ACTINOBACTERIAL TRANSFORMATION OF OLEANANE TRITERPENOIDS

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Drugs derived from secondary plant metabolites make up about 25% of the global pharmaceutical market [1]. Oleanane pentacyclic triterpenoids, in particular oleanolic (OA) and glycyrrhetinic (GA) acids, are the most of interest for researchers in medical chemistry and used to obtain derivatives with pronounced antiviral, antimicrobial, anti-inflammatory, antitumor, and hepatoprotective activities. Along with chemical synthesis, biological methods of OA and GA transformations have been actively developing, which allow to obtain valuable derivatives without the use of aggressive reagents and can be carried out under normal temperature, pressure and pH values. Furthermore, microbial conversion ensures selective modifications of triterpenic molecule sites that are either not modified or poorly modified by chemical transformations [2]. Among the known microbial biocatalysts, members of mycelial fungi are the most studied, but their use on a preparative scale is technologically impossible and dangerous due to the mycelial type of their growth and the ability to produce mycotoxins with pronounced mutagenic and carcinogenic effects. Whereas bacterial catalysts are only represented by a few species of *Bacillus*, *Nocardia* and *Streptomyces* genera, including pathogens, exhibiting catalytic activity at a concentration of OA and GA no more than 0.3 g/L [3]. In this context, it is essential to search for new non-pathogenic bacterial strains able to carry out site-directed transformations of OA and GA. One of the intensively studied groups of microorganisms in terms of biotechnological application is non-pathogenic actinobacteria. Non-mycelial growth, synthesis of biosurfactants, the ability to grow on minimal media, a flexible metabolic system and high oxygenase activity determine the prospects for actinobacteria to be used as perspective biocatalysts for biotransformation of OA and GA [4]. Moreover, the ability of actinobacteria of genus *Rhodococcus* to transform pentacyclic triterpenoid betulin with formation of betulone was previously shown [5]. In this work, 76 strains of actinobacteria from the Regional Specialized Collection of Alkanotrophic Microorganisms (official acronym IEGM; the World Federation of Culture Collections number 285; the Unique Research Facility number 73559; www.iegmcol.ru) belonging to the species *Corynebacterium ammoniagenes* (1), *C. glutamicum* (1), *Gordonia terrae* (4), *R. aetherivorans* (1), *R. cercidiphylli* (1), *R. erythropolis* (14), *R. fascians* (2), *R. jostii* (3), *R. opacus* (15), *R. qingshengii* (2), *R. rhodochrous* (6) and *R. ruber* (26) were used. OA (≥98%, Acros Organics, USA) and GA (≥98%, Shanghai Yuanye Bio-Technology Co, China), dissolved in dimethyl sulfoxide (1:10 mg/μL), were used at a concentration of 1.0 g/L.

Bacterial cells were visualized and their morphometric parameters were measured using an Axio Imager M2 microscope (Zeiss, Germany) equipped with an Axiocam 506 Color camera (Zeiss, Germany) in phase contrast mode with a magnification of x1000. To determine the localization of enzymes, crude cell extracts were obtained according to the method described by Tarasova, Grishko, Ivshina, 2017 [6]. The qualitative and quantitative analysis of residual OA and GA and their biotransformation products were carried out by thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS).

The screening of collection actinobacterial cultures for the ability to biotransform of 1.0 g/L of OA and GA revealed that only a few representatives of *R. rhodochrous* (5), *R. opacus* (4), *R. jostii* (1), *R. ruber* (1) and *R. ruber* (5), *R. erythropolis* (1), *R. opacus* (1), *R. rhodochrous* (1), respectively, used triterpenoids as the only carbon source. At the same time, only *R. rhodochrous* IEGM 1360 exhibited high catalytic activity to both OA and GA.

Biotransformation of OA using *R. rhodochrous* IEGM 1360 cells continued for 7 days and was accompanied by 78.9% OA metabolization with the formation of 0.9% product with *m/z* 468.3 ([M]+, GC-MS for methylated biotransformation product). The mass spectrum of the obtained compound corresponded to the mass spectrum of the methyl ester of 3-oxo-olean-12-en-28-oic acid. It is known that 3-oxo-olean-12-en-28-oic acid has a pronounced *in vivo* antimelanoma [7] and *in vitro* antileishmanial and antitripanosomal effects [8]. The literature describes the formation of 3-oxo-olean-12-en-28-oic acid using actinobacteria *Nocardia iowensis* DSM 45197 as a biocatalyst. However, nocardia exhibited catalytic activity at OA concentration of 0.3 g/L, while the duration of the biotransformation was 13 days [9].

