPE, a 25% increase was found. Furthermore, *B. borstelensis* strain 707 could grow on nonirradiated LDPE, as indicated by a 2·5% reduction in the dry weight (Table 2). The effect of the time of exposure to u.v. irradiation on the biodegradation of LDPE-L0235 was studied by irradiating samples for 60, 80, 100 and 120 h prior to their incubation with *B. borstelensis*. It was found that u.v. irradiation alone exerted a reduction in molecular (M_n) weight (Fig. 1). When plotting the u.v. irradiated polyethylene against biodegradation rate, for every incubation period a direct effect ($R^2 = 0·92–0·96$) of the duration of u.v. irradiation on biodegradation of the polyethylene by *B. borstelensis* strain 707 was evident. Maximal biodegradation, as reflected by upto 17% and 34% reduction in gravimetric and molecular (M_n) weights, respectively, was obtained for the combination of 120 h of irradiation followed by 90 days of incubation with the bacterium (Fig. 1).

The FTIR spectrum of u.v. photo-oxidized polyethylene (LDPE-L0235) showed a typical carbonyl peak at 1712 cm^{-1}. Incubation of the photo-oxidized polyethylene with *B. borstelensis* for 30 days showed a marked reduction in the amount of carbonyl residues (Fig. 2). The reduction in carbonyl residues was also estimated in terms of a carbonyl index, which is the ratio between the absorbance peak of carbonyl to that of CH_2 at 1462–1463 cm^{-1}. It was found that the incubation of the u.v. irradiated LDPE-L0235 with *B. borstelensis* strain 707 reduced the carbonyl index by *ca* 70% (Table 3).

Colonization of polyethylene by *B. borstelensis* strain 707

Brevibacillus borstelensis strain 707 incubated in a liquid medium (VB) containing polyethylene as the sole carbon source, colonized the polyethylene surface poorly and formed a sparse biofilm. This finding was not surprising as formation of a biofilm on polyethylene, which is a hydrophobic polymer, requires the bacterial surface also to be hydrophobic, and the BATH assay demonstrated that the hydrophobicity of *B. borstelensis* strain 707 was very low resulting in only a 10% reduction in turbidity of the bacterial suspension because of the transfer of cells from the aqueous to the organic phase (i.e. octane, data not shown).

In an attempt to improve the adherence and colonization of the polyethylene, nonionic surfactants (Tween 60, 80 and 85) and mineral oil were tested as supplements to

