**Schlegelella thermodepolymerans** gen. nov., sp. nov., a novel thermophilic bacterium that degrades poly(3-hydroxybutyrate-co-3-mercaptopropionate)

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A novel thermophilic bacterium, strain K14⁷, capable of degrading poly(3-hydroxybutyrate) as well as copolymers containing 3-hydroxybutyrate and 3-mercaptopropionate linked by thioester bonds, was isolated. 16S rDNA sequence analysis showed that strain DhA-71, a dehydroabietic acid-degrading bacterium, was the nearest phylogenetic neighbour and that both strains should be placed as members of a newly created genus, *Schlegelella* gen. nov., in the *Rubrivivax* subgroup of the *β*-Proteobacteria. Strain K14⁷ (=LMG 21644T=DSM 15344T) is proposed as the type strain of *Schlegelella thermodepolymerans* gen. nov., sp. nov. Its phylogenetic, morphological, biochemical and chemotaxonomic characteristics are described in detail.

Polyhydroxyalkanoates (PHAs) such as poly(3-hydroxybutyrate) [poly(3HB)] and other PHAs are accumulated by a wide range of bacteria as intracellular storage compounds under conditions of nutrient stress, e.g. when an external carbon source is available but the concentration of another nutrient is limiting growth. Owing to their properties as biodegradable, non-toxic thermoplastics and/or elastomers, these biopolymers have attracted much interest and have been considered for various technical applications (Hocking & Marchessault, 1994). Degradation of PHA has been investigated in different natural environments (Mergaert & Swings, 1996). PHA-degrading micro-organisms excrete specific extracellular PHA depolymerases that degrade PHAs and utilize the degradation products as carbon and energy sources for growth (Delafield et al., 1965). Many studies have been carried out with mesophilic polymer-degrading bacteria, but relatively few with thermophilic bacteria. Purification of a PHA depolymerase exhibiting optimum activity at 70 °C from the culture broth of *Comamonas testosteroni* ATSU has been described (Kasuya et al., 1994) but, generally, little information is available about thermophilic poly(3HB) degradation (Takeda et al., 1998).

Recently, polymers with a completely different linkage type were identified in the Gram-negative PHA-accumulating bacterium *Ralstonia eutropha*. In addition to 3HB, these polymers contained 3-mercaptopropionate (3MP) or 3-mercaptobutyrat (3MB) (Lütke-Eversloh et al., 2001a, b). The peculiarity of poly(3HB-co-3MP) and poly(3HB-co-3MB) was the occurrence of thioester linkages in the polymer backbone. Therefore, they represented members of a novel class of biopolymers that were referred to as polythioester.

In this study, we have isolated the thermophilic polymer-degrading bacterial strain K14⁷, which was able to use poly(3HB-co-3MP) as a carbon source for growth. 16S rDNA sequence analysis of strain K14⁷ indicated that this novel isolate was related phylogenetically to the resin acid-degrading thermophilic bacterium DhA-71, isolated by Yu & Mohn (1999), which is capable of degrading dehydroabietic acid at high temperatures. Resin acids are tricyclic diterpenes that occur in many trees and dominate in conifers and, hence, occur in pulp and mill effluents. In general, such resin acids are removed efficiently by biological treatment systems (Kahmark & Unwin, 1996; Liss et al., 1997). Based on phenotypic, chemotaxonomic and phylogenetic results for strains K14⁷ and DhA-71, we describe a novel species belonging to a new genus, for which we propose the name *Schlegelella thermodepolymerans* gen. nov., sp. nov.

Strain K14⁷ was isolated from activated sludge under aerobic and thermophilic conditions. The enrichment culture was prepared by inoculating 5 ml sample of activated sludge from Fayoum, Egypt, in 50 ml mineral salts medium (MSM) broth (Schlegel et al., 1961) supplemented with...
0.15% (w/v) poly(3HB) or poly(3HB-co-3MP) as sole carbon and energy source and incubating the cultures at 55°C for 1 week. The polymers were prepared according to the method of Pitcher et al. (1993). Serial dilutions of the enrichment culture were spread on poly(3HB-co-3MP)-overlay MSM agar plates. Isolate K14T was chosen for its ability to form clear zones. To enhance growth of strain K14T, MSM was supplemented with the following vitamins (l−1): 20 μg biotin, 20 μg folic acid, 60 μg pantoic acid, 50 μg thiamin, 50 μg riboflavin, 50 μg nicotinic acid, 100 μg pyridoxal hydrochloride, 50 μg pantothenic acid, 50 μg vitamin B12, 50 μg p-aminobenzoic acid and 200 μg naphthoquinone (Mohn, 1995).

