Plasticicumulans lactativorans sp. nov., a polyhydroxybutyrate-accumulating gammaproteobacterium from a sequencing-batch bioreactor fed with lactate

Yang Jiang,[1] Dimitry Y. Sorokin,[1,2] Helena Junicke,[1] Robbert Kleerebezem[1] and Mark C. M. van Loosdrecht[1]

Correspondence
Dimitry Y. Sorokin
soroc@inmi.ru *or*
d.sorokin@tudelft.nl

[1]Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

[2]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-let Octyabrya 7/2, 117811 Moscow, Russia

A bacterial consortium that accumulated more than 90 % (w/w) polyhydroxybutyrate (PHB) from lactate was selected in a laboratory-scale bioreactor with a 'feast–famine' regime. Bacterial strain YD[T], representing a dominant species in this enrichment, was isolated and characterized. Analysis of the 16S rRNA gene sequence revealed that the isolate is a member of the class *Gammaproteobacteria*, forming an independent phylogenetic lineage. The closest relative of the isolate was *Plasticicumulans acidivorans* TUD-YJ37[T], with 94 % 16S rRNA gene sequence similarity. Strain YD[T] was an obligate aerobe with large, ovoid, Gram-negative cells, motile by means of a polar flagellum. It utilized a relatively broad spectrum of substrates (e.g. carbohydrates, fatty acids) as carbon and energy sources. The temperature range for growth was 20–45 °C, with an optimum at 40 °C; the pH range was pH 6.0–8.0, with an optimum at pH 7.0. The major respiratory lipoquinones were Q-8 (91 %) and Q-7 (9 %). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmono-methylethanolamine and an unidentified aminolipid. The predominant fatty acids in the membrane polar lipids were $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{18:1}\omega7c$. The G + C content of the genomic DNA was 68.5 mol%. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, the isolate is proposed to represent a novel species in the genus *Plasticicumulans*, for which the name *Plasticicumulans lactativorans* sp. nov. is proposed. The type strain is YD[T] (=DSM 25287[T]=NCCB 100398[T]).

As microbe-synthesized biodegradable polymers, polyhydroxyalkanoates (PHAs) are currently attracting great commercial interest due to the fact that their physical properties are highly similar to those of conventional thermoplastics, such as polyethylene and polypropylene (Braunegg *et al.*, 1998; Rhu *et al.*, 2003; Steinbüchel, 2001; Takabatake *et al.*, 2000). The current industrial scale of PHA production is based mainly on pure microbial culture technology. Either wild-type bacteria (e.g. *Ralstonia eutropha*) or recombinant *Escherichia coli* strains are used to generate bioplastics (Steinbüchel, 2001; Pohlmann *et al.*,

2006). Though these bacteria are capable of reaching very high PHA contents (90 % of cell dry weight; Slater *et al.*, 1988), facilitating the downstream process of PHA extraction, the production of PHAs under sterile conditions with defined growth substrates results in a higher cost for PHAs compared with petroleum-based plastics (Choi & Lee, 1997, 1999).

A new concept, referred to as 'microbial community engineering', is now being actively developed. This concept aims to select microbial communities based on ecological principles rather than genetic or metabolic engineering. With this technique, wastewater can be used as substrate and the cost arising from equipment and medium sterilization can be eliminated. PHAs are common bacterial storage compounds that give bacteria a selective advantage under varying environmental conditions, especially feast–famine conditions (van Loosdrecht *et al.*, 1997). Microbial

---

community engineering technology has been applied to PHA production, and many efforts have been made to optimize PHA production by microbial enrichment (Dias *et al.*, 2006). Two enrichments with superior PHA-producing capacity have been obtained in our laboratory. Johnson *et al.* (2009) enriched a mixed culture capable of accumulating 89 % (w/w) polyhydroxybutyrate (PHB) when fed with acetate, and Jiang *et al.* (2011a) obtained another enrichment with lactate as sole carbon source that can accumulate 92 % (w/w) within 6 h. Each of these enrichments was individually dominated by a single bacterial species. The taxon that dominates the acetate-fed bioreactor has been isolated and assigned to a novel genus and species within the class *Gammaproteobacteria*, *Plasticicumulans acidivorans* (Jiang *et al.*, 2011b). The dominant bacterial species enriched in the lactate-fed bioreactor has not yet been isolated and characterized. In this paper, we describe the isolation and characterization of a novel PHA-producing bacterium that dominated the lactate-fed laboratory-scale bioreactor which is assigned to a novel species in the genus *Plasticicumulans*.

