Review

Metabolism of triacylglycerols in *Rhodococcus* species: insights from physiology and molecular genetics

Héctor M. Alvarez[1], Roxana A. Silva[1], Marisa Herrero[1,2], Martín A. Hernández[1] and María S. Villalba[1,2]

[1]Centro Regional de Investigación y Desarrollo Científico Tecnológico, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, km 4-Ciudad Universitaria 9000, Comodoro Rivadavia (Chubut), Argentina
[2]Oil m&s, Av. Hipólito Yrigoyen 4250, 9000 Comodoro Rivadavia (Chubut), Argentina.

Received on January 7, 2013; Accepted on February 12, 2013; Published on February 20, 2013

Correspondence should be addressed to Héctor M. Alvarez; Phone: +54 297 4550339, Fax: +54 297 4550339; Email: halvarez@unpata.edu.ar

Abstract

Rhodococcus bacteria possess the ability to accumulate variable amounts of triacylglycerols (TAG) during growth on diverse carbon sources. The evolution seems to have selected these microorganisms as specialists in the accumulation of TAG among bacteria, since their biochemistry is efficiently designed for the biosynthesis and mobilization of these lipids. Detailed research of rhodococcal TAG metabolism started only a few years ago; thus, the fundamental understanding of this process and its regulation remains to be clarified. However, some interesting advances in the basic knowledge on TAG metabolism in rhodococci have been made. Most studies have focused on the physiology of TAG biosynthesis and mobilization in rhodococci. Only recently, some advances in molecular biology and genetics on TAG metabolism occurred as a result of the increasing available genomic information and the development of new genetic tools for rhodococci. These studies have been focused principally on some enzymes of TAG biosynthesis, such as the wax esters/diacylglycerolacyltransferases (WS/DGAT) and TAG granule-associated proteins. In this context, the most relevant achievements of basic research in the field have been summarized in this review article.

Introduction

Rhodococcus are aerobic, non-motile and non-sporulating actinobacteria, which are widespread distributed in natural environments. The occurrence of rhodococci has been reported in a variety of environments, such as tropical and artic soils, deserts as well in marine and deep-sea sediments (Alvarez *et al.* 2004, Heald *et al.* 2001, Luz *et al.* 2004, Peng *et al.* 2008, Peressutti *et al.* 2003, Whyte *et al.* 1998). The cosmopolitan nature of these microorganisms and their ability to adapt to a wide range of environmental conditions are probably supported at least in part by their huge metabolic repertoire. Rhodococcal genomes with different sizes (between 5 to 9 Mb) are usually enriched with catabolic genes involved in the degradation of a wide range of organic compounds and pollutants. These genes are usually distributed in chromosomes as well as in plasmids of different sizes (linear and circular plasmids) (Larkin *et al.* 2010). In addition, rhodococcal genomes possess a broad repertoire of anabolic genes for the biosynthesis of secondary metabolites and reserve materials. Among other compounds and molecules, they are able to produce variable amounts of glycogen, polyhydroxyalkanoates, carotenoid pigments and polyphosphates from diverse carbon sources (Alvarez *et al.* 2000, Hernández *et al.* 2008, Hernández & Alvarez 2010, Pieper & Steinbüchel 1992). However, the main storage compounds accumulated by rhodococci are triacylglycerols (TAG). These microorganisms seem to be specialized for the biosynthesis and accumulation of TAG during growth on diverse carbon sources. Rhodococci possess the ability to conserve metabolic useful energy during catabolism of carbon sources, thus, a part of the resulting energy can be used for growth and division, and the surplus is channeled into energy storage pathways, such as TAG biosynthesis. The metabolic flexibility of rhodococci and their ability to produce diverse storage compounds are traits that enable or enhance the probability of such microorganisms surviving and reproducing in the environment. The lipid accumulation

may provide cells of energetic autonomy and a temporal independence from the environment and contribute to cell survival when they do not have access to energy resources in the soil. The study of TAG accumulation process in rhodococci is not only important for the understanding of their physiology and biology, but also for the potential application of these oleaginous microorganisms for the production of biotechnological products. Bacterial lipids may be used for the production of additives for feed, cosmetics, lubricants, oleochemicals and biofuels (Alvarez 2010, Alvarez & Steinbüchel 2010, Holder *et al.* 2011). The generated knowledge about the biosynthesis and accumulation of TAG by rhodococci and their basic aspects could provide a new production platform for microbial oils.

This article aims to discuss the current knowledge on the biochemistry, metabolism and genetics of TAG accumulation, biosynthesis and mobilization by rhodococci.

Accumulation of triacylglycerols

Some years ago, it was assumed that TAG accumulation was restricted to eukaryotic organisms (animals, plants and yeasts), but not in prokaryotes, since most bacteria store their lipid reserves in the form of polyhydroxyalkanoates (PHA) and wax esters (WS), as had been reported previously (Makula *et al.* 1975, Steinbüchel 1991, Steinbüchel & Valentin 1995). In the past, only few publications reported the occurrence of TAG in mycobacteria, but principally as a free component of cellular envelopes (Barksdale & Kim 1977). So far as we know, the first reports specifically focused on the production of TAG in bacteria were performed in the *Streptomyces* genus (Olukoshi & Packter 1994, Packter & Olukoshi 1995). At that time, during the development of a research project at Steinbüchel's lab in Germany, which included the isolation and characterization of PHA-accumulating bacteria, we isolated, after enrichment on phenyldecane, a bacterial strain with the ability to accumulate large amounts of TAG during cultivation on different carbon sources. The strain isolated from a soil sample at a gas work plant in Moringen (Germany) was later identified as *Rhodococcus opacus* PD630 (Alvarez *et al.* 1996). Strain PD630 surprisingly accumulated TAG, but not PHA. Therefore, this strain was for us an excellent model to study basic aspects on TAG biosynthesis, accumulation and mobilization in prokaryotes. Later, we demonstrated that TAG biosynthesis and accumulation was a common feature among rhodococci, and not only restricted to *R. opacus* (Alvarez 2003, Alvarez *et al.* 1997). Figure 1 shows the total fatty acid contents, which are representative of TAG contents under the used conditions, during cell cultivation of different rhodococcal species on gluconate/glucose and hexadecane as sole carbon sources under nitrogen-limiting conditions. Some of them, such as *R. opacus* and *R. jostii*, can be considered to be oleaginous microorganisms because they produce significant amounts of TAG as intracellular inclusion bodies (Figure 2).

