Bioresource Technology 100 (2009) 6033–6039

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biosynthesis of medium chain length poly(3-hydroxyalkanoates) (mcl PHAs) from cosmetic co-products by *Pseudomonas raguenesii* sp. nov., isolated from Tetiaroa, French Polynesia

C. Simon-Colin [a,*], K. Alain [b], G. Raguénès [a], S. Schmitt [a], N. Kervarec [c], C. Gouin [a], P. Crassous [d], B. Costa [e], J.G. Guezennec [a]

[a] Institut Français de Recherche pour l'Exploitation de la Mer, Centre de Brest, BIOMAR/BMM, B.P. 70, 29280 Plouzané, France
[b] Institut Universitaire Européen de la Mer, LM2E-UMR6197, Technopôle Brest-Iroise, 29280 Plouzané, France
[c] Service Commun de RMN-RPE, UBO, 6 Avenue Le Gorgeu, 29200 Brest, France
[d] Institut Français de Recherche pour l'Exploitation de la Mer, Centre de Brest, EEP/LME, B.P. 70, 29280 Plouzané, France
[e] Pacific Biotech, B.P. 140 289, 98701 Arue, Tahiti, Polynésie Française, France

ARTICLE INFO

Article history:
Received 8 April 2009
Received in revised form 18 June 2009
Accepted 22 June 2009
Available online 25 July 2009

Keywords:
Microbial mat
Pseudomonas
mcl PHAs
Polyhydroxyalkanoates
Co-products

ABSTRACT

A new bacterium, designated as strain TE9 was isolated from a microbial mat in French Polynesia and was studied for its ability to synthesize medium chain length poly-β-hydroxyalkanoates (mcl PHAs) during cultivation on cosmetics co-products. The composition of PHAs was analysed by coupled gas chromatography mass spectroscopy (GC/MS), nuclear magnetic resonance (NMR) and Fourier Transform InfraRed (FTIR) spectroscopy. PHAs were composed of C6–C14 3-hydroxyacids monomers, with a predominance of 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and 3-hydroxydodecanoate (3HDD). Differential scanning calorimetry (DSC) experiments allowed the characterization of elastomeric materials with a melting point T_m near 50 °C, enthalpy of fusion ΔH_m from 27 to 32 J/g, and glass transition temperature T_g of −43 °C. Molecular weights ranged from 175,000 to 358,000 g/mol. On the basis of the phenotypical features and genotypic investigations, strain TE9 was assigned to the *Pseudomonas* genus and the name of *Pseudomonas raguenesii* sp. nov. is proposed.

1. Introduction

"Kopara" mats are benthic microbial communities present in shallow lakes on the rims of some atolls of French Polynesia (Défarge et al., 1994; Andréfouët et al., 2003) and grow as gelatinous deposits of several tens of centimeters in thickness. These environments are known to produce a large number of viscous laminated layers in which the presence of exopolysaccharides and poly-β-hydroxyalkanoates secreted by the microbial communities has been demonstrated in response to the high fluctuation of environmental conditions of those mats (salinity, temperature, pH etc.) (Mao Che et al., 2001; Rougeaux et al., 2001; Raguénès et al., 2004; Simon-Colin et al., 2008a,b).

Poly-β-hydroxyalkanoates (PHAs) are biopolymers stored in intracellular inclusion bodies by a wide variety of bacteria as an energy reserve, in response to excess carbon under nutrient-limited conditions (Anderson and Dawes, 1990; Doi, 1990). The monomeric composition of PHAs can be related to the nature of the carbon source supplied to the bacteria. PHAs exhibit a great variety of properties and thus may have different applications. Regarding the length of their carbon chains, PHAs can be divided into three groups: short chain length PHAs (3–5 carbon atoms, scl PHAs), medium chain length (6–15 carbon atoms, mcl PHAs) and long chain length (more than 15 carbon atoms, lcl PHAs). Due to structural differences, the physical properties of mcl PHAs are generally quite different from scl PHAs such as PHB that is highly crystalline and stiff material whereas mcl PHAs have elastomeric properties. Owing to their inherent biocompatibility and biodegradability, PHAs have attracted industrial interest and have been extensively studied in the last two decades (Hocking and Marchessault, 1994; Steinbüchel and Valentin, 1995; Hankermeyer and Tjeerdema, 1999; Braunegg et al., 1998). They are regarded as promising substitutes for petrochemicals plastics, and thus for use in tackling the problem of plastic waste in the future. Moreover, PHAs can be produced from natural renewable carbon sources and represent a new way of utilizing waste from low cost carbon stocks (Solaiman et al., 2001; Tsuge, 2002; Ashby et al., 2004, 2005; Koller et al., 2005). However, PHAs remain more expensive to produce than conventional plastics and there is a need to find high value applications for them to be commercially viable. In particular mcl PHAs show great promise as thermoelastomers for biomedical applications,

* Corresponding author. Tel.: +33 (0) 298224528; fax: +33 (0) 298224757.
E-mail address: christelle.simon.colin@ifremer.fr (C. Simon-Colin).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.06.075

such as drug delivery (Pouton and Akhtar, 1996) and tissue engineering (Williams et al., 1999; Chen and Wu, 2005).