A 2.5 l bioreactor was inoculated with activated sludge from a nutrient removal (i.e. organic carbon and nitrogen) sewage treatment plant (Kralingseveer, Rotterdam, Netherlands; April 2010) and was maintained under non-sterile conditions. It was fed with sodium DL-lactate (Sigma) as the sole carbon and energy source in a sequencing-batch mode (Jiang *et al.*, 2011a). The reactor was operated at 30 °C and maintained at pH 7.0 ± 0.05 by using 1 M HCl and 1 M NaOH. The concentration of dissolved oxygen was measured with a dissolved oxygen electrode (Mettler Toledo) and the pH was monitored with a pH electrode (Mettler Toledo). The dissolved oxygen varied between 30 and 100 % during operation of the reactor. The enrichment selected under these conditions was able to accumulate 92 % (w/w) PHA within 6 h. Photomicrographs showed that the dominant bacteria were represented by relatively large coccoid cells full of storage material (Jiang *et al.*, 2011a).

In order to isolate the dominant bacterium, serial dilution to extinction and plating were used. However, initial attempts failed. The main problem was that the bacteria aggregated and formed flocs in the bioreactor. By the method of serial dilution, only untargeted bacteria (representing minor satellite populations in the bioreactor) could be selected from the highest dilution series. Pottering and mild sonication were therefore used in an attempt to obtain homogenized free target cells, but again no effective results were obtained. It was noticed that more free cells belonging to the dominant coccoid morphotype were formed in the bioreactor during the feast phase (when the external substrate is present) than during the famine phase (when the external substrate is depleted). Biomass was thereby collected during the feast phase. After removal of the flocs by gravity settling for 30 min, the remaining suspended biomass was used as the starting material for serial dilution (from 10^{0} to 10^{-7}; Fig. S1, available in

IJSEM Online). Four types of mineral medium were tested for strain purification. The only difference between the four mineral media was the carbon source. Sodium lactate, sodium succinate, sodium acetate or sodium propionate was used as the sole carbon source. The same nutrient source was used in all the mineral media. The composition of the mineral medium used for strain purification contained the following compounds: 3.3 mM sodium DL-lactate (or 2.5 mM sodium succinate, 5 mM sodium acetate or 3.3 mM sodium propionate), 2 mM NH_4Cl, 25 mM KH_2PO_4, 0.56 mM $MgSO_4 . 7H_2O$ and 0.075 % (v/v) trace element solution (Vishniac & Santer, 1957). The medium was autoclaved at 121 °C for 20 min before use. Samples from the dilution series were incubated at 30 °C at 150 r.p.m. until bacterial growth became visible. No bacterial growth was observed in the dilution series higher than 10^2 when lactate was used as the carbon source. Within this dilution series, no pure culture could be obtained. However, with the other substrates, bacteria grew up to the 10^{-6} dilution. The cultures from the highest dilution series [10^{-6}, from succinate, acetate and propionate] were further purified by one more round of dilution (from 10^{0} to 10^{-7}) with the same medium from which the cultures were harvested. The purity of the cultures from the highest dilution series in the second round of isolation was examined by adding one drop of sterilized yeast extract (10 %, w/v). No contaminants were observed and the isolate was considered pure. The isolated strain was designated YD^T. The pure culture was recultivated in lactate dilution series, and it grew up to 10^5 dilution. The difference between this dilution series and the first trial purification series was the free cell concentration. Compared with this cultivation, the substrate to cells ratio was remarkably higher in the first purification trial. The possible reason for the failure of purification of strain YD^T on lactate is its extremely high capacity for substrate uptake. Strain YD^T showed much higher respiration activity on lactate compared with the other tested substrates (Fig. S2), and the biomass dominated by strain YD^T exhibited a superior specific lactate uptake rate (Jiang *et al.*, 2011a). This type of bacterium has a tendency to produce PHA first rather than synthesizing biomass. Many lysed bacteria and released PHA granules were observed in lactate-grown cultures (Fig. S3). It is likely that growth of strain YD^T on lactate is so unbalanced that the large amounts of stored PHB burst the cells.

