Microbial Biofilms in the Bioinformatics Era

Application of High-Throughput DNA Sequencing Technologies in the Metagenomic Study of Marine Biofilms

Kemal Sanli
Department of Biological and Environmental Sciences
Faculty of Science

This thesis will be defended in public on Friday, the 17th of June, 2016, at 10:00, in the lecture hall (Hörsalen) at the Department of Biological and Environmental Sciences, Carl Skottsbergs gata 22B, Gothenburg.

Faculty opponent: Prof. Daniel Huson, Dept. of Algorithms in Bioinformatics, Tübingen University, Germany

Examiner: Prof. Adrian K. Clarke, Dept. of Biological and Environmental Sciences, University of Gothenburg, Sweden

ISBN: 978-91-85529-91-9 (PRINT)
ISBN: 978-91-85529-92-6 (PDF)
http://hdl.handle.net/2077/42424

Abstract

Adverse effects of anthropogenic impact on the environment have become conspicuous in the past century and among others include the gradual increase in the global CO_2 levels, the contamination of air, soil and water by toxic chemicals, and the emergence of antimicrobial resistance among pathogenic microbial species. Microorganisms partake in an extreme diversity of activities in the environment, and hence, constitute the prime candidates to be investigated in understanding of the progression and effects of the aforementioned environmental hazard scenarios. The spectacular rise of massively parallel sequencing (next generation sequencing, NGS) technologies in mid 2000s initiated a renaissance in microbial ecology by allowing the *in situ* investigation of environmental samples at metagenome level, largely eliminating prior laboratory culturing steps. Metagenomics has thereby been established as a new interdisciplinary field and methodology, harmonizing the accumulated knowledge in microbial ecology and genetics with the high-throughput environmental DNA sequence data through the means of bioinformatics analysis resources.

One of the emerging application areas that require a comprehensive microbial investigation is the study of the effects of toxic chemicals on biota in the environment, namely ecotoxicology. In this PhD thesis, bioinformatics software development and microbial ecological data analysis projects are integrated within the field of ecotoxicology. The objective of the thesis is to implement metagenomics as a robust tool in the field of ecotoxicology to gain both community and molecular level insights. Paper I presents FANTOM (Functional and Taxonomic Analysis of Metagenomes), a graphical user interface (GUI)-based metagenomic data analysis tool that provides various statistical analysis and visualization features for biologists with limited bioinformatics experience. PACFM (Pathway Analysis with Circos for Functional Metagenomics), another GUI-based software tool, is presented in Paper II, and it provides researchers in metagenomics with a novel plot and various biochemical pathway analysis features. Paper III is an exploratory study of the marine biofilms (also known as periphython), constituting the first study to sequence the total genomic DNA content of these microbial communities that inhabit the aquatic environment. The metagenomic analysis of the marine biofilms revealed that *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* are the most abundant organisms in these biofilm communities. In addition, the functional repertoire within the metagenome involved signatures of anaerobic processes including denitrification and methanogenesis, which suggests the presence of low-oxygen zones within the micro-ecosystem formed by the marine biofilms. Paper III also constituted the pilot study for Paper IV, where an experimental design was set up to investigate the toxic effects of the broad spectrum antimicrobial agent, triclosan, on the marine biofilms. High and low levels of triclosan exposure was shown to cause significant changes in the community structure and the functioning of the marine biofilms. A sulfur-based microbial consortium together with several algal groups were hypothesized to partake in the detoxification of triclosan. Hence, metagenomics is shown to be a powerful research tool in the field of ecotoxicology.

This PhD thesis presents novel software tools and applications in the field of metagenomics, combining a wide range of paradigms from several disciplines within a unified solution framework as an attempt to practice and transcend interdisciplinary research.

Keywords: metagenomics, bioinformatics software, microbial biofilms, Next Generation Sequencing, pathway analysis, periphyton, marine biofilms, FANTOM, PACFM