Triacylglycerol biosynthesis by rhodococci

Rhodococci are able to produce TAG from a wide diversity of carbon sources; including mono- and disac-

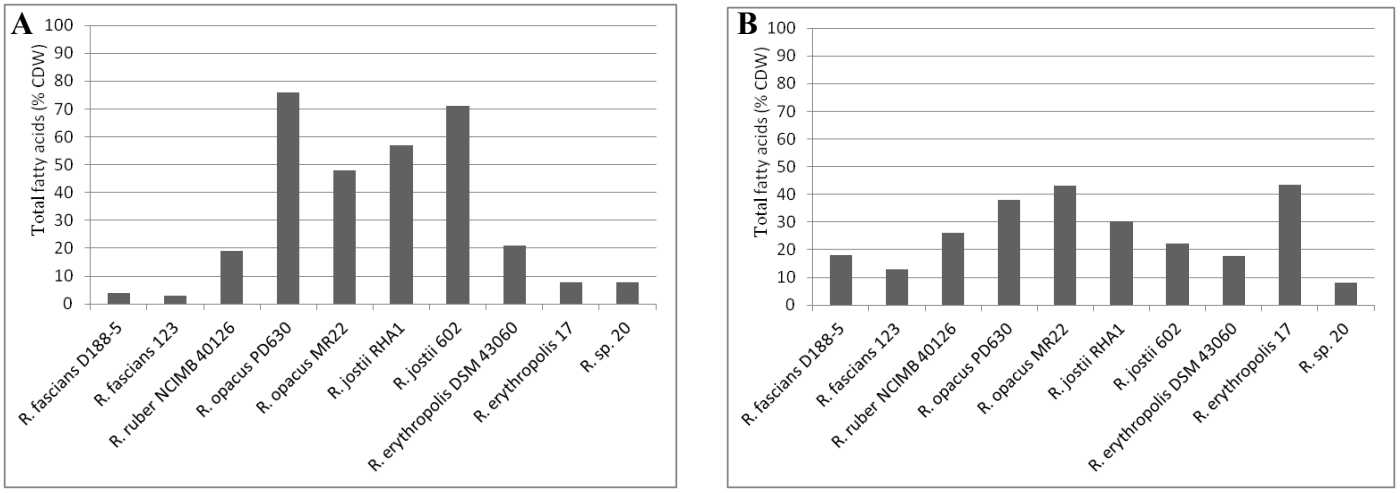

Figure 1. Total fatty acid content (%) of different rhodococcal strains after cultivation in mineral salts medium (MSM) containing 0.1 g/l ammonium chloride and the indicated carbon source at the following concentrations: (A) sodium gluconate 1 % (w/v), glucose 0.5 % (w/v) and (B) hexadecane 0.1 % (v/v). *R. fascians* 123, *R. opacus* PD630, *R. opacus* MR22, *R. jostii* RHA1, *R. jostii* 602, *R. erythropolis* DSM43060, *R. erythropolis* 17, and *R.* sp 20 were cultivated on gluconate whereas *R. fascians* D188-5 and *R. ruber* NCIMB40126 were grown on glucose, as the sole carbon source. *R.* sp 20 is taxonomically close to the *R. erythropolis* species. Abbreviations: CDW, cellular dry weight.

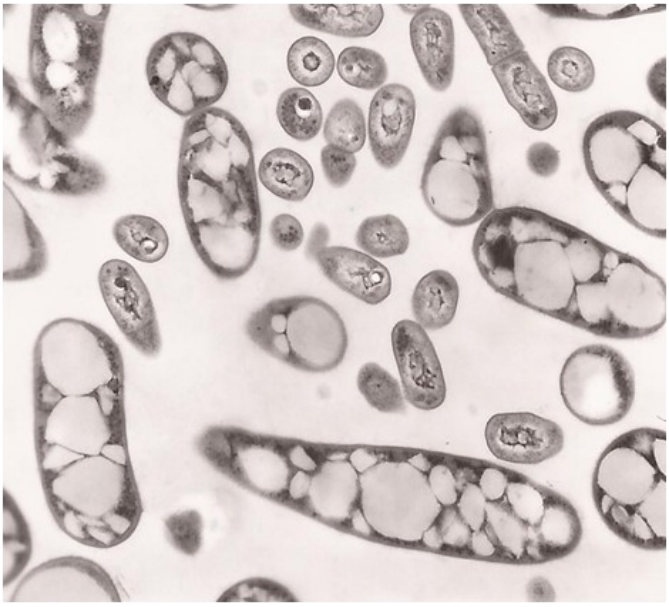

Figure 2. Micrograph showing cells of *Rhodococcus opacus* PD630 containing several TAG granules during growth on gluconate as the sole carbon and energy source. Figure taken from R. Reichelt from the Westfälische Wilhelms University of Münster, Germany.

charides, organic acids, *n*-alkanes, aromatics and polyaromatic hydrocarbons, phenyl-alkanes, and agro-industrial wastes, such as carob and orange wastes and sugar cane molasses (Alvarez *et al.* 1996, 2002, Gouda *et al.* 2008, Hernández & Alvarez, 2010, Voss & Steinbüchel 2001, Silva *et al.* 2010). Nitrogen starvation with an excess of a carbon source promotes TAG biosynthesis and accumulation by rhodococcal cells. Biosynthesis of TAG requires an efficient metabolic network able to produce the necessary precursors, reducing power and energy for the specific reactions. In general, the pathways of rhodococcal central metabolism are able to efficiently convert diverse carbon sources to key metabolic intermediates, such as pyruvate, acetyl-CoA and glycerol-3-phosphate, to create reducing equivalents that are required by lipid biosynthesis pathways and to produce the necessary energy as ATP. The conversion of acetyl-CoA from glycolysis -derived pyruvate might be the major route of carbon flux to fatty acid biosynthesis by some rhodococci; thus, sugars and related substrates support significant TAG accumulation by *Rhodococcus* members (Figure 1A). However, the total TAG content may vary noticeably among individual species or strains within rhodococci, and are ultimately controlled by the genetic make-up of individual organisms (Figure 1A and B). The capacity of rhodococcal cells for the uptake and catabolism/assimilation of the available carbon source seems to also be relevant for TAG accumulation. For instance, the biosynthesis of TAG from gluconate/glucose and hexadecane involves different metabolic pathways (Figure 3). When cells are cultivated on gluconate or glucose (unrelated carbon sources), the substrate has to be degraded to acetyl-CoA, which serves as precursor for fatty acid biosynthesis. In contrast, during cultivation with hexadecane (related carbon source) the cells utilize the preformed fatty acids derived from the mono-terminal oxidation of the alkane for TAG biosynthesis (Figure 3) (Alvarez