Cultivation of strain YD^T on agar plates was also attempted. Agar plates were prepared with the same composition as the liquid medium with the addition of 2 % (w/v) agar. After plating with strain YD^T, the agar plates were incubated. Microcolonies were only observed after incubation for long periods (2 months) at 30 °C, indicating a very limited capacity of strain YD^T to grow on solid media.

The substrate spectrum of YD^T was investigated in liquid carbon-free mineral medium [1.5 mM NH_4Cl, 25 mM KH_2PO_4, 0.56 mM $MgSO_4 . 7H_2O$, 0.075 % (v/v) trace element solution] mixed with 20 mM (carbon-based)

carbon source. Biomass growth was monitored by the increase in OD_{600}. Anaerobic growth by fermentation (of glucose) and by respiration of nitrogen oxyanions was tested in 30 ml serum bottles with 20 ml medium closed with butyl rubber stoppers. Anoxic conditions were achieved by several cycles of evacuation and flushing with argon gas. Nitrate was added at 10 mM, nitrite at 5 mM and N_2O at 100 % gas phase. Hydrolytic activity was tested by growth in liquid culture and on plates. Hydrolysis of starch was tested by flooding with iodine, hydrolysis of casein by flooding with 10 % trichloroacetic acid and hydrolysis of Tween 80 by the appearance of a precipitate halo around the colonies. Acid formation from sugars was measured as a pH change using a pH electrode.

The results demonstrated that strain YD^T could utilize volatile fatty acids (C_2–C_6) and had a broad substrate utilization spectrum on carbohydrates, organic acids and alcohols. PHA granules in cells could be observed during growth on all tested substrates, even under non-nutrient-deficiency conditions.

The phenotypic properties of strain YD^T were analysed using lactate as substrate. Depending on the PHA granule content of the cells, the morphology of strain YD^T varied from coccoid to ovoid, 1.5–5 × 2–5 μm in size. Cells were motile by means of a single polar flagellum and generally stored large globules of PHB material (Fig. 1). The alkali lysis test and electron microscopy of thin-sectioned cells revealed the Gram-negative cell wall type. With lactate as substrate and at pH 7, the organism grew at 20–45 °C (with an optimum at 40 °C) and at pH 6–8 at 30 °C (with an optimum at pH 7). The substrate affinity of strain YD^T was studied based on

respiration rates. The detailed procedure of respiration measurement was described by Sorokin *et al.* (2003). Under conditions of optimum temperature and pH, the K_s for lactate was about 40 μM (Fig. S4). Growth and respiration of cells were inhibited completely by NaCl at concentrations above 200 mM (Fig. S5). The bacterium could not grow anaerobically with nitrate, but could reduce nitrate to nitrite. Ammonia, nitrate and complex organic nitrogen (yeast extract) could be used as nitrogen sources. Oxidase [1 % (w/v) tetramethyl *p*-phenylenediamine] and catalase [3 % (v/v) H_2O_2] tests were positive.

The phylogenetic position of the isolate was studied using 16S rRNA gene sequence analysis. Genomic DNA of YD^T was extracted using the Ultra Clean Soil DNA Extraction kit (MoBio Laboratories) following the manufacturer's instructions. A 1400 bp fragment of the 16S rRNA gene was amplified by using the universal bacteria primers GM3 (5′-AGAGTTTGATCMTGGC-3′) and GM4 (5′-TACCT-TGTTACGACTT-3′). The DNA fragment obtained was sequenced by Macrogen (Korea) by using primers GM3, GM4, 341F (5′-CCTACGGGAGGCAGCAG-3′), 907F (5′-AAACTCAAAKGAATTGACGG-3′) and 907RM (5′-CCG-TCAATTCMTTTGAGTTT-3′). Sequence similarity was initially analysed using BLAST. The highest similarity (95.0 %) was obtained with an uncultured bacterial clone from activated sludge in Australia (Schroeder *et al.*, 2009). In addition, a similarity analysis was performed against sequences of type strains on the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012). The nearest cultured relatives were members of the genera *Plasticicumulans* (maximum sequence similarity of 94.4 %), *Ectothiorhodosinus* (90.6 %), *Ectothiorhodospira* (90.6 %), *Natronocella* (90.5 %), *Thiofaba* (90.2 %), *Aquimonas* (90.2 %), *Nitrosococcus* (90.1 %), *Methylococcus* (90.0 %) and *Thioalkalivibrio* (90.0 %). The phylogenetic tree was reconstructed by using the ARB software program (Ludwig *et al.*, 2004) for the alignment of 16S rRNA gene sequences and the RAxML search algorithm including rapid bootstrap analysis for the calculation of an optimized phylogenetic tree. Strains of *Escherichia fergusonii*, *Escherichia coli* and *Escherichia albertii* were chosen as the outgroup to represent species that are clearly of a different phylogenetic lineage (Fig. 2).