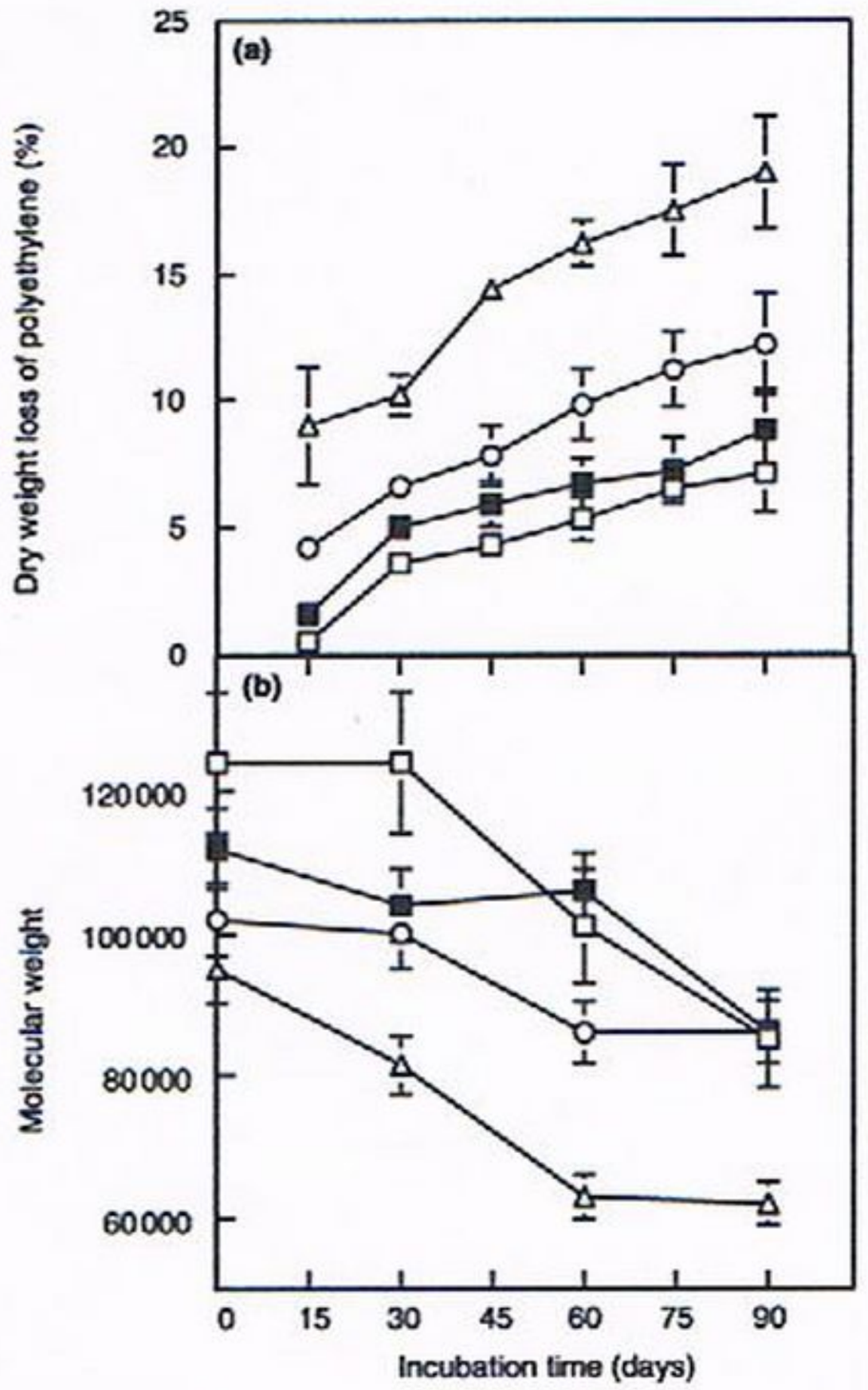

Fig. 1 Evolution of gravimetric (a) and molecular weight (M_n) (b) of u.v.-irradiated polyethylene (LDPE) as a function of incubation for 60 (□), 80 (■), 100 (○) and 120 h (△) with *Brevibacillus borstelensis* strain 707. Cultures were grown at 50°C in a mannitol-free VB medium containing 2 g l^{-1} of KNO_3. Values are mean of three replicates ± S.D. The figure depicts experimentally obtained values minus values for noninoculated control, which were normally <0·2%. The M_n of nonirradiated sterile polyethylene was 123 000

