Transforming Waste into New Antibiotics, TWIN-A

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

The occurrence of drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* was surveyed in five wastewater treatment plants, four biogas plants, and five composting processes by real-time PCR detection of *nucA*, *mecA*, *ecfX* and *gyrB* genes. Genes indicative of both bacteria were detected in the wastewater treatment processes up to digestion and in other processes especially at the beginning. Although the levels detected were generally low, the total gene quantities were enormous due to the large waste volumes. The screening of 135 *S. aureus* genomes for genes of enzymes degrading poly-*N*-acetylglucosamine (PNAG) revealed the occurrence of eight different genes in all genomes, and nine more in part of genomes. Several branches were found in the phylogenetic trees of each gene, indicating remarkable diversity in PNAG degrading genes. The expression stages of these genes during the biofilm lifecycle is ongoing using mRNA sequencing.

We are also applying orthogonal research approaches to explore waste as a source of a new type of antibiotics capable of recognizing and disrupting bacterial biofilms. The development of novel biosensors consists of engineered surfaces where the propensity to form biofilms is high and the biofilm can be securely formed, thus enhancing the sensitivity of the biofilm platform features in the biosensor. We have successfully characterized several types of substrates and optimized a proper set of microbial assays to study the bacterial biofilm composition in depth (*S. aureus* and *P. aeruginosa*). Altogether, we have identified the adequate substrate that promotes both high level of bacterial adhesion and biomatrix

production, both essential factors to facilitate the detection of biofilm disassembling agents – crucial feature of the biosensor. The next stage of this study involves exposing such a substrate (incorporated in the biosensor) to the actual waste sample.

With a view to isolating and identifying the microbes that potentially show antimicrobial characteristics, we have developed a microwell chip. The chip is made of polymer and stainless steel, comprising a microwell array 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, a previously reported microbe isolation chip design has been re-designed and optimized for this project 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 to prevent the microbes from leaking to the surroundings while allowing passage of critical nutrients from the WWTP sludge to the microbe culture. The optimizations include feasible material selection and characterization with a view to biocompatibility and leakage-free performance and trapping efficiency, as well as development of micro-optical elements to improve the detection (optical resolution) of the trapped microbes. For the time being, the chip waits for validation with real samples.

Finally, we have developed flexible printed biofilm sensors for screening biofilm-degrading compounds in active waste process sites. Three different types of sensors have been under development: a chemiresistive sensor (CRS), an electrochemical impedance spectroscopic sensor (EISS) and an optical sensor (OS). All of these sensors can 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 best results were obtained with the EISS system, showing that the biofilm growth can be monitored and, most importantly, the sensor can distinguish living biofilm samples from dead biofilm samples after exposure to antibiotic compounds. The next step is to continue developing and optimizing this most promising sensor type, the EISS, to be tested with more complex samples, including real wastewater samples.

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