The respiratory quinones and polar lipids were analysed according to methods described by Tindall (1990a, b) by the Identification Service of the DSMZ (Braunschweig, Germany). The dominant components were identified as Q-8 (91 %) and Q-7 (9 %), a characteristic trait of many other gammaproteobacteria (Yokota *et al.*, 1992). The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and an unidentified aminolipid (Fig. S6). Phosphatidylethanolamine and phosphatidylglycerol were the two major compounds. For analysis of fatty acid methyl esters, polar lipids were extracted from dry cell material with acidic methanol. The resulting methyl esters were analysed by GC-MS using the Sherlock Microbial Identification system (MIDI Inc.) according to Zhilina *et al.*
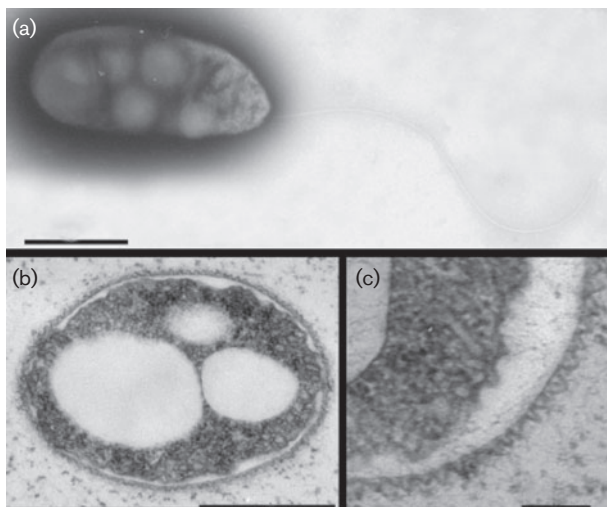

Fig. 1. Electron photomicrographs of strain YD^T. (a) Whole cell contrasted with phosphotungstic acid. (b, c) Thin sections showing ultrastructure of the cell. In the enlarged cell section (c), note the highly undulating outer membrane typical of some Gram-negative bacteria. Bars, 1 μm (a), 0.5 μm (b) and 0.1 μm (c).
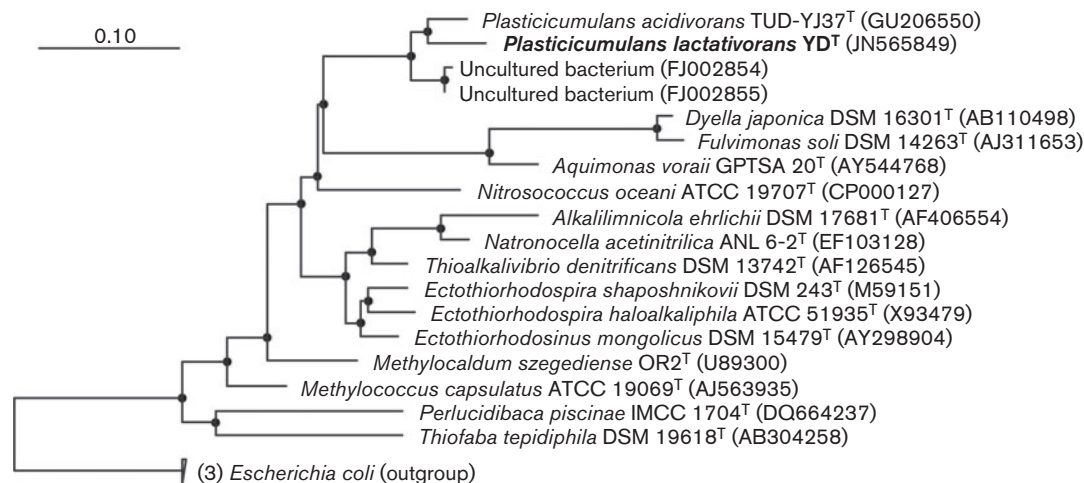
Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationships of isolate YD[T]. Filled circles represent a bootstrap value of 100 %. Sequence accession numbers are listed in parentheses. Bar, 10 % sequence difference. Strains Escherichia fergusonii ATCC 35469; Escherichia coli ATCC 11775; Escherichia albertii B090 of *Escherichia fergusonii* ATCC 35469, *Escherichia coli* ATCC 11775 and *Escherichia albertii* B090 were chosen as the outgroup to represent species that are clearly of a different phylogenetic lineage.

