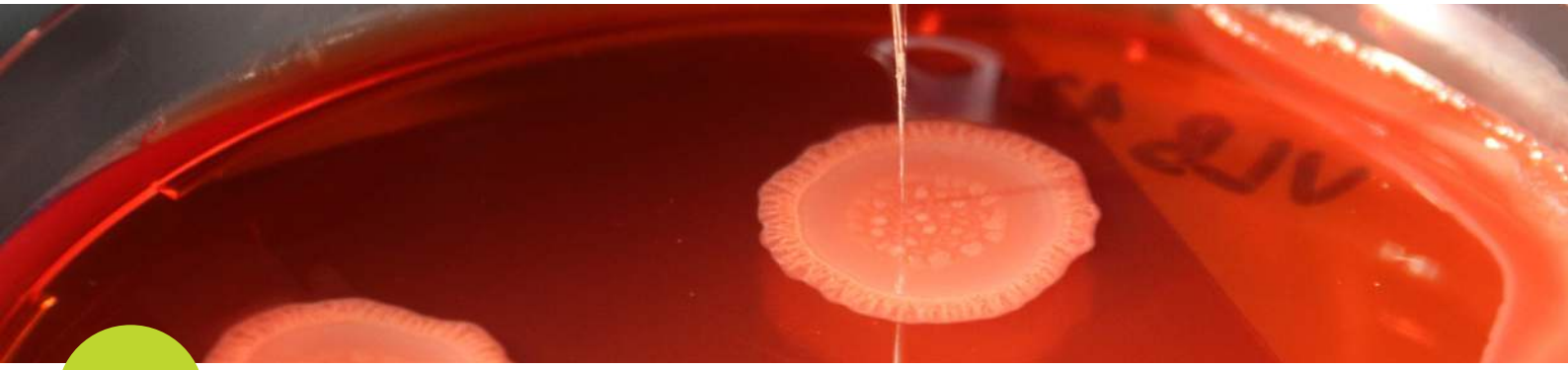


Unisense microsensors in biofilm research

Oxygen and redox potential in *Pseudomonas aeruginosa* colony biofilms

Introduction

Associate Professor Lars Dietrich and his research group at Columbia University routinely use Unisense oxygen microsensors and redox microelectrodes to characterize the chemical gradients that form in bacterial colony biofilms.

The Dietrich lab focuses mainly on Pseudomonas aeruginosa PA14 which is a gram-negative pathogen involved in e.g. lung infections. They use the Unisense microsensors together with the Unisense Microprofiling System to obtain valuable information about the biofilm microenvironment and redox metabolism.

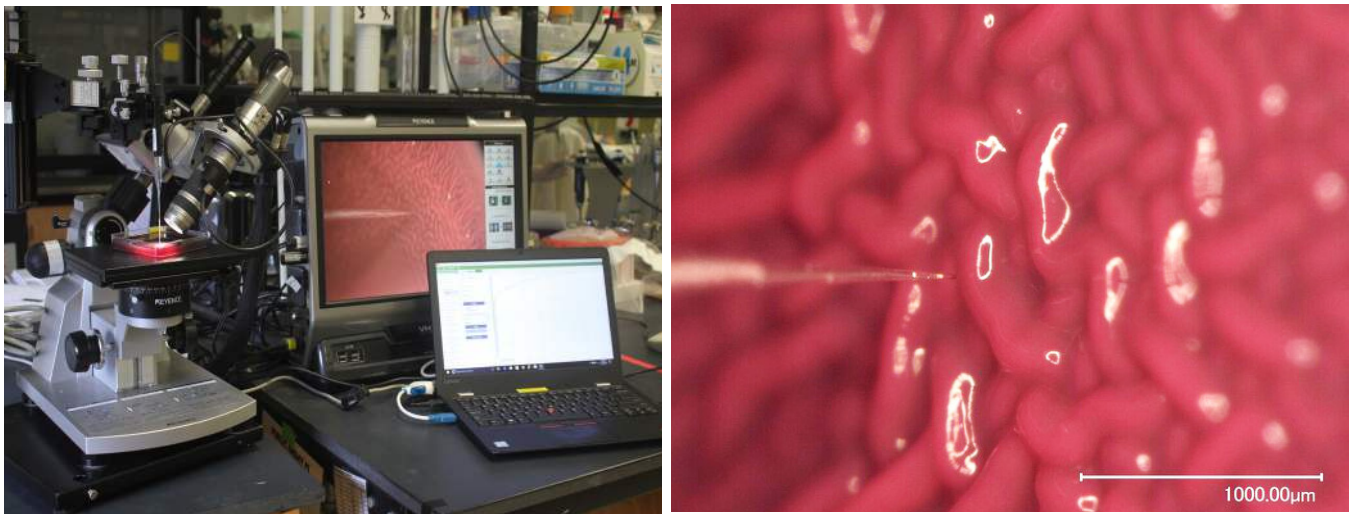
In this study, the group investigated the reduction of phenazines, which are antibiotics produced by *P. aeruginosa*.

Laboratory setup

The researchers completed oxygen and redox potential profiling in a *P. aeruginosa* PA14 colony biofilm model grown on agar-solidified media. On day two, the thickness of the wild-type *P. aeruginosa* colony and the phenazine-null mutant was ~125 µm and ~80 µm, respectively, and microprofiles were made down to a depth of 200 µm. The oxygen concentration was measured using a Unisense oxygen microsensor with a tip diameter of 25 µm (OX-25). The extracellular redox potential was measured using a Unisense redox microelectrode with a tip diameter of 25 µm (RD-25) and a reference electrode (REF-RM).