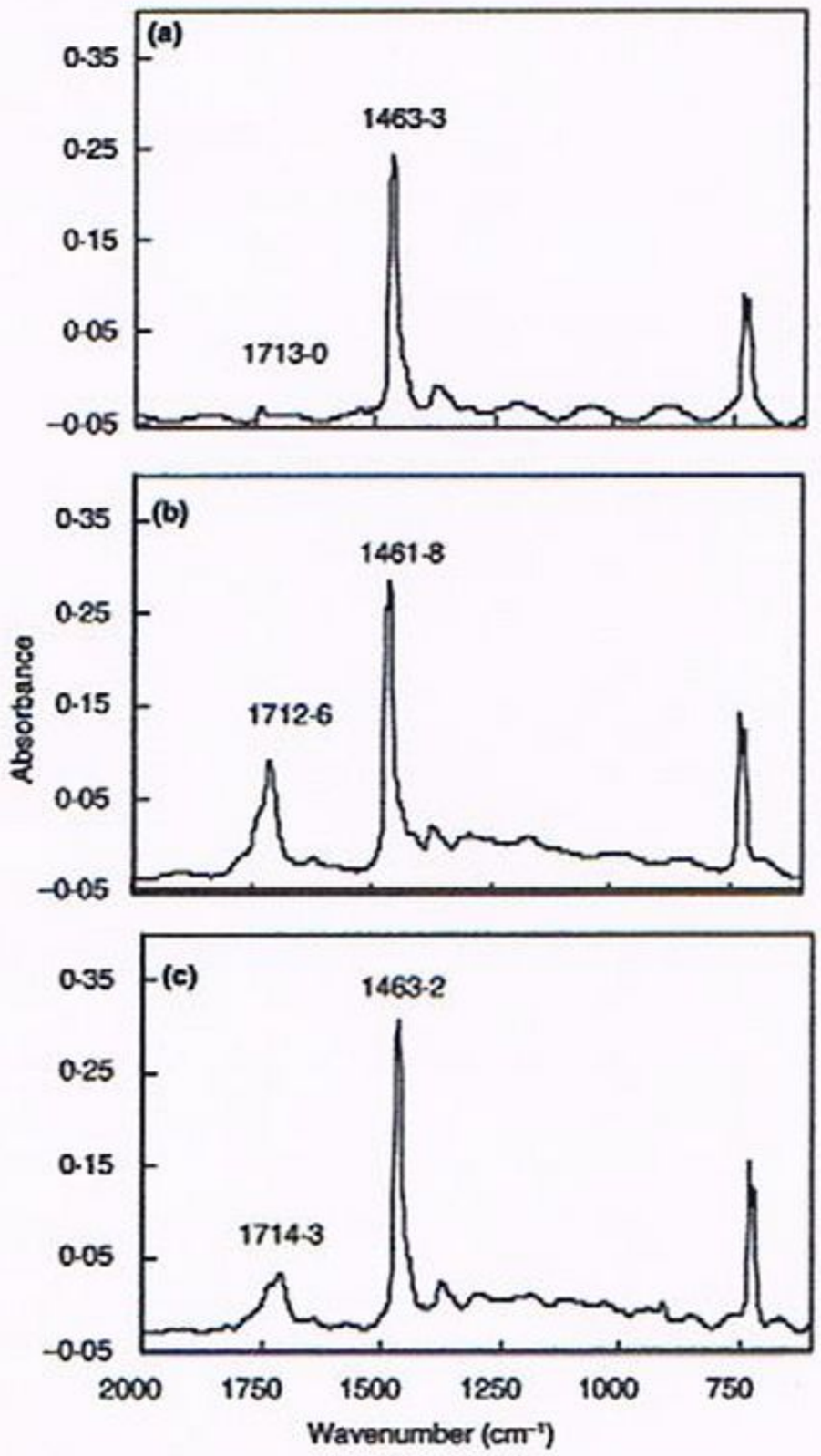

Fig. 2 FTIR spectra of LDPE-L0235: (a) untreated (noninoculated); (b) u.v. irradiated for 100 h; and (c) u.v. irradiated for 100 h followed by 30 days of incubation with *Brevibacillus borstelensis* strain 707. Cultures were grown at 50°C in a mannitol-free VB medium containing 2 g l^{-1} of KNO_3

Table 3 Carbonyl index obtained from FTIR spectra of u.v.-irradiated (LDPE-L0235) polyethylene incubated for 30 days at 50°C with *Brevibacillus borstelensis* vs noninoculated polyethylene

Polyethylene	Carbonyl index ($A_{C=O}$:A_{CH_2})*
Nontreated (control)	0·01 ± 0·01
u.v. irradiated	0·24 ± 0·05
u.v. irradiated + *B. borstelensis* strain 707	0·07 ± 0·01

*The carbonyl index expresses the ratio between the absorbance peak of the carbonyl (1712 cm^{-1}) and that of the CH_2 groups at 1462–1463 cm^{-1}. Values are means ± S.D. ($n = 3$).

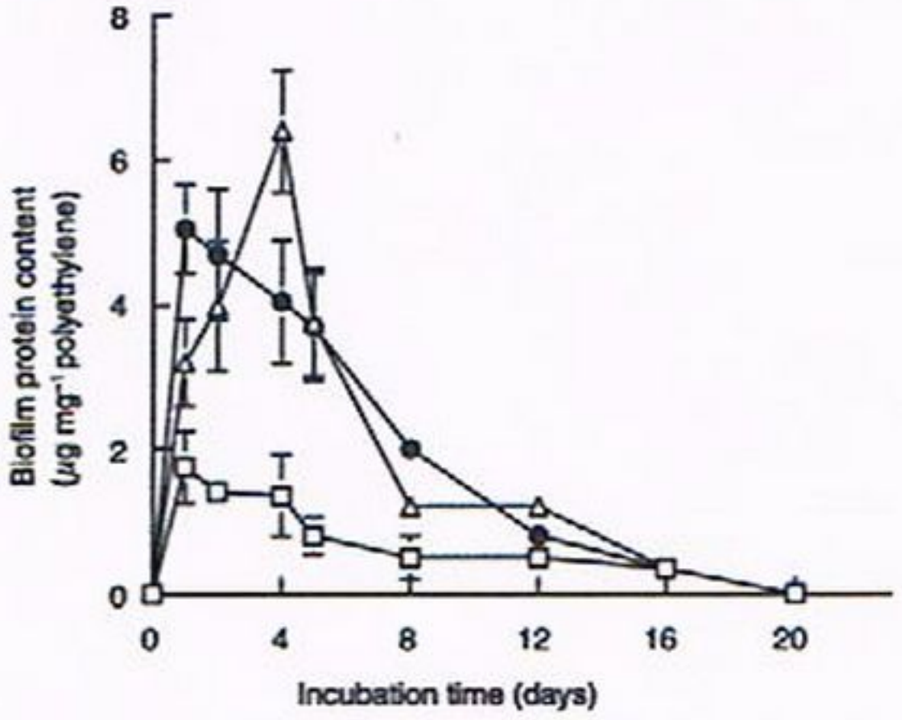

Fig. 3 Effect of 0·05% (w/v) Tween 85 (●) or mineral oil (△) and nonamended VB medium (□), on the extractable protein content of the biofilm formed by *Brevibacillus borstelensis* on u.v.-irradiated (60 h) polyethylene LDPE-L0235. Cultures were grown at 50°C in a mannitol-free VB medium containing 2 g l^{-1} of KNO_3. Values are mean of three replicates ± S.D.

