Journal of Industrial Microbiology (1996) 17, 463–469


# Biodiversity of microorganisms that degrade bacterial and synthetic polyesters

J Mergaert and J Swings

*Laboratorium voor Microbiologie, Vakgroep Biochemie, Fysiologie en Microbiologie, Universiteit Gent, KL Ledeganckstraat 35, B-9000 Gent, Belgium*

**The biodiversity and occurrence in nature of bioplastic-degrading microorganisms are exemplified by the identification of 695 strains, isolated from different environments, such as soils, composts, natural waters, and sludge, that are able to degrade the bacterial polyester poly(3-hydroxybutyrate) *in vitro*. These microorganisms belong to at least 57 different taxa, including Gram-negative and Gram-positive bacteria, streptomycetes, and moulds. The literature on the biodiversity of poly(3-hydroxybutyrate)-degrading microorganisms is reviewed. The degrading abilities of 171 streptomycete strains were investigated on four different bacterial poly(3-hydroxyalkanoates), and the synthetic polyesters poly(ε-caprolactone) and BIONOLLE, and most of these strains degraded at least three different polymers.**

**Keywords:** bioplastics; biodiversity; biodegradation; streptomycetes; polyhydroxyalkanoates; poly(ε-caprolactone); BIONOLLE

## Introduction

Plastics play an important role in human society. Because of the low cost of production, ease of processing, and outstanding mechanical and physical properties, plastics, such as polyolefins, have replaced wood, cotton, paper and other natural substances in many applications, such as packaging, building materials, and commodity and hygienic products. However, because of their biological resistance, they finally end up in the non-degradable waste stream, and account for more than 20% of the municipal waste volume [14]. Efforts have been made to design and develop biodegradable alternatives, sometimes also referred to as bioplastics. The term bioplastics may refer to biodegradable plastics and/or the biological origin of the plastic. The best known are the polyhydroxyalkanoates (PHA), which are produced intracellularly in many bacteria as carbon and energy storage products, and are intrinsically biodegradable, because of their biological origin. The metabolism and role of these polymers have been extensively reviewed [1,10,37,39]. Poly(3-hydroxybutyrate) [P(3HB)] and copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate [P(3HB-co-3HV)] are the best known members of this polymer family; they are produced industrially by *Ralstonia eutropha* [42], and commercialized as BIOPOL by Monsanto (Billingham, UK), formerly Zeneca BioProducts. Their biodegradation has been extensively investigated in different natural environments, as well as under laboratory conditions [4,5,7,22–24,26,27], and hundreds of microorganisms that are able to degrade these polymers *in vitro*, and which originate from different environments such as soils, composts, natural waters and sludges, have been isolated and identified [22–28,35]. Apart from these commercially available plastics, copolymers containing very high portions (up to 99%) of 3-hydroxyvalerate [P(3HB-co-3HV)] can be produced by *Rhodococcus ruber* [13], and poly(3-hydroxyvalerate) [P(3HV)] homopolymer can be produced on a large scale by *Chromobacterium violaceum* [38]. Copolymers of 3-hydroxalkanoates with longer side chains, ie poly(3-hydroxyoctanoate) [P(3HO)] and poly(3-hydroxyoctanoate-co-3-hydroxydecanoate) [P(3HO-co-3HD)] can be produced by pseudomonads [41]. More recently, chemically synthesized biodegradable polyesters have received attention. The biodegradability of poly(ε-caprolactone) (PCL), a synthetic poly(6-hydroxyhexanoate), has been reviewed, and has recently been combined with natural polymers, such as starch, in multicomponent biodegradable plastics, such as MaterBi (Novamont, Novara, Italy) [2,12]. A number of chemically synthesized aliphatic polyesters have been commercialized as BIONOLLE by Showa Highpolymer (Japan). In contrast to BIOPOL, only a few reports have been published on microorganisms that are responsible for the degradation of bioplastics like MaterBi, PCL and BIONOLLE [15,31,32,34].

In the present paper we review the biodiversity and occurrence of P(3HB)-degrading microorganisms in the environment, and the abilities of streptomycetes to degrade four different poly(3-hydroxyalkanoates) and the synthetic polyesters PCL and BIONOLLE *in vitro*.

