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Review

Triacylglycerol and wax ester-accumulating machinery in prokaryotes

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ABSTRACT

Gram negative bacteria as well as Gram positive actinobacteria possess the ability to accumulate variable amounts of wax esters (WE) and/or triacylglycerols (TAG) under nitrogen limiting conditions. In recent years many advances have been made to obtain insight into neutral lipid biosynthesis and accumulation in prokaryotes. The clinical and industrial relevance of bacterial WE/TAG significantly promoted basic and applied research in this field. The recent integrated omic studies as well as the functional characterization of diverse genes are contributing to unravel the composition of the WE/TAG-accumulating machinery in bacteria. This will be a valuable data for designing new drugs against bacteria with clinical importance, such as *Mycobacterium tuberculosis*, or for transferring and optimizing lipid accumulation in bacterial hosts naturally unable to produce such lipids, such as *Escherichia coli*. In this article, recent investigations addressing WE/TAG biosynthesis and storage in prokaryotes are presented. A comprehensive view of the current knowledge on the different genes/proteins involved in WE/TAG biosynthesis is included.

Contents

1. Introduction

Most bacteria are able to survive and thrive in environments with fluctuating nutritional conditions. Moreover, bacterial cells also interact with multiple stress factors that simultaneously occur in natural environments. The production of neutral lipids, such as wax esters (WE) and triacylglycerols (TAG), may be part of the complex strategic survival mechanisms evolved by some prokaryotes, which allow them to colonize and thrive in natural environments. These lipids are convenient storage compounds for carbon and energy, which can be utilized for cell survival in energy-poor environments. Since the carbon atoms of acyl moieties of TAG and WE are in their most reductive form; the degradation of these biomolecules produces a maximum yield of energy in comparison to other storage compounds produced by bacteria, such as glycogen and polyhydroxyalkanoates [1]. The energy obtained by the slow

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mobilization of stored lipids 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 environment. Lipid stored by bacteria may be important not only for their energy potential but also as a reservoir of metabolic water under desiccation conditions, since fatty acid oxidation releases large amounts of metabolic water [2]. In addition, storage lipids possess other important functions in cells, such as the regulation of the fatty acid composition of membrane lipids, as a sink for reducing equivalents and physiological active and potentially toxic metabolic intermediates for balancing the metabolism under environmental fluctuating conditions, as precursor source for biosynthesis of essential lipids, among other possible functions [3].

The biosynthesis and accumulation of TAG and/or WE are stimulated when an excess of a carbon source is available and the nitrogen source is limiting [4,5]. These special conditions are frequently found in soil and marine environments. The ability to accumulate storage lipids demands the presence of a genetic and enzymatic endowment in the microorganism and the capability for maintaining the balance of precursors and reducing equivalents since the lipid accumulation is an energy-expensive process, which compete with cellular growth. The process of neutral lipid accumulation and their involved components have been well studied in eukaryotic organisms, such as plants and yeasts [6,7]. The pioneer studies on WE and TAG accumulation in prokaryotes were mainly performed in members of *Acinetobacter* [4], *Mycobacterium* [8], *Streptomyces* [9] and *Rhodococcus* [10] genera. The important role of TAG in the pathogenesis of *Mycobacterium tuberculosis,* and the relationship of TAG metabolism with antibiotic biosynthesis by *Streptomyces coelicolor* have stimulated the basic research on such lipids in those microorganisms. On the other hand, members of *Acinetobacter* and *Rhodococcus* genera, such as *Acinetobacter baylyi* ADP1 and *Rhodococcus opacus* PD630 have been used as models for deciphering different aspects on WE/TAG biosynthesis and accumulation. More recently, other bacteria with the ability to produce WE and/or TAG have emerged as model organisms for different studies in this field, including *Marinobacter hydrocarbonoclasticus* [11], *Alcanivorax borkumensis* [12] and *Rhodococcus jostii* [13]. The potential application of such neutral lipid-producing microorganisms as a source of single cell oil useful for the production of biofuels or other derived industrial products, promoted further studies which contributed with our understanding of the process. Single cell oils are lipids extracted from microorganisms, which could serve as alternative oil sources for the production of biofuels with similar efficiency as petroleum diesel. The use of microorganisms for lipid production provides some advantage over agricultural sources with regards to the enormous variability of fatty acid composition depending on the carbon source used for cultivation of cells, and the better accessibility of microorganisms to genetic and metabolic engineering. Current research efforts are being focused on the biochemistry and genetics of oil-accumulating bacteria for designing a scalable and commercially viable oil-producing system from inexpensive feedstocks. In this context, the application of omic approaches as well as the functional identification and characterization of key genes/proteins from model bacteria, enabled significant advances in the fundamental knowledge on WE/TAG metabolism. This review article provides a comprehensive view on the composition of the WE/TAG-accumulating machinery necessary for supporting biosynthesis and accumulation of such lipids in prokaryotes.

