Doctoral Thesis

Genetics and biochemistry of Pseudomonas aeruginosa rhamnolipid biosurfactant synthesis

Author(s):
Ochsner, Urs Arnold

Publication Date:
1993

Permanent Link:
https://doi.org/10.3929/ethz-a-000926163

Rights / License:
In Copyright - Non-Commercial Use Permitted
Genetics and biochemistry of *Pseudomonas aeruginosa* rhamnolipid biosurfactant synthesis

A dissertation submitted to the Swiss Federal Institute of Technology Zürich for the degree of Doctor of Natural Sciences

presented by
URS ARNOLD OCHSNER
Dipl. Natw. ETH
born June 22, 1965
citizen of Einsiedeln SZ

accepted on the recommendation of
Prof. Dr. A. Fiechter, examiner
Prof. Dr. B. Witholt, co-examiner
PD Dr. J. Reiser, co-examiner

Zürich 1993
SUMMARY

Pseudomonas aeruginosa rhamnolipid biosurfactants are synthesized during the late-exponential and stationary phases of growth. Rhamnolipid biosynthesis proceeds by sequential glycosyl transfer reactions, each catalyzed by a specific rhamnosyltransferase with TDP-rhamnose acting as a rhamnosyl donor and β-hydroxydecanoyl-β-hydroxydecanoate or L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate acting as the acceptor. Enzyme assays for both rhamnosyltransferases based on the incorporation of [14C]-rhamnose into rhamnolipids have been established. The rhamnosyltransferases have been partially purified from P. aeruginosa cell extracts. A large proportion of the enzymatic activity was found to be localized in the cell membrane. The solubilized rhamnosyltransferases appear to be associated with lipopolysaccharides, and enzymatic activity depends on the integrity of these high-molecular weight transferase-LPS complexes.

Rhamnolipid-nonproducing strains have been isolated from a pool of transposon Tn5-GM induced mutants. A screening method based on the solubilization of the cationic detergent CTAB by the anionic rhamnolipids in the presence of methylene blue allowed the detection of rhamnolipid-deficient strains on agar plates. Three mutants strains (UO287, UO299, UO391) have been isolated which were unable to produce rhamnolipids and lacked rhamnosyltransferase activity under any of the conditions tested. However, these strains were capable of growing slowly in hexadecane-containing minimal medium without the addition of surfactants to the medium. During cultivation on n-alkanes, all of the mutant strains were found to produce the «protein-like activator for n-alkane oxidation» (PA), which is an extracellular 14 kDa protein.

The DNA regions flanking the transposon in these mutants were cloned and used as specific probes for the isolation of the corresponding wild-type genes from a P. aeruginosa wild-type cosmid gene library. Single cosmids capable of restoring rhamnolipid synthesis in the mutant strains were isolated. All of these cosmids were found to cross-complement the rhamnolipid-deficient mutants UO287, UO299 and UO391 as well as the previously described 65E12 strain. Further subcloning of the cosmid DNA resulted in a 5.8 kb DNA fragment harbouring all the genes necessary to complement the mutant strains. The rhamnolipid-nonproducing mutants constitute three complementation groups defining the rhl operon. DNA sequence analysis of the rhl operon led to the identification of three closely spaced genes, rhlA, rhlB and rhlR. Mutations within any of these genes abolished rhamnolipid production. The UO299 and UO391 mutants carry a transposon insertion within the rhlA coding sequence, while the UO287 mutant is affected in the rhlB gene. The 65E12 strain is a
double mutant, having a small deletion in its rhlR gene beside the presence of a transposon in an as yet unidentified locus. A better defined rhlR mutant strain was constructed by gene disruption and was found to lack the capacity to synthesize rhamnolipids. The rhlR gene encodes a putative protein of 26.5 kDa which shares homology with some transcriptional activators, including LuxR, LasR, and those of two-component regulatory systems. The RhlA protein (32.5 kDa) harbours a putative signal sequence, suggesting that this protein is secreted into the periplasm, while the RhlB protein (47 kDa) contains at least two putative membrane-spanning domains.

Expression of the rhl genes was studied by using rhl::lacZ fusions allowing the detection of rhl gene expression by measuring β-galactosidase activity. The rhlR gene was found to be constitutively transcribed from a σ70 promoter at low levels. The rhlA and rhlB coding regions are preceded by σ54-type promoters, but only the rhlA promoter was found to be active, resulting in a bicistronic mRNA covering the coding regions of both the rhlA and rhlB genes. The expression of the rhlAB genes is enhanced 20-fold during the stationary phase of growth under conditions of nitrogen limitation. This rhlA promoter activation was absent in the 65E12 mutant strain carrying a mutation in the rhlR locus, indicating that a functional RhlR regulatory protein is necessary for the transcriptional activation of the rhlA gene. The regulatory sequence upstream of the rhlA promoter contains two inverted repeats defining putative binding sites for the RhlR regulator. A model for the regulation of rhamnolipid biosynthesis in response to environmental stimuli involving a two-component regulatory system is presented.

The rhl genes were expressed in heterologous hosts. Rhamnolipid biosynthesis was enhanced in P. fluorescens ATCC15453 when this strain was provided with the P. aeruginosa PG201 rhl operon on a plasmid. The rhlAB genes were overexpressed in different hosts by using plasmids harbouring the rhlAB genes under inducible tac promoter control. The controlled expression of the rhlAB genes in P. aeruginosa and P. fluorescens grown in complex media led to the production of rhamnolipids independent of the environmental conditions and the overexpression of the rhlAB genes in E. coli led to the formation of active rhamnosyltransferase. This shows that the rhlAB genes encode the rhamnosyltransferase.
ZUSAMMENFASSUNG


im rhlB Gen betroffen ist. Der 65E12 Stamm ist eine Doppelmutante, die nebst einer Transposon-Insertion eine kleine Deletion im rhlR Gen trägt. Ein besser definierter rhlR Mutantenstamm, der durch Inaktivierung des rhlR Gens mittels gezielter Mutagenese konstruiert wurde, vermochte ebenfalls keine Rhamnolipide zu produzieren. Das rhlR Gen kodiert ein mutmassliches Protein von 26.5 kDa, das Sequenzhomologien zu einigen transkriptionellen Aktivatoren von Zweikomponenten-Regulationssystemen aufweist. Das RhlA Protein (32.5 kDa) verfügt über eine Signalsequenz und konnte daher ins Periplasma sekretiert werden, während das RhlB Protein (47 kDa) wenigstens zwei mutmassliche Membrandomänen enthält.