## Materials and methods

### Polymers

The following polymers were investigated: homopolymer P(3HB) (BIOPOL, high purity grade, batch No. GO8, Zeneca BioProducts, Billingham, UK), copolymer P(3HB-co-19% 3HV) (technical grade powder, batch No. PO16, Zeneca BioProducts), copolymer P(3HB-co-97%3HV) (powder, batch No. F15–31, produced in *Rhodococcus*

Correspondence: J Mergaert, Laboratorium voor Microbiologie, Vakgroep Biochemie, Fysiologie en Microbiologie, Universiteit Gent, KL Ledeganckstraat 35, B-9000 Gent, Belgium
Received 4 April 1996; accepted 17 September 1996

*ruber*, Zeneca BioProducts), P(3HO-co-70%3HD) (solution cast, rubbery film, Zeneca BioProducts), PCL (powder type TONE-P767, or granules type TONE-P787, Brenntag Eurochem GmbH, Essen, Germany), BIONOLLE (granules type 3020, polybutylene succinate adipate copolymer, batch No. TB 43010 F, Boehringer Ingelheim KG, Ingelheim/Rhein, Germany).

### Media

Bioplastic degradation was investigated on polymer overlay plates, ie solidified mineral base [9], supplemented with 0.005% yeast extract (Oxoid, Unipath Ltd, Basingstoke, UK) and 0.01% casein hydrolysate (vitamin-free salt-free) (ICN Biomedicals, Cleveland, Ohio, USA), overlaid with the same medium supplemented with polymer. Overlays containing 0.25% P(3HB) and P(3HB-co-19%3HV) were prepared by suspending powder in the medium, through sonication, prior to autoclaving the suspension. For other polymers, 1 g of polymer was dissolved in 10 ml of dichloromethane, and this solution was emulsified in 200 ml mineral medium [9], containing 4 g agar, using an UltraTurrax blender (Janke & Kunkel, Staufen, Germany). By autoclaving the solution for 15 min at 121°C, during which the solvent was evaporated, stable suspensions were obtained. The still warm suspensions were poured as overlays on minimal agar medium. On such plates degrading microorganisms can easily be recognized by the formation of clear zones around their colonies, due to degradation of the insoluble polymers to soluble degradation products.

### Biodegrading microorganisms

Almost 500 of the bioplastic-degrading microorganisms were isolated earlier [22–27], by selection on overlay plates containing P(3HB), P(3HB-co-10%3HV) or P(3HB-co-19%3HV) powder, and inoculated with dilutions of soil, compost, and sludge suspensions or natural waters. Nearly 200 additional strains were isolated using the same methods, and a few strains were kindly provided by D Jendrossek (University of Göttingen, Germany) or A Heinemann (University of Stuttgart, Germany). Most of the streptomycetes, including reference strains obtained from LMG (Culture Collection of the Laboratorium voor Microbiologie, Gent, Belgium), were listed earlier [28]. For testing of bioplastic degradation by the streptomycete cultures, strains were grown on ISP medium 3 (Difco Laboratories, Detroit, MI, USA) for the production of spores, which were harvested and suspended in sterile glycerol : water (1 : 9), supplemented with 0.5% Triton X-100 (Beckman Instruments, Fullerton, CA, USA). Plates with overlays were inoculated with drops of these suspensions and incubated at 28°C, and appearance of clearing around the inocula was observed within 30 days. Alternatively, spore masses were streaked directly on the polymer overlay plates.

### Identification of the biodegrading microorganisms

Bacteria and streptomycetes were characterized by their fatty acid composition as described earlier [26,28]. All identifications obtained for aerobic bacteria, and published earlier [22,23,26,27], were reinterpreted by comparison to library entries contained in the MIS database TSBA, version 3.90. The streptomycetes were assigned to one of the fatty acid clusters described by Mergaert *et al* [28], by comparison to a database constructed from these data. Moulds were identified by MUCL (Mycothèque de l'Université Catholique de Louvain-la-Neuve, Belgium).

## Results and discussion

### Biodiversity of poly(3-hydroxybutyrate)-degrading microorganisms in different environments

A total of 695 strains, isolated or tested by the authors [22–28] (this paper) were able to degrade P(3HB) *in vitro*, and belonged to 59 different microbial taxa (Figure 1, Table 1). Some of these microorganisms were also reported earlier to be able to degrade P(3HB). Additional species were reported by others to be able to degrade P(3HB) (Table 1). Summing up, strains able to degrade P(3HB) belong to at least 80 different taxa. These include 29 Gram-negative bacterial species, 11 species of Gram-positive bacteria, 17 fatty acid clusters of streptomycetes, and 23 fungal species.

