Structural diversity of the triterpenic hydrocarbons from the bacterium Zymomonas mobilis: the signature of defective squalene cyclization by the squalene/hopene cyclase

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Abstract

Twelve polycyclic triterpenic hydrocarbons (α- and γ-polypodatetraenes, dammara-20(21),24-diene, 17-isodammara-12,24-diene, eupha-7,24-diene, hop-17(21)-ene, neohop-13(18)-ene, 17-isodammara-20(21),24-diene, neohop-12-ene, fern-8-ene, diploptene and hop-21-ene) were detected in the hydrocarbon fraction from the bacterium Zymomonas mobilis. Some of them have never been reported from bacteria. These triterpenes were present in Z. mobilis in significant amounts, comparable to those of diploptene, which is usually the major triterpenic hydrocarbon in hopanoid-producing bacteria. The occurrence of such compounds confirms the lack of specificity of bacterial squalene cyclases and the possibility of alternative cyclization routes induced by the existence in the cyclization process of intermediate carbocations of sufficient lifetime. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Zymomonas mobilis is a strictly fermentative Gram-negative bacterium producing ethanol from sugars (glucose, fructose and sucrose) via the Entner-Doudoroff pathway [1]. It shows a tolerance to extreme sugar concentrations (currently 20%, and even up to 30–40% w/v, for some strains) and an unusual resistance to high ethanol concentrations (up to 13% w/v) [2]. Its ability to grow under such unfavorable environmental conditions was correlated to its membrane lipid composition [3,4]. Z. mobilis contains high levels of cis-vaccenic acid and belongs to the best hopanoid producers with concentrations up to 30 mg g⁻¹ (freeze-dried cells) [5,6], i.e. one order of magnitude higher than in most hopanoid producers [7]. Such membrane lipids are thought to counterbalance the destabilizing action of ethanol, playing an essential role as modulators in ethanol tolerance [4]. Continuing our lipid screening in Z. mobilis, in an effort to better understand its unusual tolerance towards membrane-unfriendly growth conditions [8], we now report the identification of triterpenic hydrocarbons (Figs. 1 and 2), which are characteristic for failures of the normal cyclization process of squalene into diploptene [9].

2. Materials and methods

2.1. Culture conditions

Z. mobilis ATCC 10988 was grown semi-anaerobically at 30°C [10]. Cells from a 160-l culture were harvested during the stationary growth phase by centrifugation (6000×g, 10 min, 4°C), washed with distilled water, lyophilized and stored at −20°C.

2.2. Lipid extraction and hydrocarbon isolation

Lyophilized cells (53 g) were extracted three times for...
of this bacterium produced mainly diploptene (12) and diplotropol (hopan-22-ol) and only trace amounts of these unexpected hydrocarbons with shorter GC retention times than that of diploptene [9]. GC-MS analysis of the total hydrocarbon mixture showed that all these novel Z. mobilis metabolites were characterized by an mlz 410 molecular ion and were accordingly isomers of squalene and diploptene. Several hydrocarbons (Fig. 2) were isolated from this fraction and identified. Hydrocarbons (4) and (9) were obtained as a mixture of two compounds with a 0.033% yield from total lipids. Their 1H-NMR and GC-MS mass spectra were identical with those respectively reported for dammara-20(21),24-diene and 17-isodammara-20(21),24-diene [13]. Hydrocarbon (5) was obtained with a 0.023% yield and was identified as 17-isodammara-12,24-diene by comparison of its 1H-NMR and mass spectra with those reported in the literature [9]. Eupha-7,24-diene (6) and neohop-13(18)-ene (8) were obtained as pure products with 0.010% and 0.050% yields respectively. Their 1H-NMR and mass spectra were identical to those reported in the literature [9]. These compounds were directly identified by GC coinjection with reference samples. α- and γ-polydadatetraines (2) and (3) were identical (GC, GC-MS) to the corresponding hydrocarbons resulting from the squalene cyclization by a mutated squalene cyclase [12]. Hop-17(21)-ene (7) and neohop-12-ene (10), which coeluted on argentation TLC, were obtained as a mixture with a 0.020% yield and characterized by the 1H-NMR spectrum of the mixture [13] as well as by GC-MS [14,15]. Fern-8-ene (11), hop-21-ene (13) as well as hop-17(21)-ene (7) were identified by GC coelution with reference samples and comparison of the mass spectra obtained by GC-MS with those of the standards or described in the literature [14,15].