2. Synthesis and accumulation of WE/TAG by bacteria

TAG as well as WE are synthesized by a diversity of bacteria. However, there are some qualitative and quantitative differences in their accumulation profiles. The synthesis and accumulation of TAG and WE have been reported for Gram negative hydrocarbon-degrading bacteria belonging to *Acinetobacter*, *Marinobacter*, *Thalassolituus* and *Alcanivorax* genera [4,14,15]. These microorganisms are able to produce TAG and WE during cultivation of cells on acetate, pyruvate or hexadecane as sole carbon sources. In general, those cells accumulate low amounts of neutral lipids during growth under nitrogen limiting conditions. However, some strains such as *A. baylyi* ADP1 and *A. borkumensis* SK2, are able to accumulate about 10–20% of cellular dry weight (CDW) of neutral lipids [15,16]. Usually, there are differences regarding the production of TAG and WE depending on the strain. In the case of *A. borkumensis* SK2 and *Alcanivorax jadensis* T9 TAG are the main neutral lipids accumulated, whereas *A. baylyi* ADP1 and *Marinobacter hydrocarbonoclasticus* SP17 and DSM 8798 accumulate mainly WE [11,15].

On the other hand, the accumulation of neutral lipids is a common feature of Gram positive actinobacteria, such as those belonging to *Streptomyces*, *Gordonia*, *Mycobacterium*, *Rhodococcus* and *Nocardia* [8,9,17]. Among them, the members of mycolic acid-containing actinobacteria seem to be the TAG-accumulating specialists. Most of these microorganisms accumulate exclusively TAG when cells are cultivated on non-related carbon sources, such as gluconate or glucose [1]. Large amounts of TAG and markedly more than Gram negative bacteria are usually accumulated by actinobacterial cells during growth on different carbon sources. *Rhodococcus opacus* PD630, which represents the paradigm of TAG-accumulating bacteria, is able to produce up to 75% (CDW) of TAG during cultivation of cells on gluconate under nitrogen limiting conditions [10]. *R. opacus* PD630 produced WE in addition to TAG, only during growth of cells on *n*-alkanes or phenylalkanes as sole carbon sources [10,18]. Altogether, the combination of the substrate used as carbon source for cell growth and the metabolism of the bacterial strain determine the type of neutral lipid accumulated by bacterial cells.

3. Key acyltransferase enzymes for TAG and WE synthesis in bacteria

The synthesis of TAG and WE in prokaryotes depends on the presence of a CoA-dependent acyltransferase enzyme known as wax ester synthase/diacylglycerol acyltransferase (WS/DGAT). This enzyme can exhibit simultaneously both, acyl-CoA:fatty alcohol acyltransferase (wax ester synthase, WS) and diacylglycerol acyltransferase (DGAT) activities (Fig. 1). The first prokaryotic WS/DGAT was reported for *A. baylyi* ADP1 by Kalscheuer and Steinbüchel [16]. Later, several WS/DGATs were identified, cloned and characterized in different strains of *Marinobacter*, *Alcanivorax*, *Mycobacterium*, *Streptomyces* and *Rhodococcus* [11,15,19–22]. In general, WS/DGATs are promiscuous enzymes that accept a broad diversity of acyl-CoA substrates for esterification of DAG or long-chain fatty alcohols for the synthesis of TAG or WE, respectively [16,23] (Figs. 1 and 2). This property confers to bacterial cells the ability to produce TAG and/or WE depending on which intermediates are present in the cellular metabolism. The presence of WS/DGAT enzymes seems to be a key feature that differentiates bacteria capable for synthesizing TAG or WE to those unable to produce such lipids. The heterologous expression of the WS/DGAT enzyme from *A. baylyi* ADP1 or that from *Streptomyces colelicolor* in *Escherichia coli* conferred the ability to produce low amounts of TAG in this bacterial host which is not naturally able to synthesize these neutral lipids [24,25].

In general, Gram negative TAG/WE-synthesizing bacteria possess low number of *ws/dgat* genes in their genomes (1–3 copies) [23]. A similar situation was observed in members of *Streptomyces* genus, such as *S. coelicolor* (3 *ws/dgat's*) [20] and *Streptomyces avermitilis* (1 *ws/dgat*) [26]. In contrast, mycolic acid-containing actinobacteria