As evidenced by Table 1, the biodiversity of P(3HB)-degrading microorganisms is considerable in most environments. This is not surprising, in view of the enormous biodiversity of the polymer-producing prokaryotes that prevail in these environments [10], and that leave these polymers as biological plastic waste in nature after their death. Moreover, the degradation of samples of P(3HB) in such different environments as soils, composts, freshwater, seawater and aerobic and anaerobic sludge could only be explained by the presence of very different degrading microorganisms, which are specially adapted to different temperature, oxygen, salinity or other conditions of their environment. The largest biodiversity was observed in soils and compost, with at least 39 and 23 different species or fatty acid groups, respectively, comprising bacteria, streptomycetes and moulds. From soils *Variovorax paradoxus* was most frequently isolated, and from composts *Acidovorax*. Moulds were not isolated from natural waters or sludges by the methods followed. From seawater all P(3HB)-degrading isolates were Gram-negative bacteria, belonging mainly to *Pseudoalteromonas haloplanktis*. Although biodegradation of P(3HB) in anaerobic sludge has been demonstrated [7,24], only two anaerobic microorganisms that are able to degrade P(3HB) *in vitro* have been isolated and described. *Ilyobacter delafieldii*, a Gram-negative, obligate anaerobe, was isolated from estuarine sediment [16], and a *Clostridium* strain was isolated from anaerobic sludge [24]. It is a challenge to gain more insights in the *in situ* interactions in these environments between bioplastics and the microorganisms present in the environments, and able to degrade these bioplastics *in vitro*.

### Versatility of streptomycetes to degrade different bacterial and synthetic polyesters

To determine the range of bioplastics that can be degraded by 171 individual streptomycete strains, the degradation of P(3HB), P(3HB-co-19%HV), P(3HB-co-97%3HV), P(3HO-co-70%3HD), PCL (Figure 2) and BIONOLLE was investigated on polymer overlay plates. Seventeen different patterns of bioplastic degradation abilities were found (Table 2). No distinctive correlation could be found between degrading ability patterns and classification

**Table 1** Biodiversity and distribution of P(3HB)-degrading microbial isolates in the environment

| Microbial taxa or groups | Source of isolates [22–28] (this paper)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Soils | Composts | Freshwater | Seawater | Sludge | Other/ unknown | Total No. of isolates | Other references |
| Total No. of P(3HB)-degrading isolates: | 392 | 122 | 57 | 26 | 25 | 73 | 695 | |
| **Gram-negative bacteria**: total No: | 163 | 70 | 36 | 26 | 19 | 12 | 326 | |
| *Acidovorax delafieldii/facilis* | +++ | +++ | +++ | | ++ | | 136 | [9, 18] |
| *Acinetobacter johnsonii* | | | | + | | | 2 | |
| *Alcaligenes faecalis* | | | | | | | | [39] |
| *Burkholderia cepacia* | | | | | + | | 1 | [30] |
| *Comamonas acidovorans* | | | | | | | | [30] |
| *Comamonas testosteroni* | + | ++ | | | | ++ | 13 | [9,29] |
| *Comamonas* sp | | | | | | | | [18] |
| *Flavobacterium johnsoniae*[b] | + | + | + | + | | | 6 | |
| *Ilyobacter delafieldii*[c] | | | | | | | | [16] |
| *Ochrobactrum anthropi* | | | | | + | | 1 | |
| *Pseudoalteromonas haloplanktis*[d] | | ++ | + | +++ | | | 34 | |
| *Pseudomonas alcaligenes* | | + | | | | | 3 | |
| *Pseudomonas chlororaphis* | | | + | | | | 2 | |
| *Pseudomonas lemoignei* | ++ | + | | | | | 10 | [9] |
| *Pseudomonas mallei* | | | | | | | | [36] |
| *Pseudomonas mendocina* | + | + | | | | | 4 | |
| *Pseudomonas pseudoalcaligenes* | | | | | + | | 2 | |
| *Pseudomonas pseudomallei* | | | | | | | | [9] |
| *Pseudomonas putida* | + | | | | | | 3 | |
| *Pseudomonas stutzeri* | + | | | | | + | 5 | [30] |
| *Pseudomonas syringae* pv *maculicola* | | | + | | | | 2 | |
| *Pseudomonas vesicularis* | | | | | | | | [30] |
| *Pseudomonas viridiflava* | | | | | | + | 1 | |
| *Pseudomonas* sp | | | | | | | | [8] |
| *Ralstonia pickettii*[e] | | | | | | | | [30] |
| *Stenotrophomonas maltophilia* | + | | | | | | 1 | [33] |
| *Variovorax paradoxus* | +++ | + | + | | ++ | + | 99 | [9] |
| *Vibrio ordalii* | | | | + | | | 1 | |
| *Zoogloea ramigera* | | | | + | | | 1 | [18] |
| **Gram-positive bacteria**: total No. | 46 | 10 | 12 | 0 | 3 | 6 | 77 | |
| *Arthrobacter ilicis* | | + | | | | | 1 | |
| *Bacillus circulans* | | | + | | + | + | 5 | |
| *Bacillus laterosporus* | + | | | | | | 2 | |
| *Bacillus megaterium* | +++ | ++ | ++ | | | + | 50 | |
| *Bacillus* sp | | | | | | | | [18] |
| *Clavibacter michiganense* subsp *insidiosum* | | | + | | | | 2 | |
| *Clostridium* sp[c] | | | | | + | | 1 | |
| *Paenibacillus polymyxa* | ++ | | | | | + | 11 | |
| *Staphylococcus aureus* | | | + | | | | 1 | |
| *Staphylococcus epidermidis* | | | + | | | | 1 | |
| Unidentified Gram-positive | + | | | | | | 3 | |
| **Streptomycetes**[f]: total No: | 86 | 37 | 9 | 0 | 3 | 55 | 190 | [8,9,35] |
| Fatty acid cluster A | + | | | | | | 2 | |
| Fatty acid cluster B | + | | | | | | 2 | |
| Fatty acid cluster C | + | | | | | + | 6 | |
| Fatty acid cluster F | | | | | | + | 2 | |
| Fatty acid cluster G | | + | | | | + | 5 | |
| Fatty acid cluster H | | + | | | | ++ | 6 | |
| Fatty acid cluster I | ++ | + | + | | | ++ | 24 | |
| Fatty acid cluster J | ++ | + | + | | | + | 17 | |
| Fatty acid cluster K | + | | + | | | + | 5 | |
| Fatty acid cluster L | + | | | | | | 2 | |
| Fatty acid cluster M | + | | | | | + | 4 | |
| Fatty acid cluster N | ++ | + | | | | + | 11 | |
| Fatty acid cluster O | + | | | | | | 2 | |
| Fatty acid cluster P | + | + | | | | + | 4 | |
| Fatty acid cluster Q | + | ++ | + | | | + | 9 | |
| Fatty acid cluster R | +++ | ++ | ++ | | + | ++ | 65 | |
| Other groups/not identified | ++ | + | | | + | ++ | 24 | |