the medium (Fig. 3). Without any supplement, bacterial colonization (measured as extractable protein) of the polyethylene by *B. borstelensis* strain 707 rose during the first day and remained stable for four additional days followed by a gradual decrease until the 20th day of incubation when no extractable protein could be detected. Tween 85 and mineral oil enhanced colonization, but the reduction in extractable protein then fell at a greater rate than that of the control (Fig. 3). Supplementing the medium with Tween 60 or 80 had a similar effect to that of Tween 85 (data not shown).

Biodegradability of polyethylene with time

Our standard biodegradation assay for polyethylene lasts for *ca* 30 days. During this period, the bacteria utilize the carbonyl residues and reduce their concentration. Therefore, it was important to verify that the biodegradability of polyethylene with a reduced level of carbonyls would not be diminished: in other words, would the bacteria consume a $[CH_2]_n$ polyethylene backbone that was almost devoid of carbonyls?. To answer this question, we tested the biodegradability of polyethylene over two consecutive 30-day periods of incubation with *B. borstelensis*. After each incubation period, the polyethylene samples were washed, dried and weighed. No significant difference was found between the biodegradation rate during the two incubation periods (Fig. 4). The total reduction in gravimetric and

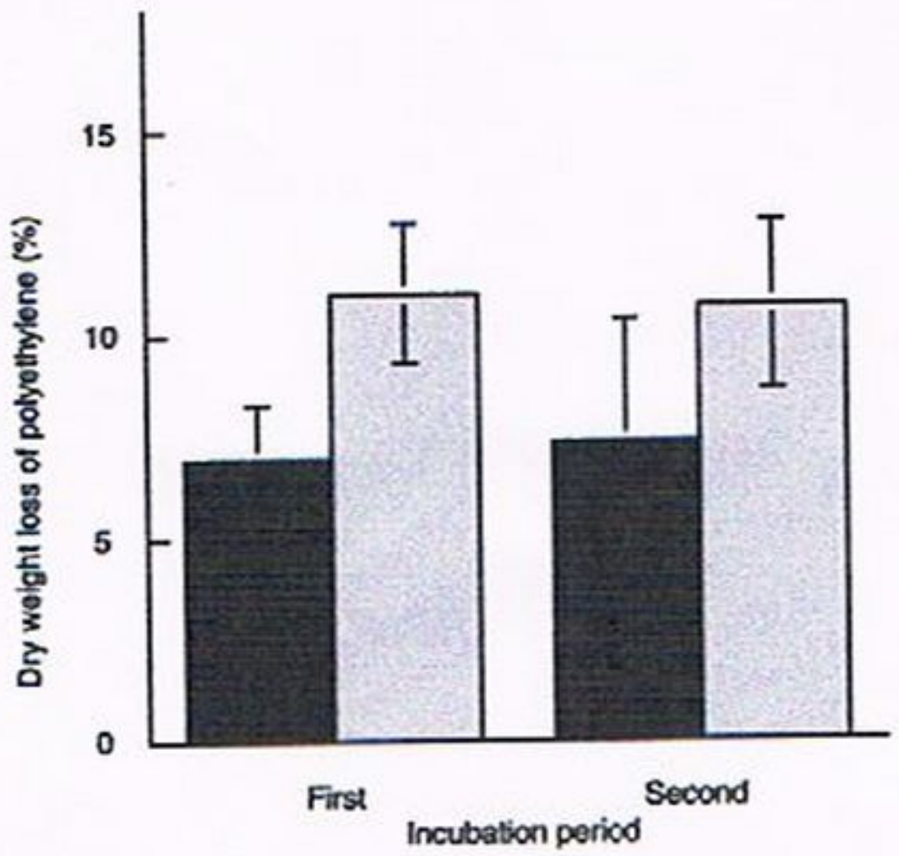

Fig. 4 Gravimetric (■) and molecular (M_n) (▥) weights of u.v.-irradiated (60 h) polyethylene (LDPE-L0235) after one or two consecutive 30-day periods of incubation with *Brevibacillus borstelensis* strain 707, at 50°C, in a mannitol-free VB medium containing 2 g l^{-1} of KNO_3. Experimentally obtained values minus values for noninoculated control, normally <0·2%, are given. The M_n of the noninoculated control was 123 000. Values are mean of three replicates ± S.D.

molecular weights of the polyethylene samples, determined at the end of each of the two incubation periods, was 14 and 21% respectively.

