HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight April 2015

☒ co-ordinated with the Director of the Institute / Research Unit

Research Unit Microbe-Plant Interactions (AMP)

PSP-Element:
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Title of the highlight:
Analysis of N-acylhomoserine lactone-dynamics in continuous cultures of *Pseudomonas putida* IsoF using ELISA and UHPLC/qTOF-MS-derived measurements and mathematical models.

Keywords:
Pseudomonas putida IsoF, continuous culture, *N*-acyl-homoserine lactones, mathematical modelling, ELISA, quorum sensing

Central statement of the highlight in one sentence:
The AHL quorum sensing (QS) system in *P. putida* IsoF shows a remarkable stability under varying environmental conditions as demonstrated in chemostat experiments, which always show a steep decline after a peak in early logarithmic growth phase due to a putatively QS regulated lactonase.

Text of the highlight:
In this interdisciplinary approach, the dynamics of production and degradation of the quorum sensing signal 3-oxo-decanoyl-homoserine lactone were studied in continuous cultures of *Pseudomonas putida* IsoF. The signal concentrations were quantified over time using monoclonal antibodies and ELISA. The results were verified with ultra-high performance liquid chromatography, thus, ELISA presents a cost-effective method for acyl-homoserine lactone-screening. Based on a mathematical model, we derived quantitative values for non-induced and induced signal production rate per cell. Noteworthy, we found rather constant values for different dilution rates in the chemostat, and the values seem to be close to those reported for batch cultures before. Thus, the quorum sensing system in *P. putida* IsoF shows a remarkable stability under varying environmental conditions. In all chemostat experiments, the signal concentration decreased strongly after a peak, caused by an emerging lactonase activity. The lactonase activity probably is quorum sensing regulated.

This observed AI dynamic in *P. putida* IsoF clearly contradicts the usual assumption of an induced AHL-production rate as long as the population density exceeds a certain threshold and does not enter the stationary phase in a batch culture. Of course, the proposed down-regulation would most likely require a modulatory mechanism on transcriptional level, quite similar to the environment dependent activity postulated for RsaL in *P. putida* WCS 358. To address this question a transcriptional analysis at varying environmental conditions would be necessary.
Alternatively, the QS system could work in an oscillating manner, e.g. to enable a kind of periodical reset. Due to the low abiotic degradation rate of AHLs for pH values around and below pH 7.0, the cells are exposed to AHL concentrations which do not just reflect the actual cell density, but also integrate about the past. This may not be desirable for actual decisions. A periodic degradation would diminish the impact of the past in a reset button-like way. The regulation system in *P. putida* IsoF consists of a positive feedback (AHL promoted AHL synthesis) coupled with a negative feedback (via lactonase). Dependent on the parameter setup, such a system can indeed show oscillation with a period longer than the duration of our experiment.

Publication:

Buddrus-Schiemann K., Rieger M., Muehlbauer M., Barbarossa M. V., Kuttler C., Hense B. A., Rothballer M., Uhl J., Fonseca J. R., Schmitt-Kopplin P., **Schmid M**., **Hartmann A**. (2014). Analysis of N-acylhomoserine lactone-dynamics in continuous cultures of *Pseudomonas putida* IsoF using ELISA and UHPLC/qTOF-MS-derived measurements and mathematical models. Anal. Bioanal. Chem. 406: 6373-6383

Taking account of the HMGU mission:

Biocontrol agents like *P. putida* IsoF are nowadays widely used in agriculture to avoid chemical fertilizers, herbicides etc. thereby ensuring the production of sufficient high quality food which beneficially affects human health. The most important life style of bacteria colonizing surfaces of e. g. roots or leaves seems to be in biofilms and in these systems the cells' gene expression is under the control of quorum sensing. Understanding the regulating mechanisms of these quorum sensing systems allows a more effective and cost efficient application of biocontrol agents in the field resulting in a more reliable and predictable impact on host plants and the interacting microbial communities.