Genomic DNA extraction, PCR amplification of the 16S rDNA and purification of PCR products were carried out using procedures described previously (Rainey et al., 1996). The PCR product was purified by using a NucleoTrap PCR extraction kit (Macherey-Nagel). The following oligonucleotides were used as primers: 27f (5′-GAGTTTGTATCCTGCGTACG-3′), 343r (5′-CTGCGAGCCGCTCCGTA-3′), 537f (5′-TACGGGAGGCAGCAG-3′), 519r (5′-GTTAGATACCTTATGGG-3′), 536f [5′-CAGC(C/A)GCCGGCGGTATATC(3′)], 803f (5′-ATTAGATACCTTATGGG-3′), 907r (5′-GGGTCAATTTGAAGTATGATCT-3′), 1114f (5′-GCACGGAGCGCAGCAGC-3′), 1385r [5′-CGTGT(GCA)CGCCGCAGGC-3′] and 1525r (5′-AGAAAAGGAGGTGATCCAGGCC-3′).

Sequencing reactions were performed using a SequiTherm Long-Read cycle sequencing kit (Epicentre Technologies) in a model 4000L semiautomatic DNA sequencer (LI-COR). Long-Read cycle sequencing kit (Epicentre Technologies) in sequencing reactions were performed using a SeqiTherm AGGTGATCCAGCC-3′ TGTGT(A/G)CAAGGCCC-3′ 1114f (5′- TTACGGGAGGCAGCAG-3′).

Fig. 1. Neighbour-joining tree based on 16S rDNA sequences showing the estimated phylogenetic relationships of strain K14T and the nearest members of the β-Proteobacteria. Accession numbers are given in parentheses. Bootstrap values are shown as percentages of 1000 replicates. Bar, 1% sequence divergence.

DNA–DNA hybridizations were carried out with photo-biotin-labelled probes in microplate wells as described by Ezaki et al. (1989), using an HTS7000 BioAssay Reader (Perkin Elmer) measuring fluorescence. The hybridization temperature was 50°C. Reciprocal experiments were performed for every pair of strains. DNA–DNA hybridization confirmed that strains K14T and Dha-A-71 are closely related, with DNA reassociation level of 94%, and thus constitute a single species according to the recommendations of Wayne et al. (1987).

Gram-staining was performed according to Gerhardt et al. (1994). Wet mounts for motility determination from various growth stages were prepared from liquid broth and also from solid media and were examined with an Ortholux II microscope (Leitz). Flagella staining was performed using the method of Heimbrook et al. (1989). Cells of strain K14T were Gram-negative rods and motile by means of one polar flagellum. The dimensions of the cells were 0.5–0.6 × 1.0–2.8 μm when grown in sodium gluconate MSM. Cells of strain Dha-A-71 were rod-shaped, motile cells, 0.6 × 1.8 μm when grown in dehydroabiotic acid-containing MSM (Yu & Mohn, 1999).

Strains K14T and Dha-A-71 exhibited some similarity in colony morphology, such as being white and smooth and exhibiting convex elevation, entire margins and butyrous texture, but they were different with regard to colony form and opacity; whereas colonies of K14T were punctiform and...
For fatty acid analysis, the strains were grown for 48 h at 50 °C on TSA medium. Fatty acids were methylated and extracted and separated by GLC using the MIDI system (Microbial ID) as described before (Mergaert et al., 1993). Strains K14 and Dha-A71 showed very similar fatty acid profiles. Their mean composition was: 1:3 % 10:0, 3:3 % 10:0 3-OH, 1:6 % 12:0, 2:0 % 12:0 3-OH, 43:1 % 16:0, 32:6 % 17:0 cyclo, 4:4 % 18:1ω7c and 2:2 % of other fatty acids (each accounting for less than 1 %).

Growth of the strains was investigated in liquid MSM and on MSM agar plates and utilization of carbon sources was determined according to Schirmer et al. (1995) and Gerhardt et al. (1994). The dehydroabietic acid-degrading bacterium Dha-A71 was cultivated as described by Mohr (1995). Utilization of resin acid was determined according to Yu & Mohn (1999). Utilization of 3′,3′-thiodipropionic acid or 3-mercaptopropionic acid was tested by using these compounds at respective concentrations of 0·2 % and 0·15 % (w/v). Activities of catalase, oxidase, lipase and urease were estimated according to Gerhardt et al. (1994). Hydrolysis of gelatin, starch and lipids, indole formation from tryptone, ammonia formation from arginine, nitrate reduction, citrate utilization and hydrogen sulfide production were investigated according to Gerhardt et al. (1994) and Harrigan & McCance (1966). Test substrates were added to MSM and the cultures were incubated at 50 °C.