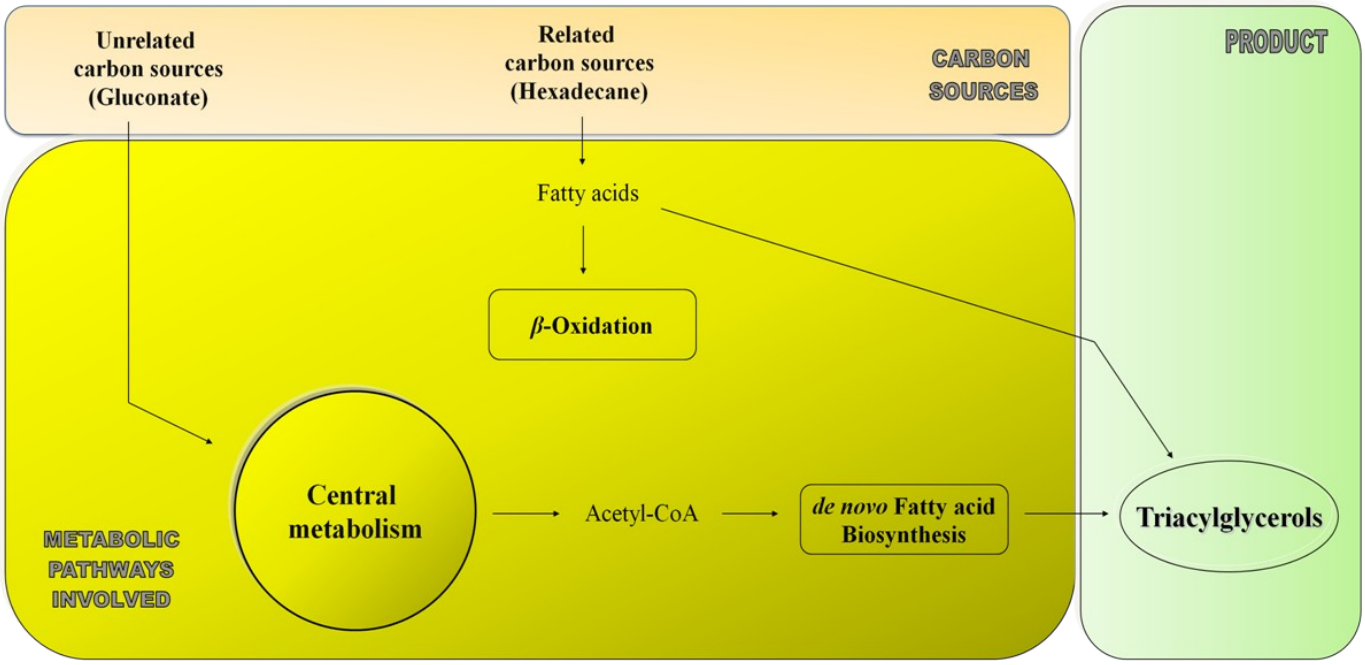

Figure 3. Pathways involved in TAG biosynthesis proposed for *Rhodococcus* sp. during growth on unrelated carbon sources (gluconate or glucose) or related carbon sources (hexadecane).

et al. 1997, Alvarez 2003).

Depending on the substrate used as the carbon source, more than one pathway of central metabolism may contribute to producing and maintaining the acetyl-CoA pool available for fatty acid and TAG biosynthesis. The production of the key metabolic precursors for TAG biosynthesis, the fatty acids, is performed in rhodococci by a multienzymatic complex known as fatty acid synthase I (FASI). This complex catalyzes the successive reaction of condensation, reduction, dehydration and reduction. The FAS type I consists in a unique large protein with different catalytic activities. FASI enzymes are found in the cytoplasm of eukaryotic cells and in a subgroup of actinobacteria, such as mycobacteria, which are used for phospholipids and TAG synthesis or for mycolic acid production after an elongation process mediated by FASII (Bloch 1977, Zimhony *et al.* 2004). The FASI multienzyme gene of mycobacteria and rhodococci seem to be structurally very similar. The main products of rhodococcal FASI may be C_{16}- C_{18} fatty acids, which may be utilized for phospholipids and TAG biosynthesis. Fatty acids, probably as acyl-CoA residues, are sequentially incorporated into the glycerol-3-phosphate backbone for TAG biosynthesis.

Key genes involved in TAG biosynthesis

The TAG biosynthesis in rhodococci has been proposed to occur via sequential acyl-CoA-dependent reactions referred to as the 'Kennedy pathway', which has been described for yeast and plants (Figure 4). The pathway involves the sequential acylation of the *sn*-1, 2 positions of glycerol-3-phosphate, resulting in the formation of phosphatidic acid. The removal of the phosphate group catalysed by the phosphatidic acid phosphatase enzyme occurs before the final acylation step. In the third acylation reaction, an acyl-residue is transferred to the vacant position of diacylglycerol, which is the final step of TAG biosynthesis (Figure 4). The three acylation reactions are catalyzed by different acyltransferases. Phosphatidic acid and diacylglycerol generated in the Kennedy pathway are also used for the synthesis of phospholipids occurring in the membranes. Thus, the third acylation step of the glycerol backbone is the unique enzymatic reaction to TAG biosynthesis (Figure 4). This reaction is catalysed by a diacylglycerol acyltransferase enzyme (DGAT or Atf). Rhodococcal DGAT's belong to a new class of TAG synthesizing enzyme, which exhibits no extended sequence similarity to any known eukaryotic acyltrans-