The image below shows the microprofiling setup with the microsensors mounted in the motorized MicroProfiling System.

The group used SensorTrace Profiling software to control the movement of the sensors and completed microprofiles with a measuring period of 3 seconds, and a waiting time between measurements of 5 seconds. Data acquisition and analysis were done with the SensorTrace Profiling software.



MicroProfiling setup (left) and microsensor inserted into a *P. aeruginosa* colony (right).
Photo by Jeanyoung Jo.

Results and conclusion

Figure 2 shows the oxygen concentration and redox potential as a function of depth in the wild-type *P. aeruginosa* colony biofilm and a phenazine-null mutant (no phenazine production). The oxygen gradient in the wild-type and mutant biofilms decreased similarly from the surface and down into the biofilm. The redox potential in the wild-type biofilm decreased with depth whereas the redox potential in the mutant remained the same throughout the biofilm. The decrease of redox potential in the wild-type indicated reduction of the phenazines. The decline in oxygen concentration was seen right from the surface of the wild-type biofilm whereas the decline in redox potential was mostly pronounced at around 50 µm depth.

The data suggested that the use of oxygen and phenazines as electron acceptors by the bacteria depends the depth in the biofilm and that oxygen is preferred. The reduction of phenazines in the hypoxic zones of the biofilm could contribute to survival of the bacteria and may be an important finding for the development of new treatment strategies.

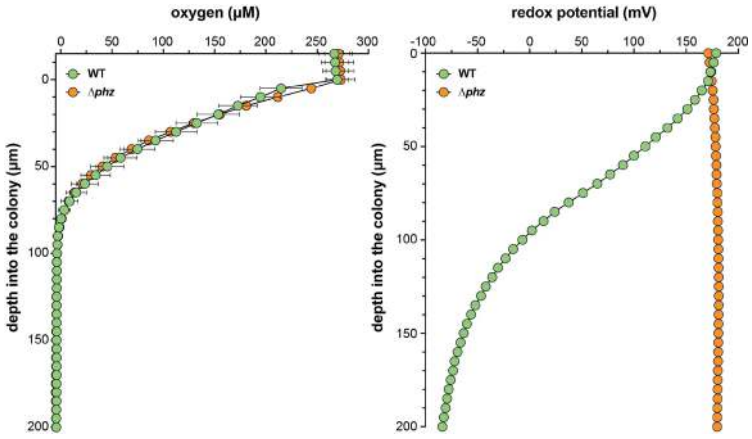
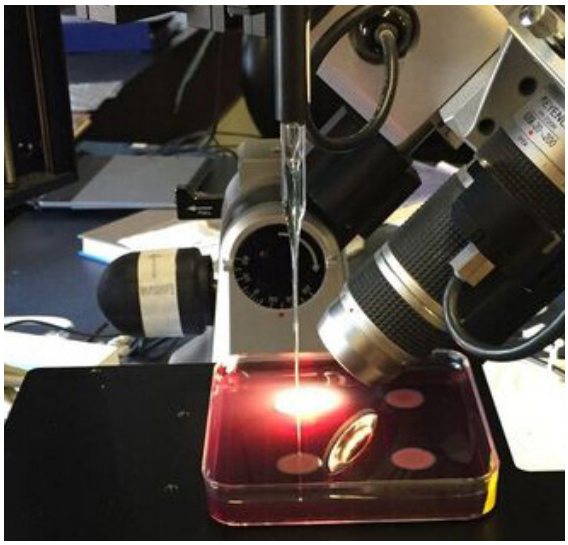


Figure 2: Oxygen microprofiling data (left) and redox potential microprofiling data (right) in the wild type (green dots) and the phenazine-null mutant (red dots) *P. aeruginosa PA14* colony biofilm. Data kindly provided by Jeanyoung Jo.

For further reading please see the article: Jo et al. (2017) An orphan cbb3-type cytochrome oxidase subunit supports *Pseudomonas aeruginosa* biofilm growth and virulence. eLife, 6: e30205



System Components	Product
Sensors	OX-25, RD-25, REF-RM
Amplifier	Microsensor Multimeter
Positioning Equipment	MicroProfiling System
Software	SensorTrace Suite (including Profiling software)

Related publications

Arnaouteli et al. (2017) Biofunctionality of a biofilm matrix protein controlled by redox state. PNAS, vol 114, no 30.

Madsen et al. (2015) Facultative Control of Matrix Production Optimizes Competitive Fitness in *Pseudomonas aeruginosa* PA14 Biofilm Models. Applied and Environmental Microibology, Vol 81, Number24.

Kempes et al. (2014) Morphological optimization for access to dual oxidants in biofilms, PNAS, vol 111, no 1.

Cowley et al. (2015) Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. mBio Volume 6, Issue 4.

Kolpen et al. (2014) Nitrous oxide production in sputum from cystic fibrosis patients with chronic pseudomonas aeruginosa lung infection. PLOS ONE Vol 9, Issue 1.

FOR MORE INFORMATION
WWW.UNISENSE.COM
SALES@UNISENSE.COM