**Table 1** Continued

| Microbial taxa or groups | Source of isolates [22–28] (this paper)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Soils | Composts | Freshwater | Seawater | Sludge | Other/ unknown | Total No. of isolates | Other references |
| **Moulds**: total No. | 97 | 5 | 0 | 0 | 0 | 0 | 102 | |
| *Acremonium* sp | + | | | | | | 1 | |
| *Aspergillus fumigatus* | +++ | + | | | | | 67 | |
| *Aspergillus penicilloides* | + | | | | | | 1 | |
| *Aspergillus* sp | | | | | | | | [20] |
| *Cephalosporium* sp | | | | | | | | [20] |
| *Cladosporium* sp | | | | | | | | [20] |
| *Eupenicillium* sp | | | | | | | | [21] |
| *Gerronema postii* | | | | | | | | [19] |
| *Gliocladium album* | | | | | | | | [19] |
| *Mucor* sp | | | | | | | | [19] |
| *Paecilomyces marquandii* | ++ | | | | | | 13 | |
| *Penicillium adametzii* | + | | | | | | 1 | |
| *Penicillium chermisinum* | + | + | | | | | 2 | |
| *Penicillium daleae* | + | | | | | | 4 | |
| *Penicillium funiculosum* | | | | | | | | [6] |
| *Penicillium ochrochloron* | + | + | | | | | 3 | |
| *Penicillium restrictum* | + | | | | | | 1 | |
| *Penicillium simplicissimum* | + | + | | | | | 6 | [19,21] |
| *Penicillium* sp | | | | | | | | [20] |
| *Polyposur circinatus* | | | | | | | | [19] |
| *Verticillium leptobactrum* | + | | | | | | 1 | |
| *Verticillium* sp | | | | | | | | [20] |
| Unidentified mould | + | | | | | | 2 | |

[a]Symbols: +, 1–4 isolates; ++, 5–19 isolates; +++ ≥20 isolates; blank, strains of the species were not encountered or tested by the authors [22–28].
[b]Formerly *Alteromonas haloplanktis* [11].
[c]Anaerobic bacterium.
[d]Formerly *Cytophaga johnsonae* [3]
[e]Formerly *Pseudomonas pickettii* [42].
[f]According to the clustering of Mergaert *et al* [28].

