BF-001

EFFECT OF NANO-MICROMETRIC TOPOGRAPHIES ON EARLY STEPS OF BIOFILM FORMATION

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Biofilms are defined as communities of microorganisms that live attached to a surface. They can include a single bacterial specie or multiple species and are formed on both abiotic and biotic surfaces. This well-known phenomenon has undesirable effects for industrial or medical surfaces. Surface properties impact on the first steps of biofilm formation. Nature offers multiple solutions to biofilm formation. An important number of biological surfaces prevent microbial colonization due to their surface topographies, e.g.: the shells of mollusks and crabs and the skin of marine mammals and sharks. These facts have encouraged research of bioinspired surface designs. The main objectives of this work were to produce micro-nanometric hierarchical topographies and to analyze the influence of the topography on the bacterial adhesion. The hierarchical surface was designed using surface plasma oxidation of uniaxial stretch of polydimethylsiloxane (PDMS) films. This method has the advantage to allow designing sub-micrometric wrinkle topographic surfaces changing the plasma time exposition and the uniaxial stretch. Different topography surfaces were obtained, surface has wrinkles with different wavelength (from 500 to 3000 nm) and amplitude (from 80 to 700 nm) parameters. The bacterial adhesion on these novel hierarchical surfaces was evaluated through exposing them to a culture of *Pseudomonas protegens* Pf-5 for different times. The bacterial attachment was evaluated taking images of the wrinkled and smooth surfaces using an Atomic Force Microscopy (AFM). The initial results of this study suggests that wrinkled surface with a wavelength of 1000 nm (aprox. bacteria size) delay the bacterial adhesion and, on the other hand, wrinkled surface with a wavelength of 3000 nm enhance and encourage bacterial adhesion. These results demonstrate the importance of the topographic surface to inhibit or stimulate the biofilm development.

BF-002

METABOLIC ENGINEERING OF A DIAZOTROPHIC BACTERIUM IMPROVES AMMONIUM RELEASE AND BIOFERTILIZATION OF PLANTS AND MICROALGAE

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The biological nitrogen fixation carried out by some Bacteria and Archaea is one of the most attractive alternatives to synthetic nitrogen fertilizers. However, with the exception of the symbiotic rhizobia-legumes system, progress towards a more extensive realization of this goal has been slow. In this study we manipulated the endogenous regulation of both nitrogen fixation and assimilation in the aerobic bacterium *Azotobacter vinelandii*. Substituting an exogenously inducible promoter for the native promoter of glutamine synthetase (GS) produced conditional lethal mutant strains unable to grow diazotrophically in the absence of the inducer. This mutant phenotype could be reverted in a double mutant strain bearing a deletion in the *nifL* gene that resulted in constitutive expression of *nif* genes and increased production of ammonium. Under GS non-inducing conditions both the single and the double mutant strains consistently released very high levels of ammonium (> 20 mM) into the growth medium. The double mutant strain grew and excreted high levels of ammonium under a wider range of concentrations of the inducer than the single mutant strain. Induced mutant cells could be loaded with GS at different levels, which resulted in different patterns of extracelular ammonium accumulation afterwards. Inoculation of the engineered bacteria into a microalgal culture in the absence of sources of C and N other than N2 and CO2 from the air, resulted in a strong proliferation of microalgae that was suppressed upon addition of the inducer. Both single and double mutant strains also promoted growth of cucumber plants in the absence of added N-fertilizer, while this property was only marginal in the parental strain. This study provides a simple synthetic genetic circuit that might inspire engineering of optimized inoculants that efficiently channel N2 from the air into crops.