Biofilm as a production platform for heterologous production of rhamnolipids by the non-pathogenic strain Pseudomonas putida KT2440 - DTU Orbit (22/12/2018)

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Background

Although a transition toward sustainable production of chemicals is needed, the physiochemical properties of certain biochemicals such as biosurfactants make them challenging to produce in conventional bioreactor systems. Alternative production platforms such as surface-attached biofilm populations could potentially overcome these challenges. Rhamnolipids are a group of biosurfactants highly relevant for industrial applications. However, they are mainly produced by the opportunistic pathogen Pseudomonas aeruginosa using hydrophobic substrates such as plant oils. As the biosynthesis is tightly regulated in P. aeruginosa a heterologous production of rhamnolipids in a safe organism can relieve the production from many of these limitations and alternative substrates could be used.

Results

In the present study, heterologous production of biosurfactants was investigated using rhamnolipids as the model compound in biofilm encased Pseudomonas putida KT2440. The rhlAB operon from P. aeruginosa was introduced into P. putida to produce mono-rhamnolipids. A synthetic promoter library was used in order to bypass the normal regulation of rhamnolipid synthesis and to provide varying expression levels of the rhlAB operon resulting in different levels of rhamnolipid production. Biosynthesis of rhamnolipids in P. putida decreased bacterial growth rate but stimulated biofilm formation by enhancing cell motility. Continuous rhamnolipid production in a biofilm was achieved using flow cell technology. Quantitative and structural investigations of the produced rhamnolipids were made by ultra performance liquid chromatography combined with high resolution mass spectrometry (HRMS) and tandem HRMS. The predominant rhamnolipid congener produced by the heterologous P. putida biofilm was mono-rhamnolipid with two C₁₀ fatty acids.

Conclusion

This study shows a successful application of synthetic promoter library in P. putida KT2440 and a heterologous biosynthesis of rhamnolipids in biofilm encased cells without hampering biofilm capabilities. These findings expands the possibilities of cultivation setups and paves the way for employing biofilm flow systems as production platforms for biochemicals, which as a consequence of physiochemical properties are troublesome to produce in conventional fermenter setups, or for production of compounds which are inhibitory or toxic to the production organisms.

General information

State: Published
Organisations: Department of Systems Biology, Infection Microbiology, Metabolomics Platform, National Food Institute, Research group for Microbial Biotechnology and Biorefining, National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Contributors: Wigneswaran, V., Nielsen, K. F., Sternberg, C., Jensen, P. R., Folkesson, A., Jelsbak, L.
Number of pages: 13
Publication date: 2016
Peer-reviewed: Yes

Publication information

Journal: Microbial Cell Factories
Volume: 15
Issue number: 181
ISSN (Print): 1475-2859
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.2 SJR 1.443 SNIP 1.227
Web of Science (2017): Impact factor 3.831
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.92 SJR 1.481 SNIP 1.228
Web of Science (2016): Impact factor 3.681
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.08 SJR 1.563 SNIP 1.265
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.25 SJR 1.757 SNIP 1.52