(1997). The major cellular fatty acids of strain YD[T] were $C_{16:1}\omega7c$ (41.6 %), $C_{16:0}$ (32.7 %) and $C_{18:1}\omega7c$ (19.9 %), which are quite typical of proteobacteria. Table S1 shows the detailed fatty acid composition of strain YD[T] in comparison with that of *P. acidivorans* TUD-YJ37[T]. The chemotaxonomic characteristics (lipoquinones, polar lipids and phospholipid fatty acid profile) of the novel isolate closely resembled those of its nearest relative, *P. acidivorans* TUD-YJ37[T] (Jiang *et al.*, 2011b).

The G + C content of the genomic DNA was determined by the thermal denaturation technique (Marmur & Doty, 1962) after extraction from 1 g wet-cell biomass according to Marmur (1961). The value obtained was 68.5 mol%, only slightly higher than in *P. acidivorans* TUD-YJ37[T] (67.4 mol%).

The most important phenotypic properties of strain YD[T] were compared directly with the characteristics of *P. acidivorans* and with more distant relatives from the gammaproteobacteria (Table 1). Although strain YD[T] strongly resembled *P. acidivorans* phenotypically, there were some important differences in the carbon source utilization spectrum. *P. acidivorans* is specialized, utilizing C_2–C_9 volatile fatty acids, while strain YD[T] exhibited a much broader substrate spectrum, indicating a more complex metabolism. Therefore, we can expect that strain YD[T] has a broader environmental distribution. Despite the relatively large phylogenetic distance, the overall results of phenotypic comparison suggest that the novel PHA-accumulating bacterium from the bioreactor, strain YD[T], is a member of the genus *Plasticicumulans* and is assigned to a novel species, for which the name *Plasticicumulans lactativorans* sp. nov. is proposed.

Description of *Plasticicumulans lactativorans* sp. nov.

Plasticicumulans lactativorans (lac.ta.ti.vo′rans. N.L. n. *lactas -atis* lactate; L. part. adj. *vorans* eating, devouring; N.L. part. adj. *lactativorans* lactate-devouring).

Cell shape varies from coccoid to ovoid, 1.5–5 × 2–5 μm. Has limited colony-forming capacity. Colonies formed after 2 months of incubation reach a maximum of 1 mm in diameter, with hard consistency, and are pearl white with irregular edges. Cell-wall type is Gram-negative. Accumulates more than 90 % (w/w) PHAs, with storage polymers in the form of large intracellular granules. Grows at 20–45 °C, with an optimum at 40 °C, and pH 6–8 (optimum pH 7). Salt-sensitive; growth and respiration are inhibited in the presence of 200 mM NaCl. Utilizes the following compounds as carbon and energy sources during aerobic growth: acetate, propionate, butyrate, valerate, pyruvate, succinate, lactate, ethanol, butanol, D-fructose, D-glucose, D-galactose, sucrose, maltose, melibiose, raffinose, D-glucosamine, glycerol and yeast extract. Growth by sugar fermentation is not detected. The following compounds are not utilized: formate, isobutyrate, heptanoate, octanoate, nonanoate, decanoate, L-arabinose, D-ribose, D-xylose, D-mannose, lactose, cellobiose, D-galacturonic acid, D-glucuronic acid, methanol and H_2. Starch, gelatin and Tween 80 are not hydrolysed. Ammonium and nitrate serve as nitrogen sources. Tests for oxidase and catalase are positive. Hydrolysis of aesculin and gelatin is negative. Nitrate is reduced to nitrite aerobically but anaerobic growth with nitrate, nitrite and N_2O with lactate as electron donor is not observed. Membrane polar lipids include diphosphatidylglycerol, phosphatidylglycerol,

