Transforming Waste into New Antibiotics, TWIN-A Consortium

We investigate low-value waste/wastewater treatment processes as unprecedented sources of antibiotics against biofilm-forming *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as their drug-resistant strains, by using advanced anti-biofilm and microsystems technologies.

Results and highlights

1. The occurrence of drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* was determined in five wastewater treatment plants by real-time polymerase chain reaction. Although both bacteria were only detected in low quantities in processes until rotting, the total gene quantities were enormous due to the large size of the plants. The screening of 135 *S. aureus* genomes for genes of enzymes degrading poly-*N*-acetylglucosamine (PNAG) revealed the occurrence of 8 different genes in all genomes, and 9 more in part of genomes. Several branches were found in the phylogenetic trees of each gene, indicating remarkable diversity in PNAG degrading genes.

2. Bacterial biofilms are a frequent cause of failure during antibiotic treatments. This is why, we are applying orthogonal research approaches to explore waste as a source of a new type of antibiotics able to recognize and disrupt bacterial biofilms. We are developing novel biosensors with engineered surfaces where stable biofilms can be securely formed. We have successfully characterized several types of substrates in which the propensity for bacterial adhesion (*S. aureus* and *P. aeruginosa*) is very high. In the next stage, inhibition of bacterial attachment and biofilm disassembling will be tested. For that purpose, we are conducting electrochemical impedance measurements, to enable label-free detection of biofilm inhibitors.

3. With a view to isolating and identifying microbes with antimicrobial efficiency from the wastewater resources, we have developed a hybrid 3D-printable polymer and stainless-steel based isolation chip, which we will use for trapping single microbes in separated agar-filled cavities. A porous membrane mechanically attached to the agar cavities prevents the microbes from leaking to the surroundings (outside), but allows passage of nutrients needed for bacterial growth (inside). At best, this approach allows us to culture microbes *in situ* in the wastewater environment even in cases these are doomed uncultivable in the laboratory environment. Optimization of the microbe isolation conditions and detection elements (e.g., integration of polydimethylsiloxane microlenses) is currently under way.

4. We develop flexible printed biofilm sensors for screening biofilm-degrading compounds in active waste process sites. Three different types of sensors are under development: a chemiresistive sensor (CRS), an electrochemical impedance spectroscopic sensor (EISS) and an optical sensor (OS). All of these sensors are manufactured on disposable cellulose-based substrates and they enable direct (real-time) and indirect detection of the metabolic activity of the biofilms grown on the substrate. Preliminary results show that EISS can resolve the kinetics of biofilm growth, and the OS consisting of an array of chemical indicators provides

More information:

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