RHODOCOCCUS ERYTHROPOLIS STRAIN A29-K1 – AN EFFECTIVE PRODUCER OF SURFACE ACTIVE COMPOUNDS

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Microbial surface-active compounds (SACs) are under great industrial interest due to their potential application in different fields, e.g. in soil and water remediation technologies, in oil industry, as well as in medicine, cosmetics, and food industries. Glycolipids are one of the best-studied microbial SACs and rhodococci are one of the most effective (i.e. trehalose lipids) producers of this class of SACs [1]. In spite of the fact that *Rhodococcus erythropolis* is a well-known surfactant producer, its genetics of SAC synthesis is at present not well understood. This information can be useful for the construction of more effective SAC producers. Our research is just the first step investigation of the genetic aspects of surfactant production in *R. erythropolis* strain A29-k1.

R. erythropolis strain A29-k1 was isolated in 2013 from a non-polluted soil sampled in Vecherniy region (Oasis Tale's Hills, Eastern Antarctica).

The spectrum of utilized substances was determined by cultivation on mineral agar plates with different carbon sources (kerosene, diesel oil, *n*-hexane, *n*-nonane, *n*-hexadecane, *o*-xylene, *m*-xylene, *p*-xylene, benzene, ethyl benzene, toluene, naphthalene – in vapor; 2,2,4,4,6,8,8-heptamethylnonane – 0.1%; anthracene, phenanthrene, fluorene, biphenyl, pyrene – 0.02%). Effectiveness of oil (4% v/v) degradation was evaluated gravimetrically after 14 days of cultivation in mineral medium. Hexadecane degradation was quantified with gas chromatography (Agilent 7890B). Surface tension measurements were performed by du Nöuy ring method using a 3S tensiometer (GBX, Romans sur Isère, France) [2]. The critical micelle dilution (CMD) was determined as a parameter proportional to the amount of the produced surfactant [3]. Emulsification assay (E_{24}) was carried out using kerosene as oil phase [2]. Oil spreading test (OS-test) was performed with crude oil [4]. Crude surfactants were extracted with methyl-tert-butyl ether [5]. Glycolipids were separated with thin-layer chromatography and their concentration was measured spectrophotometrically by phenolic-sulfuric acid method [6].

Primers for alkane monooxygenase genes were constructed manually and checked with PrimerBLAST. PCR products were cloned in suicidal vector pK18mob. For target insertion mutagenesis, rifampicin resistant mutants of *R. erythropolis* A29-k1 were crossed with *E. coli* BW19851 possessing one of the plasmid pK18mob+*alkB1*, pK18mob+*alkB2*, pK18mob+*alkB3*, or pK18mob+*alkB4*. Insertion

mutants were selected on peptone-yeast agar plates added with rifampicin (100 μg/ml) and kanamycin (250 μg/ml).

It was shown that *R. erythropolis* strain A29-k1 utilized crude oil, kerosene, diesel oil, *n*-hexane, *n*-nonane, *n*-hexadecane, *o*-xylene, ethyl benzene, toluene, and anthracene as a sole carbon source. Effectiveness of oil degradation at 28°C was about 30%. When the strain was grown on *n*-hexadecane (20 g/L) as a carbon source, near 85% of the substratum was degraded after 3 days in liquid cultures.

R. erythropolis A29-k1 effectively produced SACs when grown with hydrophobic substrates, such as *n*-hexadecane. The surface tension of the whole cultures was decreased to 28 mN/m after 3 days of cultivation while the surface tension of the cell-free filtrate was just 56 mN/m. The mean diameter of oil-free zones in OS-test was 1.85 cm. On the other hand, emulsification activity (E_{24}) was low for whole cultures and absent in the cell-free filtrates. Overall, our results demonstrated the strain A29-k1 produced cell-bound surfactants with a low emulsification activity. About 11 g/L crude surfactants were extracted from *R. erythropolis* A29-k1 whole culture with MTBE. At least two types of glycolipids (with R_f about 0.46 and 0.54) were present in crude surfactants in concentration near to 8 mM.

As far as concerned the SAC production genetics, there is a little amount of data regarding the *Rhodococcus* genus. Inaba et al. have shown that the products of three genes (*alkB, fda, tlsA*) are involved in trehalose lipids synthesis in *Rhodococcus* sp. strain SD-74 [7], but *R. erythropolis* possesses four *alkB*-genes [8]. Therefore, four insertion mutants were obtained in order to investigate the role of alkane monooxygenases in SAC production: *R. erythropolis* A29-k1 Rif^R *alkB1*::pK18mob (Km^R), *R. erythropolis* A29-k1 Rif^R *alkB2*::pK18mob (Km^R), *R. erythropolis* A29-k1 Rif^R *alkB3*::pK18mob (Km^R), and *R. erythropolis* A29-k1 Rif^R *alkB4*::pK18mob (Km^R).

All the mutants were able to use *n*-hexadecane as a sole carbon source, while the mutant *R. erythropolis* A29-k1 RifR alkB3::pK18mob (KmR) showed a lower growth rate. On the one hand, there were no statistically significant differences between surface tension, emulsification activity as well as OS-test results for whole cultures of wild type strain and the four mutants after 3 days of cultivation in mineral medium with *n*-hexadecane. Nevertheless, the surface tension of the cell-free filtrate of *R. erythropolis* A29-k1 Rif^R *alkB3*::pK18mob (Km^R) was lower (29 mN/m) than that of the wild type strain. Moreover, the CMD value of the whole cultures of *R. erythropolis* A29-k1 (CMD = 107 times) was higher than that of the four mutants with the lowest values (i.e. lowest surfactant production) measured for *R. erythropolis* A29-k1 Rif^R *alkB3*::pK18mob (Km^R) (CMD = 48 times).

Glycolipids were detected in the cell-free filtrates of the wild-type strain, as well as in that of the four mutants even if some differences in spectra were determined. In particular, wave length of the maximum for *R. erythropolis* A29-k1 Rif^R *alkB3*::pK18mob (Km^R) was 481-483 nm, whereas it was 471-475 nm for the

wild-type strain and the other three mutants. Concentration of glycolipids for all the mutants was lower than for the wild-type strain: *alkB1* and *alkB3* – 1.4 times; *alkB2* – 1.2; *alkB4* – 1.1.

Our study demonstrated *R. erythropolis* strain A29-k1 is an effective producer of cell-bound glycolipid surfactants. As it was said, this work is the first step investigation of the genetic aspects of surfactant production in *R. erythropolis* strain A29-k1. Further studies should be performed to understand how the highlighted differences between the wild type strain and mutants are connected with differences in SAC structure and growth rates.

Literature

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