The susceptibility of the strains towards antibiotics was determined according to Schirmer et al. (1995) on MSM agar plates for the following antibiotics (ml⁻¹): 15 µg tetracycline, 100 µg streptomycin, 50 µg kanamycin, 50 µg ampicillin and 34 µg chloramphenicol.

Common biochemical and physiological characteristics were examined with cells of strains K14 and Dha-A71 cultivated in liquid MSM microtitre plates and are given below in the descriptions of the genus and species. In addition, the strains grow well on Luria–Bertani and nutrient broth plates at 37, 45 and 55 °C and at pH 6, 7 and 8. They were positive (mainly strain K14) or weakly positive (mainly strain Dha-A71) for utilization of inositol, citrate and succinate, urease, catalase, growth at 60 °C and growth at pH 9. Strains K14 and Dha-A71 were negative for growth at 4 °C and at pH 10. Strain K14 differed from strain Dha-A71 in its ability to utilize arabinose, glucose, starch, pyruvate, acetate, palmitate, arachidate and poly(3HB-co-3MP), to produce hydrogen sulfate from cysteine and to reduce nitrate to nitrogen and its inability to grow at pH 5·5. In addition, strain K14 grew weakly on galactose, fructose, sucrose, trehalose, oleate, 3′,3′-thiodipropionate and 3-mercaptopropionate, while strain Dha-A71 did not utilize these substrates but grew at 30 °C and pH 5.

The phylogenetic position of strains K14 and Dha-A71 and their thermophily support their accommodation in a new genus. We propose to classify them in the same species on the basis of their high genomic relatedness, supported by their very similar fatty acid profiles, and propose the name Schlegelella thermodepolymerans gen. nov., sp. nov. In contrast to the nearest related genus, Leptothrix (Spring et al., 1996), S. thermodepolymerans does not produce pigments, grows at 45 and 55 °C and utilizes citrate, succinate and gluconate. Furthermore, total fatty acid extracts of S. thermodepolymerans are characterized by large amounts of cyclic fatty acids, which are absent in extracts from Leptothrix strains (Spring et al., 1996) and rare in other members of the β-Proteobacteria (Willems et al., 1990).

**Description of Schlegelella gen. nov.**

Schlegelella (Schlegelella. L. fem. ending -ella; N.L. fem. n. Schlegelella named in honour of H. G. Schlegel, a pioneer in PHA research).

Gram-negative, non-spore-forming aerobic rods. The temperature range for growth is 37–60 °C, with an optimum around 50 °C. Citrate, succinate and gluconate are utilized. Cells are oxidase- and catalase-positive. The predominant fatty acids are 16:0 and 17:0 cyclo. The genus belongs phylogenetically to the β-subclass of the Proteobacteria, with Schlegelella thermodepolymerans as the type species.

**Description of Schlegelella thermodepolymerans sp. nov.**

Schlegelella thermodepolymerans [the‘mo.de.po.ly’me.ans. Gr. n. therme heat; N.L. v. depolymerare to depolymerize; N.L. part. adj. thermodepolymerans depolymerizing in the heat, referring to the ability to degrade poly(3-hydroxybutyrate) at high temperatures].

Description is as for the genus. Additional characteristics are based on data obtained for strains K14 and Dha-A71. Cells are 1·0–2·8 μm long and 0·5–0·6 μm wide and are motile by means of polar monotrichous flagellation. Strains grow well on complex media at 45–50 °C. Colonies are white and smooth, with a convex elevation, entire margins and butyrous texture, either opaque or translucent. pH range for growth is 6–9 with an optimum at pH 7. Acid is not produced from glucose. Strains utilize dehydroabietic acid, gluconate, lactate, 3-hydroxybutyrate, valerate and poly(3-HB) but not xylose, mannose, hexanoate, octanoate, benzoate, ethanol or poly(3-hydroxyoctanoate). Susceptible to tetracycline, kanamycin and chloramphenicol and resistant to streptomycin and ampicillin. Aesculin and gelatin are hydrolysed. Indole is not produced. Arginine dihydrolase-positive. The G + C content of the DNA is 70·0 mol%.

The type strain, strain K14 (=LMG 21644T=DSM 15344T), was isolated from an activated sludge sample after enrichment on poly(3HB-co-3MP) as sole carbon source. A second strain of the species is strain Dha-A71 (=LMG 21645), isolated by Yu & Mohn (1999) from paper mill effluent.
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References


Pantoea stewartii and Pantoea ananas

