Transforming Waste into New Antibiotics, TWIN-A Consortium

Objectives

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. Based on the preliminary results on the occurrence of *S. aureus* and *P. aeruginosa* in composting and biogas producing plants, the bacteria were mainly detected in the early stages of composting. 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. Of note, the selected substrate also allowed to the bacteria to produce significant amounts of biofilm matrix, a crucial feature of the biosensor, which can facilitate the detection of the biomatrix degradation caused by metabolites from the waste source. Furthermore, the proteome dynamic of the bacterial biofilm onto the surfaces was also systematically analyzed as part of the functional in-depth characterization of the biosensor. The next stage of this study involves the optimization of the biosensor by exposing it to the actual waste sample.

3. With a view to isolating and identifying the microbes that potentially show antimicrobial efficiency, we have developed a microwell chip. The chip is made of polymer and stainless-steel and comprises a microwell array, which is intended for trapping single microbes in isolated agar-filled cavities (microwells). With this chip it is possible to isolate (single) microbes from the samples collected at different stages of the wastewater treatment process. However, these microbes are not necessarily cultivable under regular laboratory conditions. Therefore, in the context of this project, a previously published microbe isolation chip design has been re-designed and optimized so as to enable culturing of the trapped microbes in situ (in the sludge of the wastewater treatment plant, WWTP): A semipermeable, porous membrane is attached on top of the agar cavities in order to (i) prevent the microbes from leaking to the surroundings, while (ii) allowing passage of critical nutrients from the WWTP sludge to microbe culture. The optimizations include feasible material selection and characterizations with a view to biocompatibility and leakage-free performance and trapping efficiency, as well as development of microoptical elements to improve the detection (optical resolution) of the trapped microbes. For the time being the chip awaits for validation with real samples.

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 could be manufactured on disposable cellulose-based substrates and they enable direct real-time (CRS and EISS) and indirect (OS) detection of the metabolic activity of the biofilms grown on the substrate. The results show that the OS consisting of an array of chemical indicators provides characteristic fingerprint patterns for the metabolic products of biofilms. The results with the EISS indicate that we can monitor the biofilm growth and we can distinguish the living biofilm samples from the dead biofilm samples after exposure to antibiotic compounds. The next step is to improve the EISS system and start testing the system with more complex samples, including wastewater samples.

More information:

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