Engineering mediator-based electroactivity in the obligate aerobic bacterium Pseudomonas putida KT2440.

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Abstract

Pseudomonas putida strains are being developed as microbial production hosts for production of a range of amphiphilic and hydrophobic biochemicals. P. putida's obligate aerobic growth thereby can be an economical and technical challenge because it requires constant rigorous aeration and often causes reactor foaming. Here, we engineered a strain of P. putida KT2440 that can produce phenazine redox-mediators from Pseudomonas aeruginosa to allow partial redox balancing with an electrode under oxygen-limited conditions. P. aeruginosa is known to employ its phenazine-type redox mediators for electron exchange with an anode in bioelectrochemical systems (BES). We transferred the seven core phenazine biosynthesis genes phzA-G and the two specific genes phzM and phzS required for pyocyanin synthesis from P. aeruginosa on two inducible plasmids into P. putida KT2440. The best clone, P. putida pPhz, produced 45 mg/L pyocyanin over 25 h of growth, which was visible as blue color formation and is comparable to the pyocyanin production of P. aeruginosa. This new strain was then characterized under different oxygen-limited conditions with electrochemical redox control and changes in central energy metabolism were evaluated in

comparison to the unmodified P. putida KT2440. In the new strain, phenazine synthesis with supernatant concentrations up to 33 µg/mL correlated linearly with the ability to discharge electrons to an anode, whereby phenazine-1-carboxylic acid served as the dominating redox mediator. P. putida pPhz sustained strongly oxygen-limited metabolism for up to 2 weeks at up to 12 µA/cm(2) anodic current density. Together, this work lays a foundation for future oxygen-limited biocatalysis with P. putida strains.

Involved units

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