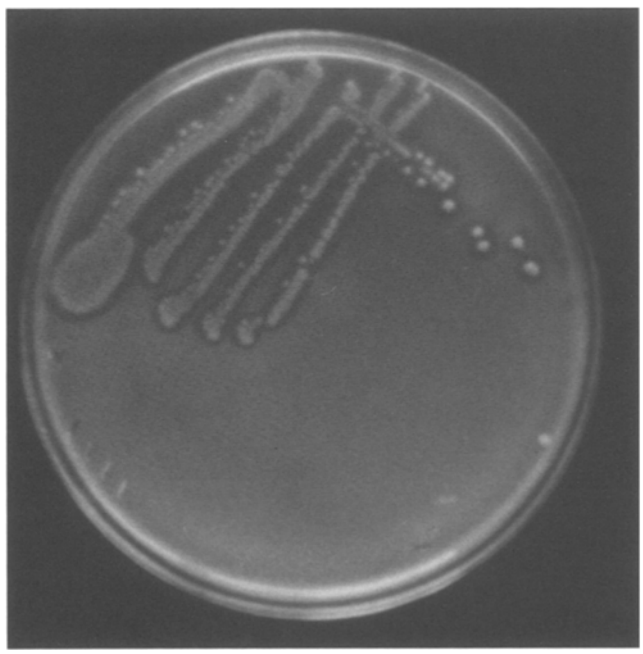

**Figure 1** Colonies of *Acidovorax* sp PHA 216, isolated from clay soil (Belgium), surrounded by degradation zones on overlay plate containing P (3HB). Diameter of the plate is 10 cm.

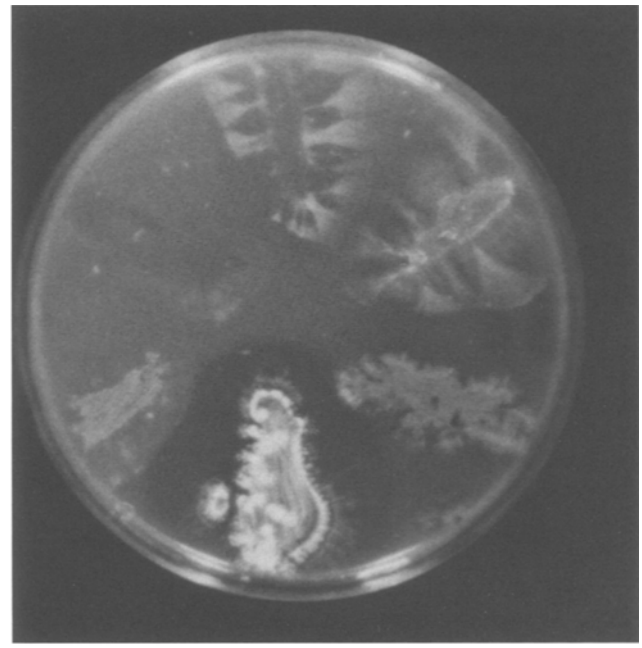

**Figure 2** Growth of streptomycete PHA 172 (fatty acid cluster R) (at 6 o'clock), isolated from hardwood forest soil (Belgium), and formation of degradation zone on overlay plate containing PCL. Diameter of the plate is 10 cm.

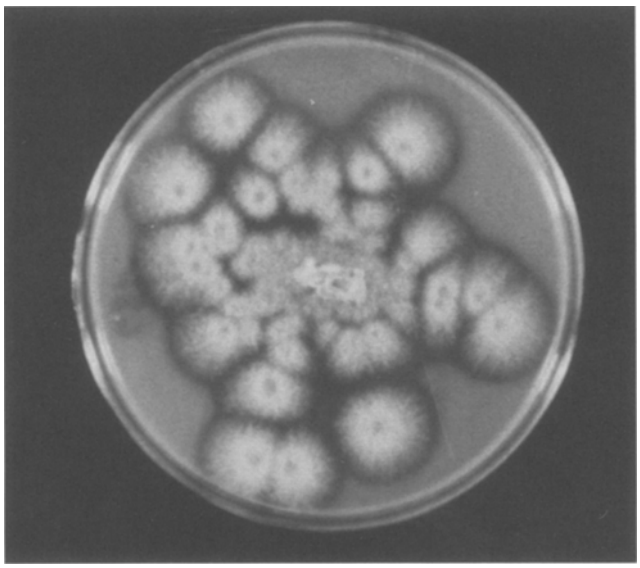

**Figure 3** Colonies of *Paecilomyces marquandii* PHA 439, isolated from sandy soil (Belgium), surrounded by degradation zones on overlay plate containing BIONOLLE. Diameter of the plate is 10 cm.

according to fatty acid analysis [28]. All 144 strains that degraded P(3HB) also degraded P(3HB-co-19%HV). One of these strains degraded all polymers tested (biodegradation pattern I), 23 strains degraded five different polymers (biodegradation patterns II and III), 37 strains were able to degrade four polymers (biodegradation patterns IV, V, and VIII), 78 strains degraded three polymers (biodegradation patterns VI, VII, IX and XI), 11 strains degraded two polymers (biodegradation patterns X, XII, and XIII), and nine strains degraded only a single polymer (biodegradation patterns XIV, XV, and XVI). Twelve reference strains from the LMG culture collection did not degrade any of the polymers tested under the test conditions used (biodegradation pattern XVII). A total number of 139 strains degraded at least three different plastics. This shows that streptomycetes are extremely versatile in their ability to degrade the polymers tested.