So far, only pentacyclic triterpenes of the hopane series including diploptene, diplotropol, bacteriohopenatetrol, a
bacteriohopanetetrol ether, bacteriohopanetetrol glycoside and 32-oxobacteriohopanetriol glycoside were known as squalene cyclization products in this bacterium [5,7,16,17]. Twenty-one different triterpenic hydrocarbons were formerly reported from another Z. mobilis strain, but were not identified [18].

Triterpenic hydrocarbons other than diploptene have rarely been reported from bacteria. Tetracyclic triterpenes of the dammarane, isodammarane and euphane series, as well as neohop-13(18)-ene have been previously described as very minor components in cells of A. acidocaldarius or were formed in trace amounts by the enzymic cyclization of squalene by the purified cloned squalene cyclase of the same bacterium [13]. Neohop-13(18)-ene, hop-17(21)-ene, fern-7-ene and fern-9(11)-ene were found in the purple non-sulfur bacterium Rhodomicrobium vanniellii [19], and hop-17(21)-ene, neohop-13(18)-ene and eupha-7,24-diene accompanied diploptene in trace amounts in Frankia sp. [20]. The composition of the Z. mobilis hydrocarbon fraction is very striking, containing triterpenes that have never

Fig. 2. Triterpenic hydrocarbons from Z. mobilis. (1) squalene, (2) α-polypodatetraene, (3) γ-polypodatetraene, (4) dammara-20(21),24-diene, (5) 17-iso-dammar-12,24-diene, (6) eupha-7,24-diene, (7) hop-17(21)-ene, (8) neohop-13(18)-ene, (9) 17-isodammar-20(21),24-diene, (10) neohop-12-ene, (11) fern-8-ene, (12) diploptene, (13) hop-21-ene.
previously been observed in prokaryotes, such as the bicyclic α- and γ-polypodatetraenes (2 and 3) and a complete series of rearranged hopanes, including hop-21-ene (13), hop-17(21)-ene (7), neo-hop-13(18)-ene (8), neo-hop-12-ene (10) and the fully rearranged fern-8-ene (11). All these hydrocarbons were present in Z. mobilis in larger amounts than those found in the formerly mentioned bacteria, at concentrations of the same order of magnitude as that of diploptene. This precludes any abiotic proton-catalyzed isomerization of diploptene for the formation of the rearranged hopanes; their formation requires drastic conditions, which were not fulfilled under the mild conditions utilized for their extraction and separation.

The low concentration of these exotic hydrocarbons in bacterial cells excludes any structural role for them, e.g. in membrane stabilization. Their presence was interpreted as a failure of the normal cyclization process leading from squalene to diploptene or diploptol. The squalene cyclases display a much lower substrate specificity and do not control the cyclization process as efficiently as the eu karyotic oxidosqualene cyclases involved in the formation of lanosterol and cycloartenol [21,22]. These diverse hydrocarbons represent the signature of intermediate carbocations that are sufficiently long-lived to present the classical reactions of carbocation chemistry: incomplete cyclizations, Wagner–Meerwein rearrangements and final trapping of the cation by proton elimination [9]. In Z. mobilis, such hydrocarbons resulting from defective squalene cyclization, with no clear physiological role, were accumulated to a much larger extent, at least as compared to diploptene, than in A. acidocaldarius, Rh. vannielii or Frankia sp. Diploptene was postulated to be the putative substrate for the insertion of the n-pentose derived C5 side chain found in the C35 bacteriohopane polyols [23], which always represent the major triterpenoids in hopanoid-producing bacteria [7,23]. According to the very high bacteriohopanepolyol concentration in Z. mobilis, an intense squalene cyclase activity yielding diploptene is expected in this bacterium. Diploptene production is accompanied in Z. mobilis by the continuous formation of small amounts of the minor wrong cyclization products (2–11 and 13). Whereas diploptene (12) is characterized, like all metabolic intermediates, by a high turnover and a low intracellular concentration, the other hydrocarbons, which represent metabolic dead ends with no physiological role, are slowly, but steadily accumulated.

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