Table 1. Comparison of the properties of strain YD[T] and its nearest phylogenetic relatives

Taxa: 1, strain YD[T]; 2, *P. acidivorans* TUD-YJ37[T] (data from Jiang *et al.*, 2011b); 3, *Ectothiorhodospira* (ten species; numbers in parentheses represent species giving the result shown) (data from Asselineau & Trüper, 1982; Imhoff, 2006; Gorlenko *et al.*, 2009). Strain YD[T] and *P. acidivorans* TUD-YJ37[T] were grown under exactly the same growth conditions (medium composition, growth phase, temperature). All taxa are negative for denitrification and growth at 50 °C. LI, Lithotrophic; PH, phototrophic; ND, no data available. Strain YD[T] and *P. acidivorans* TUD-YJ37[T] were isolated from wastewater, whereas species of the genus *Ectothiorhodospira* have been isolated from anoxic, light-penetrated, aquatic habitats.

Characteristic	1	2	3
16S rRNA gene sequence similarity to strain YD[T] (%)	(100)	94.4	90.6
Cell shapes*	C, OD	CD, OD	O, R
Nutritional type†	COH	COH	ANP
Growth at/in:			
0.5 M NaCl	−	−	+
pH 10	−	−	+ (4)
Reduction of nitrate to nitrite	+	+	ND
Anaerobic growth	−	−	+ (PH)
Autotrophy	−	−	+ (PH, LI)
Utilization of sugars	+	−	−
Major quinone(s)	Q-8, Q-7	Q-8	MK-7, Q-7 (or Q-8)
Dominant phospholipid fatty acids‡	$C_{16:1}$, $C_{16:0}$, $C_{18:1}$	$C_{16:1}$, $C_{16:0}$, $C_{18:1}$	$C_{18:1}$, $C_{16:0}$, $C_{16:1}$
Major polar lipids§	DPG, PMME, PG, PE, AL	DPG, PG, PE, 3PL, AL, L	CL, PG, PE, PC
DNA G+C content (mol%)	68.5	67.4	61.4–68.4

*C, Coccus; CD, coccoid; O, oval; OD, ovoid; R, rod.
†ANP, Anaerobic phototroph; COH, chemo-organoheterotroph.
‡In decreasing order of abundance.
§CL, Cardiolipin; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PMME, phosphatidylmonomethylethanolamine; AL, unidentified aminolipid; PL, unidentified phospholipid; L, unidentified lipid.

phosphatidylethanolamine, phosphatidylmonomethylethanolamine and an unidentified aminolipid. The major cellular fatty acids are $C_{16:1}\omega 7c$, $C_{16:0}$ and $C_{18:1}\omega 7c$. The major respiratory lipoquinones are Q-8 and Q-7 (~9:1).

The type strain is YD[T] (=DSM 25287[T]=NCCB 100398[T]), isolated from a sequencing-batch bioreactor fed with lactate. The G+C content of the DNA of the type strain is 68.5 mol%.

References

Asselineau, J. & Trüper, H. G. (1982). Lipid composition of six species of the phototrophic bacterial genus *Ectothiorhodospira*. *Biochim Biophys Acta* **712**, 111–116.

Braunegg, G., Lefebvre, G. & Genser, K. F. (1998). Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects. *J Biotechnol* **65**, 127–161.

Choi, J. & Lee, S. Y. (1997). Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Eng* **17**, 335–342.

Choi, J. & Lee, S. Y. (1999). Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. *Appl Microbiol Biotechnol* **51**, 13–21.

Dias, J. M. L., Lemos, P. C., Serafim, L. S., Oliveira, C., Eiroa, M., Albuquerque, M. G. E., Ramos, A. M., Oliveira, R. & Reis, M. A. M. (2006). Recent advances in polyhydroxyalkanoate production by mixed aerobic cultures: from the substrate to the final product. *Macromol Biosci* **6**, 885–906.