### *Diversity of extracellular depolymerizing enzymes*

The ability of individual microbial strains to degrade different bioplastics was also investigated by Schirmer *et al* [35]. They classified more than 53 microorganisms, including some streptomycetes, into 11 groups, depending on their polymer-degrading ability, and showed that some strains could degrade up to five different polyhydroxyalkanoates,

**Table 2** Bioplastics degradation patterns of 171 streptomycete strains on polymer overlayer plates

| Biodegradation pattern | Number of strains | Clusters[b] | Clear zone formation on polymer overlay plates containing[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | P(3HB) or P(3HB-co-19%3HV) | P(3HB-co-97%3HV) | P(3HO-co-70%3HD) | BIONOLLE | PCL |
| I | 1 | R | + | + | + | + | + |
| II | 2 | F, u | + | + | + | – | + |
| III | 21 | C, E, I, J, L, N, P, R, u | + | + | – | + | + |
| IV | 15 | B, C, G, I, J, M, Q, R | + | + | – | + | – |
| V | 21 | C, J, N, P, Q, R | + | + | – | – | + |
| VI | 73 | A, E, G, H, I, J, K, L, M, N O, P, Q, R, u | + | + | – | – | – |
| VII | 1 | G | + | – | + | – | – |
| VIII | 1 | M | + | – | – | + | + |
| IX | 1 | Q | + | – | – | + | – |
| X | 8 | A, H, I, Q, R, u | + | – | – | – | – |
| XI | 3 | E, G, O | – | + | – | + | + |
| XII | 1 | E | – | + | – | + | – |
| XIII | 2 | E, R | – | + | – | – | + |
| XIV | 4 | Q, R, u | – | + | – | – | – |
| XV | 2 | D, P | – | – | – | + | – |
| XVI | 3 | E, J, u | – | – | – | – | + |
| XVII | 12 | D, E, J, M, Q, R, u | – | – | – | – | – |
| Totals | 171 | | 144 | 143 | 4 | 45 | 54 |

[a]+, Strains positive; –, strains negative within 30 days.
[b]According to the clustering of Mergaert *et al* [28]; u, unclustered.

including PCL, while the degrading ability of many strains was limited to a single polymer. Some bacteria, such as *Stenotrophomonas maltophilia*, *Pseudomonas* sp and *Pseudomonas fluorescens*, degrade specifically P(3HO) and P(3HO-co-3HD) or PCL, and a *Xanthomonas campestris* strain could only degrade PCL [33,34]. *Pseudomonas lemoignei* strains are able to degrade P(3HB) and P(3HV), and can be selectively enriched in the presence of the latter polymer [25]. We have recently isolated an *Acidovorax avenae* subsp *avenae* strain that degrades PCL, MaterBi and BIONOLLE, but not P(3HB), in polymer overlay plates and in liquid medium, and a *Paecilomyces marquandii* strain is able to degrade both P(3HB) and BIONOLLE (Figure 3).

The biodiversity of the bioplastic-degrading microorganisms is thus not only apparent on a taxonomic level, but also with regard to the degrading enzymes they produce. *Pseudomonas lemoignei* produces at least five different extracellular depolymerases, one of which is specifically synthesized during growth on P(3HV), while other PHA-degrading bacteria apparently synthesize only one depolymerase [17,40]. At least one streptomycete possesses two different depolymerases [35]. Some strains degrade different bioplastics, including synthetic PCL and BIONOLLE. The latter bear ester functions in their backbone, that are quite similar to those of the bacterial PHA, and one could assume that these functions are also recognized by PHA depolymerases. Jaeger *et al* [15] studied the substrate specificities of nine different PHA depolymerases and six bacterial lipases, and found that some of these depolymerases hydrolysed BIONOLLE, or PCL, or both, while the lipases hydrolysed poly($\omega$-hydroxyalkanoates), such as poly(4-hydroxybutyrate) and PCL, and BIONOLLE, but not P(3HB) or P(3HV). This may explain why some strains that degrade PCL or BIONOLLE, did not degrade PHA. Apparently these strains produce only bacterial lipases and not PHA depolymerases. It is concluded that in nature the biodiversity of the degrading microorganisms has to be multiplied by the diversity in extracellular polymer-degrading enzymes that they produce.

## Acknowledgements

This work was supported by the European Union (ECLAIR grant AGRE.0006.C (H), and AIR2 grant CT93-1099). We are indebted to Zeneca BioProducts (Billingham, UK) for the supply of P(3HA) polymers.