In this study, we described a new PHA-producing strain designated as TE9 which was isolated from a "kopara" mat of the Tetiaroa atoll located in French Polynesia. Under stress conditions in favour of PHAs production, this bacterium was shown to produce different mcl PHAs from various carbon co-products of the cosmetic industry.

2. Methods

2.1. Isolation procedure and cytological characterization of strain TE9

Strain TE9 was isolated from a sample of "kopara" collected from a microbial mat located on the atoll of Tetiaroa, in French Polynesia. Enrichment cultures were purified on Marine Agar 2216E (Difco Laboratories, Detroit, USA). Strain TE9 was selected because of its capacity to exhibit a different phenotype on Minimum Marine Agar medium (Sigma sea salts 27 g/L, agar 15 g/L and pH 7.6) supplemented with 20 g/L of glucose, as compared to colonies obtained on Minimum Marine Agar medium. Moreover, using the PCR method developed by Solaiman et al. (2000), strain TE9 showed the amplicon size (540pb) specific of the *pha*C1 and *pha*C2 genes involved in the synthesis of mcl PHAs.

Gram staining was carried out as described by Conn et al. (1957).

For scanning electron microscopy (SEM) cells were rinsed with seawater/distilled water (50/50, v/v) to eliminate culture medium and a drop of the sample was placed on a polycarbonate filter (0.2 μm, Nucleopore). The filter was immediately placed in a 13 mm diameter coverglass containing 10% formaldehyde (v/v) and cells were fixed overnight. The dried filter was mounted onto a scanning electron microscopy sample stub and coated in a vacuum with two layers of gold (SCD040, Balzers), each of a thickness of 100 Å. Samples were observed using a FEI ESEM Quanta 200 scanning electron microscope.

2.2. Physiological and biochemical characteristics

Growth experiments were performed in 20 mL tubes containing 5 mL of 2216E Liquid medium. The tubes were inoculated at 5% (v/v) and incubated on a table rotary shaker (Infors, Massy, France) at 200 rpm. Turbidity was measured directly on culture tubes by spectrophotometry at 520 nm (Uvikon Xs Secoman, Ales, France) from 0.05 to 0.5 OD values. Growth rates were determined over a temperature range from 15 to 45 °C. The effect of pH on the growth rate was determined from 5.5 to 8.5, using 50 mM MES (pH 5.5–6.5), 50 mM MOPS (pH 6.5–8), and 50 mM AMPSO (pH 8.5) buffers. Growth in the presence of different concentrations of Na^+ was examined by using NaCl concentration between 10 and 70 g/L. Three replicates were studied. Specific growth rates were calculated by performing a linear regression analysis with five to seven points along the logarithmic part of the resulting growth curves.

API 20NE, API 50CH, API ZYM, and ATB 7 ANTIBIOGRAM strips (Biomérieux SA, Craponne, France) were used to detect metabolic properties and antibiotic susceptibilities according to the manufacturer's instructions. For these tests, the suspending medium was adjusted to an NaCl concentration of 15 g/L, and the preparations were incubated at 25 °C for 48 h. The tests results are compared with those from five *Pseudomonas* species closely related to strain TE9 on the phylogenetic tree (Table 1).

2.3. DNA base composition

For the determination of DNA base composition, genomic DNAs were isolated by the method described by Charbonnier et al. (1992). The G + C content of the DNA was determined by thermal denaturation using a Kontron spectrophotometer (Uvikon 940)

Table 1
Differential phenotypic characteristics of strain TE9 and related *Pseudomonas* species Strain/species: 1, strain TE9; 2, *P. mendocina*; 3, *P. pseudoalcaligenes*; 4, *P. alcaligenes*; 5, *P. aeruginosa*; 6, *P. stutzeri*. Data are from the present study and from Ramanenko et al. (2005) +, positive; –, negative; d, different reaction between strains. All the strains were positive for oxidase and catalase.

Characteristics	1	2	3	4	5	6
Production of:						
Water-insoluble pigments	–	+	d	–	–	d
Fluorescence pigments	–	–	–	–	d	–
Denitrification	–	+	+	d	+	+
Arginine dihydrolase	–	+	+	d	+	–
Growth at:						
4 °C	–	–	–	–	–	–
41 °C	+	+	+	+	+	+
Hydrolysis of:						
Gelatin	–	–	d	d	+	–
Starch	–	–	–	–	–	+
Tween 80	+	+	d	–	d	+
Utilization of:						
D-Glucose	+	+	–	–	+	+
Maltose	–	–	–	–	–	+
D-Galactose	+	–	–	–	–	–
Fructose	–	d	–	+	+	d
D-Sorbitol	+	–	–	–	–	–
D-Lactose	+	–	–	–	–	–
Trehalose	+	–	–	–	–	–
Glycérol	–	d	–	d	+	+
D-Mannitol	–	–	–	–	+	d
Gluconate	+	+	–	d	+	d
Malate	+	–	+	+	+	+
L-Valine	–	+	–	–	d	+
L-Lysine	–	–	–	–	+	–
L-Tyrosine	–	+	d	d	+	d
DNA G + C content (mol%)	60.4	62.8–64.3	64–68	62–64	67.2	60.6–66.3