Gorlenko, V. M., Bryantseva, I. A., Rabold, S., Tourova, T. P., Rubtsova, D., Smirnova, E., Thiel, V. & Imhoff, J. F. (2009). *Ectothiorhodospira variabilis* sp. nov., an alkaliphilic and halophilic purple sulfur bacterium from soda lakes. *Int J Syst Evol Microbiol* **59**, 658–664.

Imhoff, J. F. (2006). The family *Ectothiorhodospiraceae*. In *The Prokaryotes*, 3rd edn, vol. 6, pp. 874–886. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Jiang, Y., Marang, L., Kleerebezem, R., Muyzer, G. & van Loosdrecht, M. C. M. (2011a). Polyhydroxybutyrate production from lactate using a mixed microbial culture. *Biotechnol Bioeng* **108**, 2022–2035.

Jiang, Y., Sorokin, D. Y., Kleerebezem, R., Muyzer, G. & van Loosdrecht, M. C. M. (2011b). *Plasticicumulans acidivorans* gen. nov., sp. nov., a polyhydroxyalkanoate-accumulating gammaproteobacterium from a sequencing-batch bioreactor. *Int J Syst Evol Microbiol* **61**, 2314–2319.

Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G. & van Loosdrecht, M. C. M. (2009). Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. *Biomacromolecules* **10**, 670–676.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Marmur, J. (1961). Procedure for isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–214.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Pohlmann, A., Fricke, W. F., Reinecke, F., Kusian, B., Liesegang, H., Cramm, R., Eitinger, T., Ewering, C., Pötter, M. & other authors (2006). Genome sequence of the bioplastic-producing ''Knallgas'' bacterium *Ralstonia eutropha* H16. *Nat Biotechnol* **24**, 1257–1262.

Rhu, D. H., Lee, W. H., Kim, J. Y. & Choi, E. (2003). Polyhydroxyalkanoate (PHA) production from waste. *Water Sci Technol* **48** (8), 221–228.

Schroeder, S., Petrovski, S., Campbell, B., McIlroy, S. & Seviour, R. (2009). Phylogeny and in situ identification of a novel gammaproteobacterium in activated sludge. *FEMS Microbiol Lett* **297**, 157–163.

Slater, S. C., Voige, W. H. & Dennis, D. E. (1988). Cloning and expression in *Escherichia coli* of the *Alcaligenes eutrophus* H16 poly-beta-hydroxybutyrate biosynthetic pathway. *J Bacteriol* **170**, 4431–4436.

Sorokin, D. Y., Banciu, H., van Loosdrecht, M. C. M. & Kuenen, J. G. (2003). Growth physiology and competitive interaction of obligately chemolithoautotrophic, haloalkaliphilic, sulfur-oxidizing bacteria from soda lakes. *Extremophiles* **7**, 195–203.

Steinbüchel, A. (2001). Perspectives for biotechnological production and utilization of biopolymers: metabolic engineering of polyhydroxyalkanoate biosynthesis pathways as a successful example. *Macromol Biosci* **1**, 1–24.

Takabatake, H., Satoh, H., Mino, T. & Matsuo, T. (2000). Recovery of biodegradable plastics from activated sludge process. *Water Sci Technol* **42** (3–4), 351–356.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

van Loosdrecht, M. C. M., Pot, M. A. & Heijnen, J. J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Sci Technol* **35** (1), 41–47.

Vishniac, W. & Santer, M. (1957). The thiobacilli. *Bacteriol Rev* **21**, 195–213.

Yokota, A., Akagawa-Matsushita, M., Hiraishi, A., Katayama, Y., Urakami, T. & Yamasato, K. (1992). Distribution of quinone systems in microorganisms: Gram-negative eubacteria. *Bull Jpn Fed Culture Coll* **8**, 136–171.

Zhilina, T. N., Zavarzin, G. A., Rainey, F. A., Pikuta, E. N., Osipov, G. A. & Kostrikina, N. A. (1997). *Desulfonatronovibrio hydrogenovorans* gen. nov., sp. nov., an alkaliphilic, sulfate-reducing bacterium. *Int J Syst Bacteriol* **47**, 144–149.