

The Research Group
Microbiology

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

Amber Bernauw

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

High-throughput screening and detailed characterization of Lrp-type transcription factors for the development of beta-alanine responsive biosensors

Promotors:

Prof. dr. ir. Eveline Peeters (VUB)

Dr. ir. Indra Bervoets (VUB)

Prof. dr. Sophie de Buyl (VUB)

The defense will take place on

Thursday, September 28, 2023 at 17h in auditorium D.2.01

Members of the jury

Prof. dr. ir. Wim De Malsche (VUB, chair)

Prof. dr. Wim Vranken (VUB, secretary)

Dr. Daria Ezerina (VUB)

Prof. dr. Kim Roelants (VUB)

Prof. dr. ir. Marjan De Mey (Universiteit Gent)

Prof. dr. Julia Frunzke (Forschungszentrum Jülich)

Curriculum vitae

Amber Bernauw obtained a degree of Master of Science in Bioengineering Sciences: Cell and Gene Biotechnology at the Vrije Universiteit Brussel in 2018. After graduating, she obtained a FWO fellowship “strategic basic research” and in January 2019, she started a PhD trajectory in the Research Group of Microbiology.

Amber’s research resulted in two peer-reviewed journal articles and a book chapter. She has presented her work at several international conferences and scientific meetings. During her PhD trajectory, she supervised four master thesis students, one bachelor thesis student and guided different practical courses for bachelor and master students.

Abstract of the PhD research

For all microorganisms, adaptation of gene expression is essential to maintain a general fitness and to survive and thrive in changing environments. Different regulatory mechanisms exist to control gene expression. On the transcriptional level, regulation is performed by transcription factors. An interesting transcription factor family is the abundant and versatile Leucine-responsive Regulatory Protein (Lrp) family. These proteins typically regulate genes involved in amino acid metabolism, transport and virulence, and interact with amino acid ligands to monitor and respond to the nutritional state. However, to date the majority of Lrp-type regulators is not yet characterized, which hampers the fundamental understanding of Lrp-mediated regulation processes as well as the development of transcription factor-based applications, such as biosensor modules. Interestingly, an Lrp-type regulator has been found with specificity for the ligand β -alanine. β -alanine is a key intermediate in the production pathway of the platform molecule 3-hydroxypropionic acid. This link between an Lrp-type regulator and an important microbial production process makes the Lrp family an alluring target for sourcing of β -alanine-responsive regulators in context of biosensor design and production pathway optimization.

In this PhD research, a method was developed for the screening and initial characterization of Lrp-type regulators mined from unconventional microorganisms, with the intention of discovering novel β -alanine responsive regulators. A set of 26 transcription factors and their predicted target promoters were selected for in vivo analysis in *Escherichia coli*. A well-thought-out reporter system, consisting of two plasmids, was designed and validated to use for cloning and expression of both the transcription factors and promoters in *E. coli*. By using an automated platform, fluorescence measurements were performed with strains each containing a transcription factor-promoter pair. While inducing the transcription factor’s expression at different levels, the functionality was determined for the heterologous promoters, as well as the transcription factor’s regulatory effect. Furthermore, reporter assays were performed in the presence of amino acids, to learn more about ligand specificity and sensitivity for each regulator. For one of the β -alanine responsive transcription factors discovered in the screening, Ah-BarR from *Acidianus hospitalis*, regulatory mechanisms were further unravelled by performing in vitro experiments. In vivo results of Ah-BarR were also compared with those of other β -alanine responsive transcription factors, whether or not belonging to the Lrp family, to obtain more insights in β -alanine interaction.

To conclude, this work does not only lead to the initial characterization of numerous Lrp-type regulators, but also contributes to the general knowledge currently available on the Lrp family and to the development of β -alanine-responsive biosensors.