DISCUSSION

We have isolated a thermophilic bacterial strain 707, identified as *B. borstelensis* that was found capable of utilizing standard and photo-oxidized polyethylene as the sole carbon source. During the 1-month incubation with *B. borstelensis*, the maximal biodegradation of the polyethylene measured in terms of gravimetric and molecular weight loss was *ca* 11 and 30% respectively. This biodegradation level is higher than the values reported for polyethylene incubated in soil for 10 years, ranging from 3·5 to 8·4%, reported by Albertsson and Karlsson (1990). These low rates are in agreement with the argument of Otake *et al.* (1995) that 10 years is a relatively short period for the biodegradation of synthetic polymers such as polyethylene.

Despite the fact that *B. borstelensis* was isolated from a culture containing polyethylene as the sole carbon source, it was even capable of degrading polyethylene (albeit at a lower rate) in the presence of another carbon source such as mannitol (the standard carbon source in VB medium). This finding raises the question whether co-metabolism may take place in the biodegradation of polyethylene. A similar phenomenon was reported by Volke-Sepulveda *et al.* (2002), who showed that addition of ethanol to fungal cultures containing polyethylene improved the biodegradation of polyethylene.

The combination of u.v. photo-oxidation and biodegradation had an additive effect on the degradation of polyethylene containing a u.v. pro-oxidant. As expected, increasing the u.v. irradiation time resulted in a parallel increase in biodegradation. Surprisingly, no difference was found in the biodegradation of u.v.-irradiated polyethylene containing pro-oxidant and that of the nonamended polyethylene.

It is widely accepted that the short fragments formed in u.v.-irradiated polyethylene are readily utilized by various micro-organisms. For example, incubation of polyethylene with *Arthrobacter paraffineus* resulted in a small increase in the average molecular weight of the polyethylene, apparently because of the consumption of the low molecular weight fragments by the bacteria (Albertsson *et al.* 1998). However, the fact that the M_n of polyethylene incubated with *B. borstelensis* was significantly reduced indicates that strain 707, unlike most other tested micro-organisms, is also capable of degrading the high molecular weight fragments. Furthermore, the fast utilization of the carbonyl residue formed by u.v. irradiation, probably enables further degradation of the polyolefin backbone $(CH_2)_n$. This assumption is supported by the similar degradation level of polyethylene films during two consecutive incubations, 30 days each, with *B. borstelensis*. Presumably, if *B. borstelensis* strain 707 had been able to degrade only the carbonyl residues, the biodegradation rate in the second incubation period would have been significantly lower. Moreover, the biodegradation of nonirradiated polyethylene by *B. borstelensis* indicates that it is capable of degrading unmodified polyethylene.

Recently, we have reported the isolation of a strain of *R. ruber* that colonized polyethylene surface and formed a massive biofilm on it: a process that seemed to be a prerequisite for biodegradation (Gilan *et al.* 2004). In contrast, strain 707 of *B. borstelensis*, despite being a weak biofilm producer, was more efficient in polyethylene biodegradation than *R. ruber*. The low degree of biofilm production of *B. borstelensis* is probably because of the nonhydrophobic nature of its cell surface. Indeed, several cell-surface hydrophobicity tests confirmed the nonhydrophobic nature of *B. borstelensis*. Recently, we have found that colonization of u.v.-irradiated polyethylene exceeds that of nonirradiated (Sivan, A. and Pavlov, V., unpublished results). This may be because of the formation of carbonyl groups that contribute to the hydrophilicity of polyethylene. It may be hypothesized that biodegradation of photo-oxidized polyethylene by *B. borstelensis* is mediated by a

small number of cells that adhere strongly to the polyethylene surface and are not detected in the protein extract or FDA-hydrolysis test. Indeed, staining of the polyethylene samples with the sensitive Live/Dead kit showed (even in 6-week-old cultures) a very sparse, but viable, biofilm (data not shown) that could be responsible for the polyethylene biodegradation.

Plans to employ selected micro-organisms in the biodegradation of polyethylene waste often focus on compostation processes. It appears that *B. borstelensis* is a suitable candidate for this purpose, as like other species of *Brevibacillus*, it is thermophilic, capable of growing and degrading polyethylene at 50–60°C.

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