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Accumulation and mobilization of storage lipids by
Rhodococcus opacus PD630 and Rhodococcus ruber NCIMB 40126

Abstract The time course of the accumulation of triacylglycerols (TAGs) in Rhodococcus opacus PD630 or of TAGs plus polyhydroxyalkanoates (PHA) in Rhodococcus ruber NCIMB 40126 with gluconate or glucose as carbon source, respectively, was studied. In addition, we examined the mobilization of these storage compounds in the absence of a carbon source. R. opacus accumulated TAGs only after the exhaustion of ammonium in the medium, and, with a fixed concentration of the carbon source, the amounts of TAGs in the cells increased with decreasing concentrations of ammonium in the medium. When these cells were incubated in the absence of an additional carbon source, about 90% of these TAGs were mobilized and used as endogenous carbon source, particularly if ammonium was available. R. ruber accumulated a copolyester consisting of 3-hydroxybutyrate and 3-hydroxyvalerate already during the early exponential growth phase, whereas TAGs were synthesized and accumulated mainly during the late exponential and stationary growth phases. In the stationary growth phase, synthesis of TAGs continued, whereas PHA was partially mobilized. In the absence of an additional carbon source but in the presence of ammonium, mobilization of TAGs started first and was then paralleled by the mobilization of PHA, resulting in an approximately 90% and 80% decrease of these storage compounds, respectively. During the accumulation phase, interesting shifts in the composition of the two storage compounds occurred, indicating that the substrates of the PHA synthase and the TAG synthesizing enzymes were provided to varying extents, depending on whether the cells were in the early or late exponential or in the stationary growth phase.

Introduction

Lipophilic storage compounds are found in many eukaryotic and prokaryotic organisms (Ratledge 1989). Bacteria usually accumulate specialized lipids, such as poly(3-hydroxybutyrate) [poly(3HB)] and other polyhydroxyalkanoates (PHA), as intracellular inclusions (Steinbüchel 1991; Steinbüchel and Valentín 1995). PHAs are thermoplastic and/or elastomeric polymers that are readily biodegradable (Jendrossek et al. 1996) and may be used for various technical applications (Steinbüchel 1991). In contrast, triacylglycerols (TAGs) normally occur only in eukaryotic organisms and are not common storage compounds in bacteria. Only very few bacteria, belonging to the genera Acinetobacter (Makula et al. 1975; Scott and Finnerty 1976; Alvarez et al. 1997a), Mycobacterium (Barksdale and Kim 1977), and Streptomyces (Olkoski and Packter 1994; Packter and Olkoski 1995), are able to accumulate variable amounts of TAGs. Recently, we isolated from a soil sample a bacterial strain, identified as Rhodococcus opacus PD630, that is able to accumulate substantial amounts of TAGs from different carbon sources, which can contribute up to approximately 70% (w/w) or even more of the cellular dry matter; by contrast, PHA was not accumulated by this R. opacus strain (Alvarez et al. 1996). Several other members of this genus are also able to synthesize and accumulate TAGs; however, in addition to the neutral lipids, R. opacus strain MR22, R. erythropolis and R. fascians accumulate small amounts of poly(3HB), whereas R. ruber and Nocardia corallina accumulate significant amounts of a copolyester consisting of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) in addition from glucose (Haywood et al. 1991; Alvarez et al. 1997b).
The physiology of the biosynthesis and mobilization of TAGs in *Rhodococcus* sp. is not yet well-understood. At present, some physiological aspects of the accumulation of TAGs in bacteria have been described only for *Streptomyces* sp. (Okusho and Packter 1994). These bacteria accumulate substantial amounts of TAGs, containing only saturated fatty acids, during the stationary growth phase. In the present study, we investigated *R. opacus* PD630, which is able to accumulate only neutral lipids but not PHAs, and *R. ruber* NCIMB 40126, which accumulates both PHAs and TAGs, in order to better understand the physiological aspects of the biosynthesis and mobilization of these lipophilic storage compounds. The composition of the TAGs accumulated by *R. opacus* PD630 is unusual and was recently analyzed in detail (Wältermann et al. 2000). It was shown that a high proportion of the TAGs consist of odd-numbered and unsaturated fatty acids. Since for *R. opacus* strain PD630 a method to transfer foreign DNA has recently been established (Kalscheuer et al. 1999), and since this strain accumulates TAGs with a rather unusual composition from simple carbon sources, such as glucose, the wild-type and genetically engineered derivatives of this strain will be interesting candidates for the biotechnological production of highly valuable lipids.