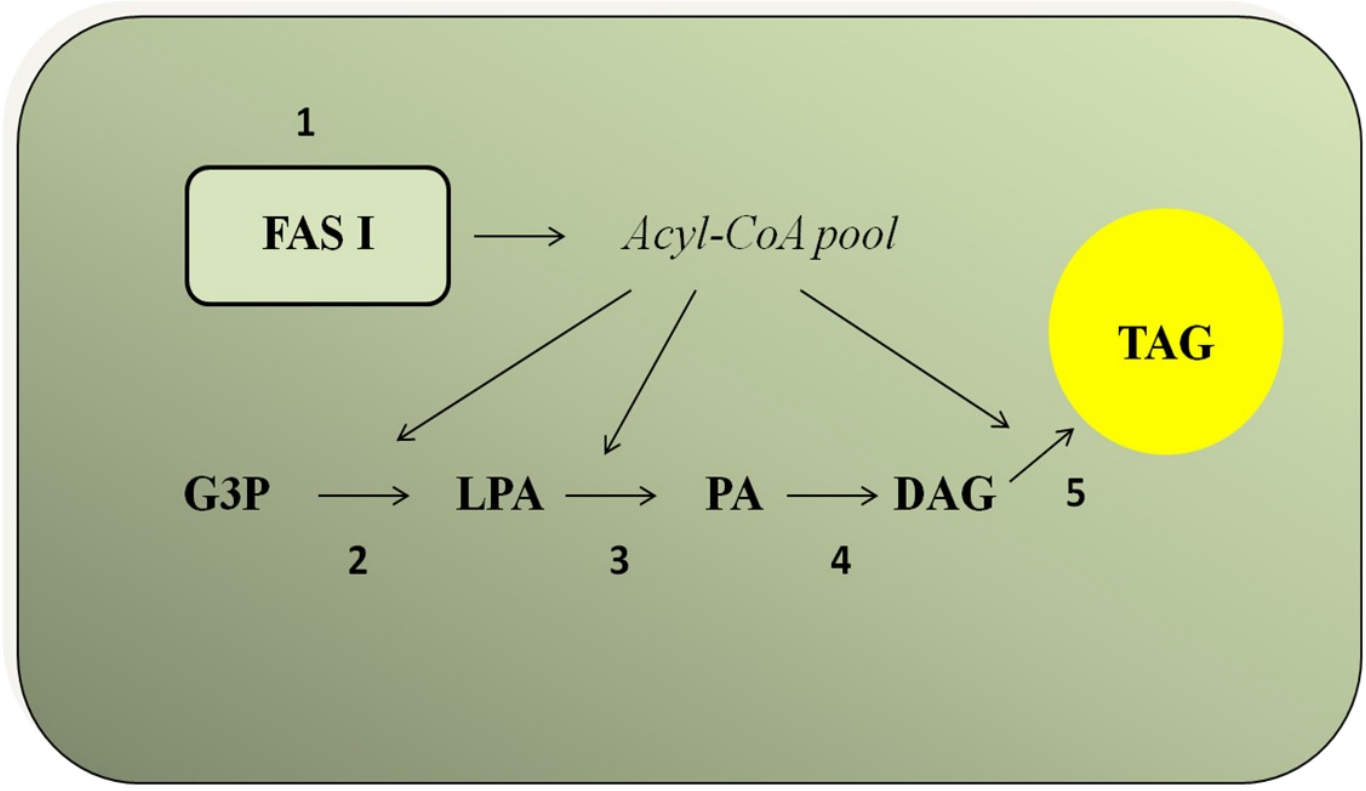

Figure 4. *De novo* TAG biosynthesis occurring in rhodococci (Kennedy Pathway). Each reaction in this figure is catalyzed by the following enzymes: (1) Multifunctional fatty acid synthase Type Ia (FASI); (2) Glycerol-3-phosphate *O*-acyltransferase; (3) 1-acylglycerol-3-phosphate *O*-acyltransferase; (4) Phosphatidic acid phosphatase (PAP); (5) Diacylglycerol-acyltransferases (DGAT). Abbreviations: G3P, glycerol-3-phosphate; LPA, lyso-phosphatidic acid; PA, phosphatidic acid; DAG, diacylglycerols; TAG, triacylglycerols.

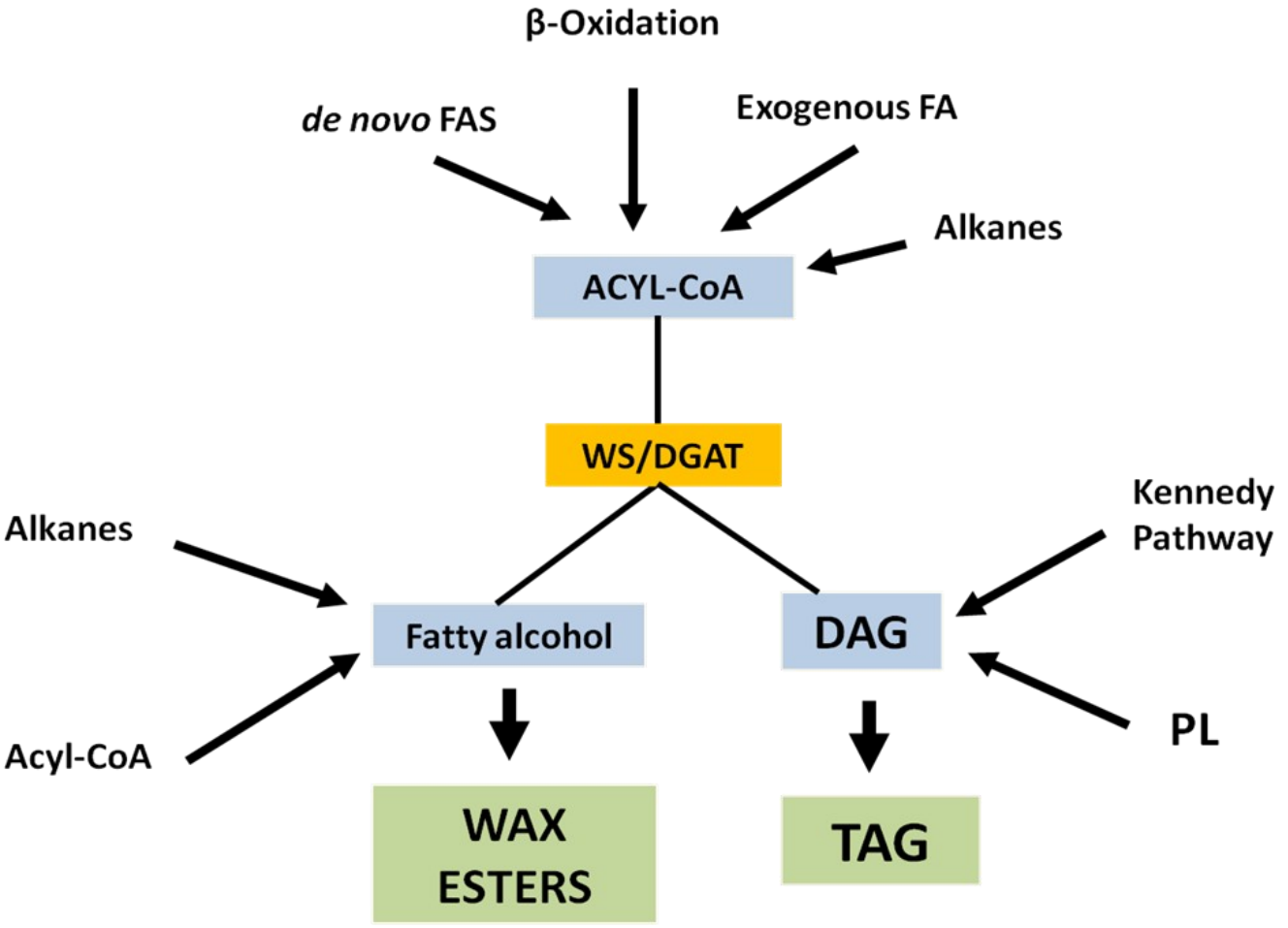

Figure 5. Biosynthesis of TAG or wax esters by bifunctional WS/DGAT's depending on the availability of metabolic intermediates (acyl-CoA, DAG or fatty alcohols) in the metabolic context of the cell. Abbreviations: FAS, fatty acid synthesis; FA, fatty acids, PL, phospholipids; DAG, diacylglyerols; TAG, triacylglyerols; WS/DGAT, wax ester synthase/diacylglyerol acyltransferase.