## References

1 Anderson AJ and EA Dawes. 1990. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol Rev 54: 450–472.
2 Bastioli C, A Cerutti, I Guanella, GC Romano and M Tosin. 1995. Physical state and biodegradation behavior of starch-polycaprolactone systems. J Environ Polym Degrad 3: 81–96.
3 Bernardet J-F, P Segers, M Vancanneyt, F Berthe, K Kersters and P Vandamme. 1996. Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom rev (basonym, *Cytophaga aquatilis* Strohl and Tait) 1978. Int J Syst Bacteriol 46: 128–148.
4 Brandl H, R Bachofen, J Mayer and E Wintermantel. 1995. Degradation and applications of polyhydroxyalkanoates. Can J Microbiol 41 (Suppl 1): 143–153.
5 Brandl H and P Püchner. 1992. Biodegradation of plastic bottles made from 'Biopol' in an aquatic ecosystem under *in situ* conditions. Biodegradation 2: 237–243.
6 Brucato CL and SS Wong. 1991. Extracellular poly(3-hydroxybutyrate) depolymerase from *Penicillium funiculosum*: general characteristics and active site studies. Arch Biochem Biophys 290: 497–502.
7 Budwill K, PM Fedorak and WJ Page. 1992. Methanogenic degradation of poly(3-hydroxyalkanoates). Appl Environ Microbiol 58: 1398–1401.
8 Chowdbury AA. 1963. Poly-$\beta$-hydroxybuttersäure abbauende Bakterien und Exoenzym. Arch Mikrobiol 47: 167–200.
9 Delafield FP, M Doudoroff, NJ Palleroni, J Lusty and R Contopoulos. 1965. Decomposition of poly-$\beta$-hydroxybutyrate by pseudomonads. J Bacteriol 90: 1455–1466.
10 Doi Y. 1990. Microbial Polyesters. VCH Publishers, New York.
11 Gauthier G, M Gauthier and R Christen. 1995. Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen nov, and proposal of twelve new species combinations. Int J Syst Bacteriol 45: 755–761.
12 Goldberg D. 1995. A review of the biodegradability and utility of poly(caprolactone). J Environ Polym Degrad 3: 61–68.
13 Haywood GW, AJ Anderson, DR Williams, EA Dawes and DF Ewing. 1991. Accumulation of a poly(hydroxyalkanoate) copolymer containing primarily 3-hydroxyvalerate from simple carbohydrate substrates by *Rhodococcus* sp NCIMB 40126. Int J Biol Macromol 13: 83–88.
14 Hrabak O. 1992. Industrial production of poly-$\beta$-hydroxybutyrate. FEMS Microbiol Rev 103: 251–256.
15 Jaeger K-E, A Steinbüchel and D Jendrossek. 1995. Substrate specificities of bacterial polyhydroxyalkanoate depolymerases and lipases: bacterial lipases hydrolyse poly($\omega$-hydroxyalkanoates). Appl Environ Microbiol 61: 3113–3118.
16 Janssen PH and CG Harfoot. 1990. *Ilyobacter delafieldii* sp nov, a metabolically restricted anaerobic bacterium fermenting PHB. Arch Microbiol 154: 253–259.
17 Jendrossek D, A Frisse, A Behrends, M Andermann, HD Kratzin, T Stanislawski and HG Schlegel. 1995. Biochemical and molecular characterization of the *Pseudomonas lemoignei* polyhydroxyalkanoate depolymerase system. J Bacteriol 177: 596–607.
18 Jendrossek D, I Knoke, RB Habibian, A Steinbüchel and HG Schlegel. 1993. Degradation of poly(3-hydroxybutyrate), PHB, by bacteria and purification of a novel PHB depolymerase from *Comamonas* sp. J Environ Polym Degrad 1: 53–64.
19 Matavulj M and HP Molitoris. 1992. Fungal degradation of polyhydroxyalkanoates and a semiquantitative assay for screening their degradation by terrestrial fungi. FEMS Microbiol Rev 103: 323–332.
20 Matavulj M, ST Moss and HP Molitoris. 1993. Degradation of poly-$\beta$-hydroxyalkanoate-based plastics in natural environments. In: Proceedings of the International Symposium on Bacterial Polyhydroxyalkanoates, Göttingen, 1992 (Schlegel HG and A Steinbüchel, eds), pp 465–466, Goltze-Drück, Göttingen.
21 McLellan DW and PJ Halling. 1988. Acid-tolerant poly(3-hydroxybutyrate) hydrolases from moulds. FEMS Microbiol Lett 52: 215–218.
22 Mergaert J, C Anderson, A Wouters and J Swings. 1994. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) in compost. J Environ Polym Degrad 2: 177–183.
23 Mergaert J, C Anderson, A Wouters, J Swings and K Kersters. 1992. Biodegradation of polyhydroxyalkanoates. FEMS Microbiol Rev 103: 317–322.
24 Mergaert J, G Glorieux, L Hauben, V Storms, M Mau and J Swings. 1996. Biodegradation of poly(3-hydroxyalkanoates) in anaerobic sludge and characterization of a poly(3-hydroxyalkanoates) degrading anaerobic bacterium. Syst Appl Microbiol (in press).
25 Mergaert J, A Schirmer, L Hauben, M Mau, B Hoste, K Kersters, D Jendrossek and J Swings. 1996. Isolation and identification of poly(3-hydroxyvalerate) degrading strains of *Pseudomonas lemoignei*. Int J Syst Bacteriol 46: 769–773.
26 Mergaert J, A Webb, C Anderson, A Wouters and J Swings. 1993. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-

