SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Microbiology

has the honor to invite you to the public defense of the PhD thesis of

Md Muntasir ALI

to obtain the degree of Doctor of Bio-Engineering Sciences

Novel metal resistance mechanisms of *Cupriavidus metallidurans*

Promotors:
Prof. dr. ir. Eveline Peeters
Prof. dr. Daniël Charlier

Co-promotor:
Dr. ir. Rob Van Houdt (SCK•CEN)

The defence will take place on

Monday November 18 2019 at 17.00h

in Auditorium D.2.01 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene, and will be followed by a reception.

Members of the jury:
Prof. dr. Serge Muyldermans (chairman)
Prof. dr. Henri De Greve (secretary)
Dr. Antonella Fioravanti
Prof. Dr. Marc Elskens
Prof. dr. Isabelle George (ULB)
Prof. dr. Jan Michiels (KUL)

Curriculum vitae

Md Muntasir Ali obtained his Bachelor in Biochemistry and Molecular Biology at the University of Dhaka (Bangladesh) in 2012, and his Master of Science in Molecular Biology at KU Leuven (2015; VLIR-UOS scholarship). He pursued a PhD (SCK•CEN fellowship) on the topic of bacterial metal resistance mechanisms. He published three research papers in international peer-reviewed journals, presented at several (inter)national conferences (poster and oral) and supervised four MSc students. He obtained an early career scientist attendance grant from the Federation of European Microbiological Societies (FEMS).

Abstract of the PhD research

Cupriavidus metallidurans bacteria, belonging to the *Burkholderiaceae* family, are characterized by their resistance to multiple metals. In this work, the rapid evolution of *C. metallidurans* towards significantly increased metal ion resistance was investigated by performing adaptive laboratory evolution experiments with silver and platinum.
For silver, the canonical resistance mechanisms did surprisingly not participate in this adaptive evolution. In contrast, a novel and unique resistance mechanism, with a pivotal role for small periplasmic proteins partially controlled by a two-component regulatory system, was discovered. As the formation of silver nanoparticles was observed, we hypothesize that this formation prevents ionic silver to exert its action in the cytoplasm and provides the ability to withstand much higher silver concentrations than efflux-mediated resistance. Binding and interaction of the involved two-component regulatory system AgrRS (and CzcR2S2) with DNA target sites was further scrutinized. Interestingly, the unphosphorylated response regulators were found to be the active form and high-resolution contact maps were obtained by various enzymatic and chemical footprinting and premodification binding interference techniques. These analyses led to a general regulation scheme in which AgrS inactivation mainly results in unphosphorylated AgrR that subsequently activates target genes, including that coding for a pivotal small periplasmic protein. Furthermore, our results show that there is crosstalk between the different two-component regulatory systems and redundancy in the adaptation to silver.
An evolved strain with increased resistance to platinum was also obtained. Since no canonical resistance mechanisms to platinum are known, it was studied using high-throughput genomic and transcriptomic sequencing. The latter showed pleiotropic alterations in membrane-related processes, such as pili, peptidoglycan turnover and electron transfer.
To conclude, this work unravelled the potential of *C. metallidurans* to adapt to toxic metal ions and an unanticipated mechanism of resistance, showed the functionality of a novel protein family, and scrutinized the role of signal transduction.