ferase (Alvarez et al. 2008, Kalscheuer & Steinbüchel 2003, Wältermann *et al.* 2007). These bacterial enzymes possess simultaneously both DGAT and acyl-CoA:fatty alcohol acyltransferase (wax ester synthase, WS) activities. This property confers to rhodococci the ability to produce TAG and/or wax esters depending on which intermediates are present in the metabolic network of cells. Some members of this genus were able to produce wax esters, in addition to TAG, during growth on *n*-alkanes and phenylalkanes using fatty alcohols occurring as intermediates during the degradation of those hydrocarbons (Alvarez *et al.* 1996, 2002, Hernández *et al.* 2008). In general, bacterial WS/DGAT's are promiscuous enzymes, which accept a broad diversity of fatty acyl-CoA's and fatty alcohols as substrates for TAG and wax esters biosynthesis (Figure 5) (Stöveken & Steinbüchel 2008). The acyl composition of stored lipids generally reflects the composition of the available acyl-CoA pool in the cells. The flexibility of the TAG and wax ester biosynthetic enzymes has also been demonstrated *in vivo* in rhodococci (Alvarez *et al.* 2002, Silva *et al.* 2010). In this context, the indigenous *Rhodococcus* sp. strain 602 produced novel TAG containing only short chain-length fatty acids (C_8, C_{10} and C_{12}) during cultivation of cells on napthyl-1-dodecanoate (Silva *et al.* 2010). On the other hand, *R. opacus* PD630 accumulated TAG and wax esters with novel chemical structures, composed by phenyldecanoic acid and phenyldecanol residues, when cells were cultivated on phenyldecane as the sole carbon source (Alvarez *et al.* 2002). The ability to produce and accumulate TAG and wax esters containing unusual fatty acids and alcohols is shared with other related actinobacteria, such as those belonging to *Nocardia* (Alvarez *et al.* 2001) and *Mycobacterium* genera (Silva *et al.* 2007). Such microorganisms with a broad catabolic capacity may possess a protective mechanism for channelling acyl intermediates with unusual chemical structures towards TAG biosynthesis, rather than towards membrane lipid formation. Some of the DGAT isoenzymes with broad specificities may ensure that unusual and potentially membrane

-toxic acyl residues are channelled away from the membrane formation and towards the biosynthesis and accumulation of TAG or wax esters.

The WS/DGAT enzyme coded by the gene *ro01601* from *R. jostii* is the only enzyme which has been analyzed *in vitro* for testing specificity for a range of straight chain fatty acids and alcohols. This approach revealed a high selectivity of the Ro01601 enzyme for palmitoyl-CoA (C_{16}), myristoyl-CoA (C_{14}) and lauroyl-CoA (C_{12}) and it produced predominantly nonanol- to tetradecanol-containing wax esters (Barney *et al.* 2012). Detailed studies on different rhodococcal DGAT's may reveal the physiological role of each isoenzyme. In this context, experimental detailed studies on WS/DGAT's were performed only in *R. opacus* PD630 (Alvarez *et al.* 2008, Hernández *et al.* 2012). Alvarez *et al.* (2008) cloned and expressed ten WS/DGAT's of strain PD630 in *E. coli* for analyzing their acyltransferase activities. In general, all crude extracts of recombinant *E. coli* strains exhibited only low enzymatic activities compared to those obtained from the *R. opacus*. Only the WS/DGAT's genes called *atf1* and *atf2* exhibited wax ester as well as significant DGAT activities, when expressed in *E. coli* (Alvarez *et al.* 2008). The heterologous expression of rhodococcal WS/DGAT's in *E. coli* does not, however, reflect their activities and functionalities *in vivo* in their original host. The heterologous expression of genes from rhodococci in *E. coli* could yield incorrect folded proteins or the enzymes may not react with the routinely used substrate palmitic acid ($C_{16:0}$).

Before any rhodococcal genome was available, Alvarez *et al.* (2008) identified and cloned the first WS/DGAT gene (called *atf*1) in *R. opacus* PD630. The disruption of the *atf1* gene caused a significant impact in the lipid biosynthesis in *R. opacus* PD630, which resulted in a substantial reduction of DGAT activity in crude extracts, and in a significant decrease of the total fatty acids (up to 50 %) as well as of the cellular TAG content. However, it was clear that additional WS/DGAT's contributed to TAG biosynthesis in strain PD630, since disrupted cells still accumulated significant amounts of TAG. Another interesting effect of the mutation of the *atf1* gene was that the mutant exhibited a modified fatty acid profile, with reduced relative amounts of oleic and palmitoleic acids. The same pattern was observed with fatty acids from both whole-cell extracts and TAG fractions (Alvarez *et al.* 2008). These results indicated that rhodococcal WS/DGAT isoenzymes may be specialized for selective incorporation of different fatty acyl residues into TAG. In another study, the disruption of the *atf2* gene resulted in a decrease of the total fatty acids and TAG contents (up to 25-30 %, w/w), in comparison to the wild type (Hernández *et al.* 2012). In contrast to *atf1* gene, the disruption of *atf*2 in PD630 did not result in any evident modification in the fatty acid composition of TAG. The *atf2* mutant was partially complemented due to the low expression rates of recombinant genes induced by *ace* promoters (pPR27ace/*atf*2) when cloned in *Rhodococcus* species (Hernández *et al.* 2012) (Figure 6). Over-expression of *atf*1 and *atf*2 genes in strain PD630 promoted an increase of approximately 10 % (w/w) in TAG accumulation, when cells were grown on glucose as well as hexadecane, as the sole carbon sources. Figure 6 summarizes the modification of TAG accumulation of the different mutant strains in comparison with the wild type (strain PD630). These results confirmed the active role of *atf1* and *atf2* genes in TAG biosynthesis in the oleaginous *R. opacus* PD630. Both genes were transcribed during all stages of growth and TAG accumulation under nitrogen-limiting conditions, as revealed by RT-PCR analyses (Alvarez *et al.* 2008).