**Materials and methods**

**Bacterial strains, media and growth conditions**

*Rhodococcus opacus* strain PD630 (DSMZ 44193, Alvarez et al. 1996) and *Rhodococcus ruber* (NCIMB 40126, Haywood et al. 1991) were used in this study. Cells were grown aerobically at 30 °C in nutrient broth (NB) medium (0.8%, w/v) or in mineral salts medium (MSM), according to Schlegel et al. (1961), with the indicated carbon source. To allow the accumulation of lipids, the concentration of ammonium chloride in the mineral medium was reduced to 0.05 g/l. To obtain solidified media, 1.8% (w/v) agar was added.

**Physiological studies**

Cells were cultivated for 24 h at 30 °C in NB medium, harvested, resuspended, and incubated for several days in low-nitrogen MSM medium with gluconate or glucose as sole carbon source. Samples were withdrawn at different times and analyzed for the contents of PHA and fatty acids. In order to study mobilization of the stored lipids, cells were first cultivated under storage conditions to obtain the maximum content of the storage compounds, harvested, washed in phosphate buffer (pH 7), and resuspended in MSM medium containing 1 g/l ammonium chloride but lacking an available carbon source. The cells were subjected to chemical analysis by gas chromatography (GC) and thin layer chromatography (TLC).

**Analysis of fatty acids and PHA**

For qualitative and quantitative determination of fatty acids and PHA, 3–5 mg of lyophilized cells were subjected to methanolysis in the presence of 15% (v/v) sulfuric acid, and the acyl- or hydroxyacyl-methylesters were analyzed by GC, as described in detail previously (Alvarez et al. 1996, 1997a, b).

Lipid extraction and thin-layer chromatography

In order to determine the identity of lipids, samples of whole cells were extracted with a mixture of methanol-chloroform (1:1, v/v) and were subjected to TLC on 60F254 silica gel plates (Merck, Darmstadt, Germany) applying the solvent system hexane/diethyl ether/acetic acid 80:20:1 (by volume). Lipid fractions were visualized by iodine vapor. Palmitic acid, stearic acid, dipalmitin, tricaplin, 1,2-dipalmitoyl-3-myrystoyl-rac-glyceryl, cetylalcohol (Merck) were used as reference substances.

**Results**

**Biosynthesis and accumulation of lipids by *R. opacus* strain PD630**

Cells of *R. opacus* PD630 were cultivated in MSM with gluconate as sole carbon source and containing a reduced amount of ammonium. The fatty acids content was determined at different times (Fig. 1). During the first 12 h of incubation, cells utilized the carbon source and ammonium for the synthesis of compounds essential for growth. Since during this period no TAGs were detected, the fatty acids synthesized were probably used principally for phospholipid biosynthesis. After approximately 12 h, when ammonium was completely exhausted from the medium, the proliferation of the cells was restricted and growth deceased, and the bacteria began to enter the stationary growth phase. They were able to continue to utilize the surplus carbon source for the synthesis of neutral lipids, principally TAGs, which were accumulated as insoluble inclusions in the cytoplasm. After approximately 24 h cultivation, the cells reached the maximum density and had the maximum content of neutral lipids (Fig. 1). Mobilization of the stored lipids started after 40 h incubation. The fatty acid composition of the stored lipids remained constant during the time course of the experiment, with hexadecanoic acid (C16:0) and octadecenoic acid (C18:1) as the predominant fatty acids occurring in the TAGs, as revealed previously (Wältermann et al. 2000).

![Fig. 1 Accumulation of fatty acids as triacylglycerols by cells of *Rhodococcus opacus* PD630 cultivated on gluconate under nitrogen-limiting conditions. Cells were cultivated in mineral salts medium (MSM) containing 0.05 g/l ammonium chloride and 1% (w/v) sodium gluconate. • ammonium, ▲ fatty acids, ◆ optical density](image)

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219