hydroxybutyrate-*co*-3-hydroxyvalerate) in soils. Appl Environ Microbiol 59: 3233–3238.

27 Mergaert J, A Wouters, C Anderson and J Swings. 1995. *In situ* biodegradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) in natural waters. Can J Microbiol 41 (Suppl 1): 154–159.

28 Mergaert J, A Wouters and J Swings. 1994. Estimation of the intrinsic biodiversity among poly(3-hydroxyalkanoates) degrading streptomycetes using gas chromatographic analysis of fatty acids. Syst Appl Microbiol 17: 601–612.

29 Mukai K, K Yamada and Y Doi. 1993. Enzymatic degradation of poly(hydroxybutyrate) by a marine microorganism. Polym Degrad Stab 41: 85–91.

30 Mukai K, K Yamada and Y Doi. 1994. Efficient hydrolysis of polyhydroxyalkanoates by *Pseudomonas stutzeri* YM1414 isolated from lake water. Polym Degrad Stab 43: 319–327.

31 Nishida H and Y Tokiwa. 1993. Distribution of poly($\beta$-hydroxybutyrate) and poly($\epsilon$-caprolactone) aerobic degrading microorganisms in different environments. J Environ Polym Degrad 1: 227–233.

32 Nishida H and Y Tokiwa. 1994. Degradation of poly(2-oxepanone) by phytopathogens. Chem Lett 1994: 1547–1550.

33 Ramsay BA, I Saracovan, JA Ramsay and RH Marchessault. 1994. A method for the isolation of microorganisms producing extracellular long-side-chain poly($\beta$-hydroxyalkanoate) depolymerase. J Environ Polym Degrad 2: 1–7.

34 Schirmer A, D Jendrossek and HG Schlegel. 1993. Degradation of poly(3-hydroxyoctanoic acid) [P(3HO)] by bacteria: purification and properties of a P(3HO) depolymerase from *Pseudomonas fluorescens* GK13. Appl Environ Microbiol 59: 1220–1227.

35 Schirmer A, C Matz and D Jendrossek. 1995. Substrate specificities of poly(hydroxyalkanoate) degrading bacteria and active site studies on the extracellular poly(3-hydroxyoctanoic acid) depolymerase of *Pseudomonas fluorescens* GK13. Can J Microbiol 41 (Suppl 1): 170–179.

36 Stanier RY, NJ Palleroni and M Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J Gen Microbiol 43: 159–271.

37 Steinbüchel A. 1991. Polyhydroxyalkanoic acids. In: Biomaterials (Byrom D, ed), pp 123–213, Macmillan Press, London.

38 Steinbüchel A and G Schmack. 1995. Large-scale production of poly(3-hydroxyvaleric acid) by fermentation of *Chromobacterium violaceum*, processing, and characterization of the homopolyester. J Environ Polym Degrad 3: 243–258.

39 Steinbüchel A and HE Valentin. 1995. Diversity of bacterial polyhydroxyalkanoic acids. FEMS Microbiol Lett 128: 219–228.

40 Tanio T, T Fukui, T Saito, K Tomita, T Kaiho and S Masamune. 1982. An extracellular poly(3-hydroxybutyrate) depolymerase from *Alcaligenes faecalis*. Eur J Biochem 124: 71–77.

41 Timm A and A Steinbüchel. 1990. Formation of polyesters consisting of medium-chain length 3-hydroxyalkanoic acids from gluconate by *Pseudomonas aeruginosa* and other fluorescent pseudomonads. Appl Environ Microbiol 56: 3360–3367.

42 Yabuuchi E, Y Kosako, I Yano, H Hotta and Y Nishiushi. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen nov: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb nov, *Ralstonia solanacearum* (Smith 1896) comb nov, and *Ralstonia eutropha* (Davis 1969) comb nov. Microbiol Immunol 39: 897–904.