Based on our results on *atf1* and *atf2* genes that showed a significant contribution of both genes to TAG accumulation in *R. opacus* PD630, we decided to generate a double *atf1*/*atf2* knockout mutant of PD630,

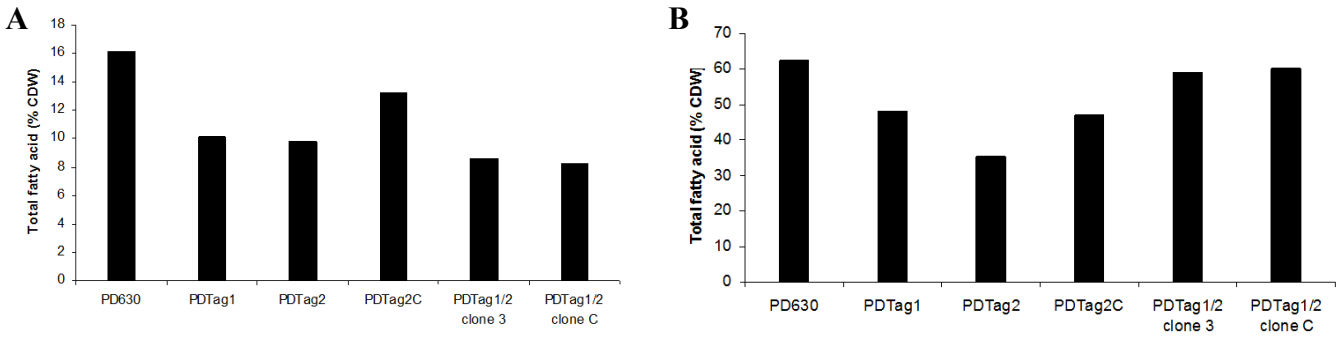

Figure 6. Total fatty acid content (%, CDW) in *R. opacus* PD630 and *atf* mutants during cultivation in: (A) LB medium during 24 h at 28 ºC; and (B) MSM0.1 plus sodium gluconate 1 % (w/v) during 48 h at 28 ºC. PD630: Wild Type strain; PDTag1: *atf*1 mutant; PDTag2: *atf*2 mutant; PDTag2C: complemented *atf*2 mutant; PDTag1/2: *atf*1-*atf*2 double mutant. Abbreviations: CDW, cellular dry weight.

aiming to significantly reduce its TAG content in comparison to the wild type. Contrary to our expectations, PDTag1/2 only accumulated reduced amounts of TAG when cultivated in nitrogen-rich media (LB medium); while in nitrogen-limiting conditions, which promotes lipid accumulation, the TAG content was similar to the wild type strain (Figure 6). The same phenotype was confirmed for two independent double mutant clones (PDTag1/2 clones 3 and C in Figure 6). Analysis of fatty acid composition revealed only slight differences between the wild type, the double *atf1*/*atf2* mutant strains and the single mutants, with a reduced oleic acid content in the double *atf1*/*atf2* mutant similar than the single *atf1* mutant. Interestingly, the cell morphology of the double mutant PDTag1/2 was similar to that of the wild type PD630, with short and wide cells showing lipid inclusion bodies; in contrast to the more elongated and thinner cells observed for the PDTag2 mutant. It is difficult to explain the phenotype of the double mutant of PD630 regarding lipid accumulation, since the regulation of large amounts of genes involved in TAG accumulation in *R. opacus* is not known yet. Cells of PD630 may possess an alternative unknown mechanism to compensate the negative effect of the disruption of both *atf* genes, which might be activated during TAG accumulation; but not when those genes are disrupted individually. A hitherto unknown regulatory mechanism may be involved in activating the additional DGAT's in order to avoid the drastic reduction of TAG accumulation when cells are cultivated under conditions that promote lipid accumulation. These results suggested that storage lipids may play a key role in the physiology and the survival of these soil microorganisms and probably in their ability to cope with diverse environmental stresses.

Genes involved in TAG accumulation

Recently, MacEachran *et al.* (2010) used a genetic approach to identify essential genes for TAG accumulation in *R. opacus* PD630. They generated and analyzed a library of 5,000 random Ez-Tn5 PD630 mutants in order to select those with reduced capacity for accumulating TAG. A gene predicted to encode a putative heparin-binding hemagglutinin homolog, which was called *tadA* (*t*riacylglycerol *a*ccumulation *d*eficient), was identified (MacEachran *et al.* 2010). The Tn5 insertion seemed to specifically negatively affect TAG accumulation but not the growth rate, sugar metabolism or nitrogen utilization. This mutant exhibited a 38% reduction in TAG accumulation during cultivation on glucose under nitrogen limiting conditions. In addition, cells of the *tad*A mutant exhibited changes in their morphology in comparison to the wild type. The mutant presented fewer and slightly larger lipid bodies compared to the appearance of the lipid inclusions of the wild type. Moreover, overexpression of the TadA protein in PD630 resulted in the production of very large inclusion bodies. Finally, the authors demonstrated that TadA protein was localized in the purified lipid bodies (MacEachran *et al.* 2010). In this context, Ding *et al.* (2012) obtained highly purified lipid bodies from *R. jostii* RHA1 and comprehensively characterized the lipid body proteome. They identified two major proteins, *ro02104* and PspA, that constituted about 15% of the total lipid body protein. The *ro02104* gene from *R. jostii* RHA1 encodes the homolog protein of TadA from *R. opacus* PD630. The structure predicted for both proteins (Ro02104 and TadA) resembles that of apolipoproteins, which are the structural proteins of plasma lipoproteins in mammals. Deletion of *ro02104* resulted in the formation of supersized lipid bodies as has been reported by TadA from *R. opacus*. Taken together, these results suggested that the TadA protein from *R. opacus* and its homolog Ro02104 from *R. jostii* may mediate lipid body formation, probably in concert with additional proteins. These proteins may play a structural role in the lipid bodies resembling the role of apolipoproteins of eukaryotes.

Mobilization of triacylglycerols

Rhodococci are able to mobilize TAG during the late stationary growth phase or when cells are incubated in the absence of a carbon source (Alvarez *et al.* 2000). The degradation of stored TAG may be a relevant process for cells in natural environments, since these microorganisms and other related actinobacteria usually habit energy-poor environments, such as deserts. It is known that TAG are excellent reserve materials due to their extremely hydrophobic properties, which allow their accumulation in large amounts in cells without changing the osmolarity of the cytoplasm. In addition, oxidation of TAG produces the maximum yield of energy in comparison to other storage compounds such as carbohydrates and PHA, since the carbon atoms of the acyl moieties of TAG are in their most reductive form (Alvarez & Steinbüchel 2002). The energy obtained by the slow mobilization of stored TAG may support the necessary biochemical and physiological adaptation mechanisms. This process may provide cells of energetic autonomy and a temporal independence from the environment and contribute to cell survival when they do not have access to energy resources in the soil. In this context, we demonstrated that TAG serves as a carbon and energy source during incubation of *R. opacus* PD630 cells under carbon starvation and water stress conditions (Alvarez *et al.* 2000, 2004).

