Synthetic biology for the design of antimicrobial peptides

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The number of multi drug resistant pathogens is constantly growing and novel antibiotic substances are desperately needed in order to at least maintain the status quo. Ribosomally synthesized antimicrobial peptides are not yet exploited for human applications despite an indisputable potential. Among those, lantibiotics, a class of posttranslationally modified and thioether-ring bearing antimicrobial peptides, show desirable properties as high but specific antimicrobial activity and excellent stability.

We employ a synthetic biology approach for the generation of novel lantibiotics employing the blueprint of natural lantibiotics. Based on their structural and functional features, natural lantibiotics are dissected into modular subunits. These subunits are then shuffled to generate thousands of novel, putative active, chimeric lantibiotics. The lantibiotic genes are subsequently produced by combinatorial DNA de novo synthesis.

These libraries will then be screened for molecules with high antimicrobial activity. To enable for high screening rates we developed a platform based on nL-sized reaction vessels (nL-reactors) that are used for peptide production and activity-screening in a single step and at rates of 10^5 variants per day. During screening, library cells are grown to microcolonies within nL-reactors along with a sensor strain serving as a model for a pathogen. Library cells secreting an active antimicrobial peptide will deactivate the sensor cells within the encircling nL-reactor. Clearance of an nL-reactor from the sensor thus indicates the presence of a strain secreting a highly active peptide. We use large particle flow cytometry and fluorescently labeled cells in order to isolate promising candidates.

We will present results from the screening of a peptide library containing 10^5 different chimeric variants derived from the module-shuffling of ten natural lantibiotics. Several interesting antimicrobials have been isolated, demonstrating for the first time the generation of new-to-nature lantibiotics by module-shuffling.

Keywords: lantibiotics; peptide-modules; module-shuffling; nL-reactor; screening; antimicrobial peptides; AMP

The value of morphological characterisation of bacterial colonies in microbial diagnosis and clinical decision-making

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During the course of infection, microorganisms go through genetic and physiological changes to survive the selective pressures imposed by the human immune system and the antibiotic treatments. Colony morphological manifestations of such antimicrobial responses are fairly immediate and inexpensive to obtain experimentally, and can be a very useful tool in clinical decision making. Several morphotypes have already been associated to chronic infections and device-associated infections. For example, P. aeruginosa mucoid variants are typically isolated from cystic fibrosis lungs at chronic stages. These colony variants are markedly resistant to common antibiotics, such as gentamicin, aminoglycosides, ciprofloxacin and imipenem. Likewise, S. aureus small colony variants, often isolated from several chronic device-associated infections, display augmented resistance to several classes of antibiotics and, able to live intracellularly, and therefore surviving the action of both antibiotics and host immune defences.

Therefore, the aim of this work is to introduce a novel computer-assisted microbial morphotyping platform in support of microbial diagnosis and further clinical decision-making. A dataset of morphotypes, extracted from the publicly available at MorphoCol database (http://morphocol.org), exemplifies how the platform assists in the manual morphological characterisation, collects data from automatic image processing tools, clusters colonies that show observable similar morphologies and describes the antibiotic susceptibility of the individual groups. Results show that key colony features, such as size, consistency and texture, can be in fact predictors of pathogenic potential of bacteria. Therefore, new colonies may be matched against the described groups, enabling the formulation of a preliminary diagnosis and therapies based on the previous reports.

Keywords: clinical decision making; data mining; colony morphology; antibiotic susceptibility

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