Biotransformation of GA using *R. rhodochrous* IEGM 1360 cells continued for 7 days as well and was accompanied by 72.2% triterpenoid metabolization and the formation of 26.1% of oxidized derivative with *m/z* 482.4 ([M]+, GC-MS for methylated biotransformation product). The mass spectrum of the obtained compound corresponded to that of the methyl ester of 3,11-dioxo-olean-12-en-29-oic acid previously obtained using mycelial fungi *Fusarium lini* (product yield was 4.0%, process duration was 12 days) and having inhibitory activity against lipoxygenase [10].

Using crude cell *R. rhodochrous* IEGM 1360 extracts, the participation of enzymes associated with the cell membrane in the biotransformations of OA and GA was experimentally proved.

Using phase contrast microscopy, the formation of separate cell aggregates on the surface of crystalline particles of triterpenoids was shown. At the same time, no significant changes in the size of cells and the relative area of their cell surfaces were recorded. Probably, the formation of aggregates determines the stable metabolic activity of rhodococci towards complex hydrophobic substrates, providing high catalytic activity under conditions in which single cells are not capable of division and biotransformation of the substrate.

The findings expand the understanding of the catalytic activity of actinobacteria of the genus *Rhodococcus* and their possible use as biocatalysts for biotransformation of hydrophobic polycyclic substrates, including for obtaining of biologically active derivatives of OA and GA.

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НОВЫЕ БАКТЕРИИ СЕМЕЙСТВА *SALINISPHAERACEAE* ИЗ СОЛЯНОЙ ШАХТЫ ВЕРХНЕКАМСКОГО МЕСТОРОЖДЕНИЯ

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Ключевые слова: Верхнекамское месторождение калийно-магниевых солей, галофильные бактерии, семейство *Salinisphaeraceae.*

В настоящее время галофильные микроорганизмы являются приоритетным объектом исследований ввиду их способности адаптироваться к широкому диапазону солености и местообитаний на основе различных механизмов приспособления к солевому стрессу [1]. Среди галофильных бактерий описаны деструкторы углеводородных загрязнителей, что свидетельствует о возможности использования данных микроорганизмов для биоремедиации загрязненных объектов окружающей среды [2].

Бактерии семейства *Salinisphaeraceae* (класс *Gammaproteobacteria*, порядок *Nevskiales*) являются гетеротрофными, галотолерантными или галофильными грамотрицательными бактериями, способны существовать при концентрации NaCl от 10 до 300 г/л [3-11]. Семейство включает три рода: *Salinisphaera*, *Abyssibacter* и *Salifodinibacter*. Представители семейства были обнаружены в средах с высоким содержанием соли, в морских или океанических средах: глубоководном рассоле [3], глубоководных гидротермальных источниках [4], морской воде [5, 6], солончаках [7], воде из бассейна для добычи соли [8], глубоководной рыбе [9] и морской воде из Марианской впадины [10]. В ходе исследования глинистых отложений ВКМКС, отобранных непосредственно в шахтах, было выделено четыре штамма галофильных микроорганизмов, обозначенных SHV1, SHV6, SWV1, RV14. Анализ последовательностей гена 16S рРНК показал, что все выделенные бактерии принадлежат семейству *Salinisphaeraceae*.

Цель исследования – проведение сравнительной характеристики новых бактерий, выделенных из соляной шахты ВМКМС, с ранее описанными представителями семейства *Salinisphaeraceae*. Бактериальные штаммы SWV1, RV14 имели сходство по гену 16S рРНК с ближайшим типовым штаммом *Salinisphaera hydrothermalis* EPR70^T на уровне 95,94% и 96,63%, соответственно. Данные низкие проценты сходства позволяют сделать вывод о том, что эти штаммы представляют собой новый таксон. Штаммы SHV1, SHV6 имели сходство по гену 16S рРНК с ближайшим типовым штаммом *Salinisphaera hydrothermalis* EPR70^T на уровне 99,89%.