The degradation pathways of fatty acids and TAG have been only poorly studied in rhodococci, in comparison to other bacteria, such as *E. coli* and *B. subtillis*. Holder et al. (2011) sequenced and performed a comparative analysis of the *R. opacus* PD630 genome. They reported that a total of 261 genes were involved in the TAG cycle of this strain. A large gene family was reported to be involved in TAG degradation and β -oxidation of fatty acids; including TAG lipases, FAD dependant acyl-CoA dehydrogenases, enoyl-CoA hydratases and acyl-CoA synthetases, among others. On the other hand, in a genome-wide bioinformatic analysis of key genes involved in TAG metabolism in *R. jostii* RHA1, we identified a total of 54 genes coding for putative lipase/esterase proteins (34 lipases and 20 esterases) in the RHA1 genome (Hernández et al. 2008). Twelve of these genes were located on plasmids, nine on pRHL1 and three on pRHL2, while the rest of the putative lipid hydrolases were located in the RHA1 chromosome. Finally, Letek et al. (2010) reported the occurrence of 36 lipases and many fatty acid β-oxidation enzymes, with 40 acyl-CoA synthetases, 48 acyl-CoA dehydrogenases and 23 enoyl-CoA hydratases, in the genome of *R. equi* strain 103S. All these studies showed that rhodococci are deeply enriched for TAG metabolism genes, including a wide repertoire of genes involved in TAG and fatty acid degradation. This uncommon large genetic and enzymatic machinery for TAG metabolism suggests a key role of these lipids in rhodococcal physiology. Further studies are necessary in order to unravel the molecular biology of TAG catabolism in these fascinating actinobacteria.

Concluding remarks

Our knowledge about the genes and enzymes involved in TAG metabolism in rhodococci is still very limited. Only a few proteins/enzymes of storage lipid formation have been experimentally identified and/or their genes cloned. Most of the studies have been focused on the genes coding for WS/DGAT enzymes, which catalyze the last acylation step for the conversion of DAG to TAG. Recently, some proteins associated with lipid granules in *Rhodococcus* members have been characterized in more detail. An improved understanding of the storage lipid formation in rhodococci will allow us to not only advance the knowledge on the biology of these actinobacteria, which play an important role in the environment, but also to develop more rational and directed strategies for their manipulation, as a valuable source of cosmetic or pharmaceutical products, fine chemicals, biofuels and high-grade lubricant oils. Attention must be directed towards the additional pathways and reactions involved in lipid biosynthesis and mobilization, as well as the understanding of its regulation.

Acknowledgements

The studies on this field were supported by the SCyT of the University of Patagonia San Juan Bosco, the Agencia Comodoro Conocimiento (MCR), the Oil m&s Company, and the Project PFIP2008 CHU25. H.M. Alvarez and R.A. Silva are career investigators and M.A. Hernández a scholarship holder of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. We would like to thank Alexander Steinbüchel for his significant contribution to the academic and scientific formation of our group in the past.

Authors' contributions

RAS, OMH, MSV and MAH participated in the design and helped to draft the manuscript. HMA conceived the manuscript and participated in its design, coordination and draft. All authors read and approved the final manuscript.

References

Alvarez HM 2003 Relationship between b-oxidation pathway and the hydrocarbon-degrading profile in actinomycetes bacteria. *Intern Biodeter & Biodeg* **52** 35-42

Alvarez HM 2006 Bacterial triacylglycerols. In: Welson LT (ed) Triglycerides and Cholesterol Research, Nova Science Publishers Inc New York **6** 159–176

Alvarez HM 2010 Biotechnological production and significance of triacylglycerols and wax esters. In: Kenneth N. Timmis (ed) Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds. Springer Verlag, Heidelberg, Germany, Volume 3 Chapter 44 pp 2995-3002

Alvarez HM & Steinbüchel A 2002 Triacylglycerols in prokaryotic microorganisms. *Appl Microbiol Biotechnol* **60** 367-376

Alvarez HM & Steinbüchel A 2010 Physiology, biochemistry and molecular biology of triacylglycerol accumulation by *Rhodococcus*. In: Alvarez HM (ed), Biology of *Rhodococcus*. Microbiology Monographs Series. Springer Verlag, Heidelberg, Germany, Chapter 10, pp 263-290

Alvarez HM, Mayer F, Fabritius D & Steinbüchel A 1996 Formation of intracytoplasmic lipid inclusions by *Rhodococcus opacus* strain PD630. *Arch Microbiol* **165** 377-386

Alvarez HM, Kalscheuer R & Steinbüchel A 1997 Accumulation of storage lipids in species of *Rhodococcus* and *Nocardia* and effect of inhibitors and polyethylene glycol. *Fett/Lipid* **99** 239-246

Alvarez HM, Kalscheuer R & Steinbüchel A 2000 Accumulation and mobilization of storage lipids by *Rhodococcus opacus* PD630 and *Rhodococcus ruber* NCIMB 40126. *Appl Microbiol Biotechnol* **54** 218-223

Alvarez HM, Souto MF, Viale A & Pucci OH 2001 Biosynthesis of fatty acids and triacylglycerols by 2,6,10,14-tetramethyl pentadecane-grown cells of *Nocardia globerula* 432. *FEMS Microbiol Lett* **200** 195-200

Alvarez HM, Luftmann H, Silva RA, Cesari AC, Viale A, Wältermann M & Steinbüchel A 2002 Identification of phenyldecanoic acid as constituent of triacylglycerols and wax ester produced by *Rhodococcus opacus* PD630. *Microbiology* **148** 1407-1412

Alvarez HM, Silva RA, Cesari AC, Zamit AL, Peressutti SR, Reichelt R, Keller U, Malkus U, Rasch C, Maskow T, Mayer F & Steinbüchel A 2004 Physiological and morphological responses of the soil bacterium *Rhodococcus opacus* strain PD630 to water stress. *FEMS Microbiol Ecol* **50** 75-86

Alvarez AF, Alvarez HM, Kalscheuer R, Wältermann M & Steinbüchel A 2008 Cloning and characterization of a gene involved in triacylglycerol biosynthesis and identification of additional homologous genes in the oleogenous bacterium *Rhodococcus opacus* PD630. *Microbiology*. **154** 2327-2335

Barksdale L & Kim KS 1977 *Mycobacterium*. *Bacteriol Rev* **41** 217-372

Barney BM, Wahlen BD, Garner E, Wei J & Seefeldt LC 2012 Differences in substrate specificities of five bacterial wax ester synthases. *Appl Environ Microbiol* **78** 5734-5745

Bloch K 1977 Control mechanisms for fatty acid synthesis in *Mycobacterium smegmatis. Adv Enzymol Relat Areas Mol Biol* **45** 1-84

Ding Y, Yang L, Zhang S, Wang Y, Du Y, Pu J, Peng G, Chen Y, Zhang H, Yu J, Hang H, Wu P, Yang F, Yang H, Steinbüchel A & Liu P 2012 Identification of the major functional proteins of prokaryotic lipid droplets. *J Lipid Res* **53** 399-411

Gouda MK, Omar SH & Aouad LM 2008 Single cell oil production by *Gordonia* sp. DG using agroindustrial wastes. *World J Microbiol Biotechnol* **24** 1703–1711

Heald, SC, Brandão PF, Hardicre R & Bull AT 2001 Physiology, biochemistry and taxonomy of deep-sea nitrile metabolising *Rhodococcus* strains. *Antonie Van Leeuwenhoek* **80** 169-183

