

RHAMNOLIPIDS BIOSYNTHESIS IN *PSEUDOMONAS AERUGINOSA* IN THE PRESENCE OF THE EXOGENOUS AUTOINDUCERS OF INTERCELLULAR COOPERATION

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Rhamnolipids biosurfactants are one of the most important secondary metabolites in *P. aeruginosa* controlled by quorum sensing system. The aim of this work was discovering of the rhamnolipids biosynthesis in *P. aeruginosa* in presence of exogenic autoinducers and their synthetic analogs. Study of rhamnolipids biosynthesis was performed on four *Pseudomonas aeruginosa* strains: ATCC 9027, ATCC 27853, ATCC 15692 and ATCC 10145 in presence of quorum sensing exogenous autoinducers: N-(3-oxo-dodecanoyl)homoserinlacton (3-oxo-C₁₂-HSL), N-butyryl-homoserinlacton (C₄-HSL), 2-heptyl-3-hydroxy-4-quinolon (PQS) and its synthetic analogs (2-octyl-, 2-nonyl- or 2-lauryl-3-hydroxy-4-quinolon). It was shown that biosurfactants biosynthesis in *P. aeruginosa* strains activated in late log-phase, and after transition into stationary phase rhamnolipids level increase permanently, depending on incubation time. Rhamnolipids biosynthesis in "planctonic-biofilm" system was more effective than in suspension.

The use of mass-spectrometry method showed that the main rhamnolipids components are L-rhamnosyl- β -hydroxydodecanoyl- β -hydroxydodecanoat (Rha-C₁₀-C₁₀) and L-rhamnosyl-rhamnosyl- β -hydroxydodecanoyl- β -hydroxydodecanoat (Rha-Rha-C₁₀-C₁₀). The influence of exogenous cell-to-cell communication autoinducers on rhamnolipids biosynthesis was studied for the first time. It was shown that 3-oxo-C₁₂-HSL has no effects on rhamnolipids biosynthesis. C₄-HSL increase summary rhamnolipids level in 2,5-4 times, but show no effects on dirhamnolipids/monorhamnolipids ratio. PQS stimulate biosurfactants biosynthesis depend the concentration (in the range of 20-80 μ M) in 2-6 times. It was shown that PQS synthetic analogs activity depends on the acyl chain length: octyl-quinolon (C₈) > nonyl-quinolon (C₉) > lauryl-quinolon (C₁₁) and their activity was lower than PQS activity. PQS contributes to the enrichment of rhamnolipids mixture with dirhamnolipids, increase Rha-Rha-C₁₀-C₁₀/Rha-C₁₀-C₁₀ ratio from 2:1 to 4,5:1.

It was shown for the first time that PQS, depend the concentration, increase rhamnosyltransferase 2 activity in 30-93%. Planctonic and biofilm cell biomass almost no change that allows to conclude, that PQS directly induces RhIC. Rhamnosyltransferase 2 kinetic study shown that in presence of PQS an affinity of enzyme to substrate increase, as evidenced by the decreasing of K_m from 12,5 to 8,3 μ M. The results serve as a biochemical rationale of improving the efficiency of biotechnological processes for production of biosurfactants *P. aeruginosa*. Data shows rhamnolipids biosynthesis stimulation with PQS in "plancton-biofilm" system substantiates the promise of technology development based on biofilms using. The possibility of enriching *P. aeruginosa* with dirhamnolipids helps to

ensure more effective drugs for medical purposes. Obtained results deepen the understanding of the rhamnolipids biosynthesis regulation mechanisms and show perspectives for using autoinducers for increasing the biosynthesis of other metabolites in *P. aeruginosa*.