The internal HMGU co-operation partners with whom the highlight was compiled, if appropriate:

ICB, BGC

Analysis of N-acylhomoserine lactone-dynamics in continuous cultures of *Pseudomonas putida* IsoF using ELISA and UHPLC/qTOF-MS-derived measurements and mathematical models

Helmholtz Zentrum München, AMP
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Introduction

- Quorum sensing (QS) plays a fundamental role in adapting to environmental changes via small diffusible signal molecules called autoinducer (AI)
- Gram-negative bacteria mainly communicate via *N*-acylhomoserine lactones (AHLs)
- *Pseudomonas putida* IsoF contains only one known QS system (PpuI/PpuR)and therefore serves as a model organism for understanding AHL regulation
- In a previous work with *P. putida* IsoF, the production and degradation of AHLs were analysed in batch cultures
- The changing environmental conditions in batch cultures (e.g. nutrients, waste products) may not represent adequately natural scenarios
- Therefore a continuous culture was established with a chemostat and time resolved AHL concentrations were measured with ELISA and UPLC
- All data was subjected to mathematical modelling

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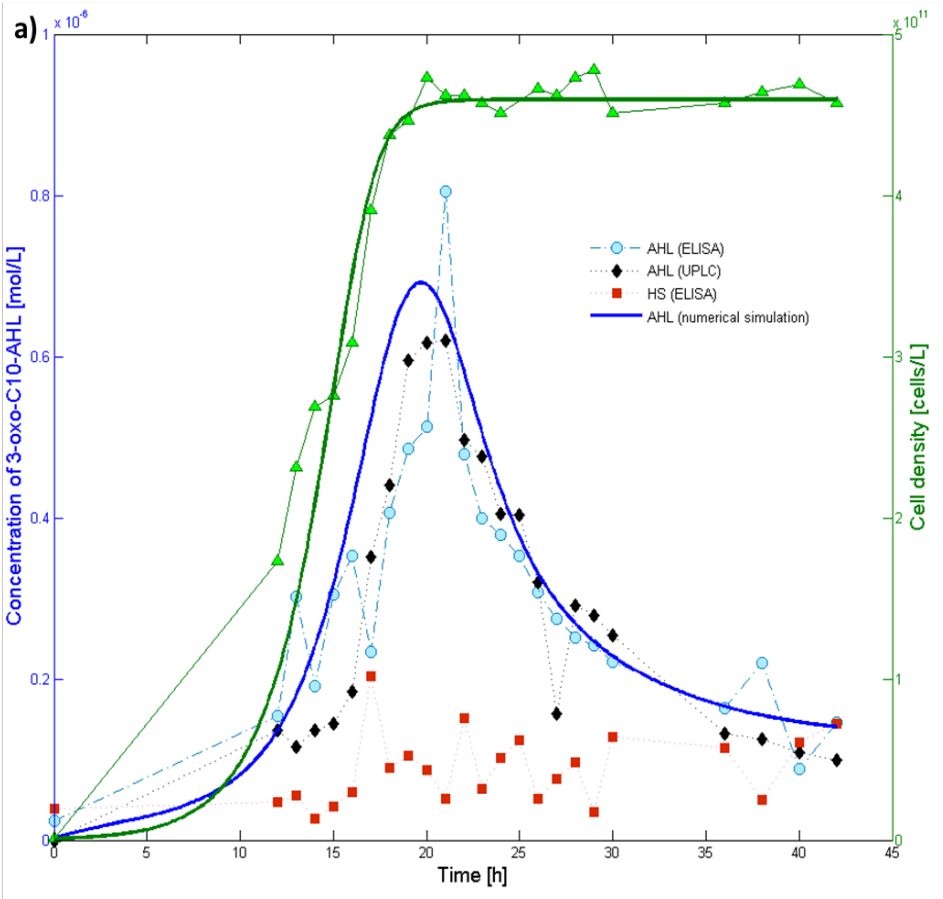
Experimental data and numerical simulations of the continuous culture

Figure: Experimental data and numerical simulations (continuous curves) of the continuous culture with the dilution rates of 0.2 L/h. Bacterial cell density (triangles), ELISA-based (circles) and UPLC-based (diamonds) 3-oxo-C10-AHL concentrations as well as ELISA-based 3-oxo-C10-HS concentrations (squares) in the *Pseudomonas putida* IsoF culture supernatants.

Main result: The AHL quorum sensing (QS) system in *P. putida* IsoF proves to be remarkably stable under varying environmental conditions, always showing a steep decline after a peak in early logarithmic growth phase due to a putatively QS regulated lactonase.



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