Hernández MA, Mohn WW, Martínez E, Rost E, Alvarez AF & Alvarez HM 2008 Biosynthesis of storage compounds by *Rhodococcus jostii* RHA1 and global identification of genes involved in their metabolism. *BMC Genomics* **12** 9:600

Hernández MA & Alvarez HM 2010 Glycogen formation by *Rhodococcus* species and effect of inhibition of lipid biosynthesis on glycogen accumulation in *R. opacus* PD630. *FEMS Microbiol* Lett **312** 93-99

Hernández MA, Arabolaza A, Rodríguez E, Gramajo H & Alvarez HM 2012 The *atf2* gene is involved in triacylglycerol biosynthesis and accumulation in the oleaginous *Rhodococcus opacus* PD630. *Appl Microbiol Biotechnol* DOI 10.1007/s00253-012-4360-1

Holder JW, Ulrich JC, DeBono AC, Godfrey PA, Desjardins CA, Zucker J, Zeng Q, Leach AL, Ghiviriga I, Dancel C, Abeel T, Gevers D, Kodira CD, Desany B, Affourtit JP, Birren BW & Sinskey AJ 2011 Comparative and functional genomics of *Rhodococcus opacus* PD630 for biofuels development. *PLoS Genet* **7(9)** e1002219

Kalscheuer R & Steinbüchel A 2003 A novel bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase mediates wax ester and triacylglycerol biosynthesis in *Acinetobacter calcoaceticus* ADP1. *J Biol Chem* **278** 8075–8082

Kelly BG, Wall DM, Boland CA & Meijer WG 2002 Isocitrate lyase of the facultative intracellular pathogen *Rhodococcus equi*. *Microbiology* **148** 793-798

Larkin, MJ, Kulakov LA & Allen CCR 2010 Genomes and Plasmids in *Rhodococcus*. *In*: H.M. Alvarez (ed) Biology of *Rhodococcus*. Microbiology Monographs Series. Springer Verlag, Heidelberg, Germany. pp 73-90

Letek M, González P, Macarthur I, Rodríguez H, Freeman TC, Valero-Rello A, Blanco M, Buckley T, Cherevach I, Fahey R, Hapeshi A, Holdstock J, Leadon D, Navas J, Ocampo A, Quail MA, Sanders M, Scortti MM, Prescott JF, Fogarty U, Meijer WG, Parkhill J, Bentley SD & Vázquez-Boland JA 2010 The genome of a pathogenic *Rhodococcus*: cooptive virulence underpinned by key gene acquisitions. *PLoS Genet* **6(9)** e1001145

Luz AP, Pellizari VH, Whyte LG & Greer CW 2004 A survey of indigenous microbial hydrocarbon degradation genes in soils from Antarctica and Brazil. *Can J Microbiol* **50** 323-333

MacEachran DP, Prophete ME & Sinskey AJ 2010 The *Rhodococcus opacus* PD630 heparin-binding hemagglutinin homolog TadA mediates lipid body formation. *Appl Environ Microbiol* **76** 7217-7225

Makula RA, Lockwood PJ & Finnerty WR 1975 Comparative analysis of lipids of *Acinetobacter* species grown on hexadecane. *J Bacteriol* **121** 250–258

Olukoshi ER & Packter NM 1994 Importance of stored triacylglycerols in *Streptomyces*: possible car-

bon source for antibiotics. *Microbiology* **140** 931–943
Packter NM & Olukoshi ER 1995 Ultrastructural studies of neutral lipid localisation in *Streptomyces*. *Arch Microbiol* **164** 420-427
Peng F, Wang Y, Sun F, Liu Z, Lai Q & Shao Z 2008 A novel lipopeptide produced by a Pacific Ocean deep-sea bacterium, *Rhodococcus* sp. TW53. *J Appl Microbiol* **105** 698-705
Peressutti SR, Alvarez HM & Pucci OH 2003 Dynamic of hydrocarbon-degrading bacteriocenosis of an experimental oil pollution on patagonic soil. *Intern Biodeter & Biodeg* **52** 21-30
Petersen TN, Brunak S, von Heijne G & Nielsen H 2011 SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature Methods* **8** 785-786
Pieper U & Steinbüchel A 1992 Identification, cloning and sequence analysis of the poly(3-hydroxyalkanoic acid) synthase gene of the Gram-positive bacterium *Rhodococcus ruber*. *FEMS Microbiol Lett* **96** 73-80
Ruiz C, Pastor FI & Diaz P 2005 Isolation of lipid- and polysaccharide-degrading micro-organisms from subtropical forest soil, and analysis of lipolytic strain *Bacillus* sp. CR-179. *Lett Appl Microbiol* **40** 218-227
Silva RA, Grossi V & Alvarez HM 2007 Biodegradation of phytane (2,6,10,14-tetramethylhexadecane) and accumulation of related isoprenoid wax esters by *Mycobacterium ratisbonense* strain SD4 under nitrogen-starved conditions. *FEMS Microbiol Lett* **272** 220-228
Silva RA, Grossi V, Olivera N & Alvarez HM 2010 Characterization of the indigenous *Rhodococcus* sp. 602, a strain able to accumulate triacylglycerides from naphthyl-compounds under nitrogen-starved conditions. *Res Microbiol* **161** 198-207
Steinbüchel A 1991 Polyhydroxyalkanoic acids. In: Byrom D (ed) Biomaterials. MacMillan, London, pp 123–213
Steinbüchel A & Valentin HE 1995 Diversity of bacterial polyhydroxyalkanoic acids. *FEMS Microbiol Lett* **128** 219-228
Stöveken T & Steinbüchel A 2008 Bacterial acyltransferases as an alternative for lipase-catalyzed acylation for the production of oleochemicals and fuels. *Angew Chem Int Ed* **47** 3688-3694
Voss I & Steinbüchel A 2001 High cell density cultivation of *Rhodococcus opacus* for lipid production at a pilot scale. *Appl Microbiol Biotechnol* **55** 547–555
Wältermann M, Stöveken T & Steinbüchel A 2007 Key enzymes for biosynthesis of neutral lipid storage compounds in prokaryotes: Properties, function and occurrence of wax ester synthases/acyl-CoA:diacylglycerol acyltransferases. *Biochimie* **89** 230 –242
Whyte LG, Hawari J, Zhou E, Bourbonniere L, Inniss WE & Greer CHW 1998 Biodegradation of variable-chain length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. *Appl Environ Microbiol* **64** 2578–2584
Zimhony O, Vilchèze C & Jacobs WR Jr 2004 Characterization of *Mycobacterium smegmatis* expressing the *Mycobacterium tuberculosis* fatty acid synthase I (fas1) gene. *J Bacteriol* **186** 4051-4055