WETENSCHAPPEN &
BIO-INGENIEURSWETENSCHAPPEN

The Research Group of
Industrial Microbiology and Food Biotechnology (IMDO)

has the honour to invite you to the public PhD defense of

ir. David Van der Veken

to obtain the degree of Doctor of Bioengineering Sciences

Exploring the ecological functioning of an anticlostridial *Mammaliicoccus sciuri* strain in view of its application as a meat starter culture

Promotor:
Prof. Dr. ir. Frédéric Leroy

The defence will take place on

Friday, April 21, 2023 at **17h00**

in **Auditorium D.0.08** 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. Steven Ballet (VUB, Chair)
Prof. Dr. ir. Jo Van Ginderachter (VUB, Secretary)
Prof. Dr. Bruno Pot (VUB)
Prof. Dr. Joske Ruytinx (VUB)
Prof. Dr. ir. Chris Michiels (KU Leuven)
Dr. Monique Zagorec (INRAE, Nantes, France)
Prof. Dr. ir. Frédéric Leroy (VUB, Promotor)

Curriculum vitae

David Van der Veken graduated as Master in Bioengineering Sciences – Chemistry and Bioprocess Technology – at the Vrije Universiteit Brussel (VUB) in 2017. Within the same year of graduation, he started his PhD at the Research Group of Industrial Microbiology and Food Biotechnology (IMDO) under the supervision of Prof. Dr. ir. Frédéric Leroy, with financial support of the Research Council of the VUB and Flanders' FOOD (BotulinSafe project). His research dealt with the characterization of microorganisms that could replace chemical preservatives in fermented meats, with the focus on the inhibition of the food pathogen *Clostridium botulinum*. David Van der Veken is co-author of twelve scientific papers published in peer-reviewed international journals, among which four times as first author. He attended two national scientific conferences at which he gave two oral presentations. Throughout his PhD, he was also responsible for the coordination of student practicals and the supervision of three Master thesis students.

Abstract of the PhD research

Nowadays, fermented meats are mostly produced on an industrial scale and with the use of starter cultures. In most cases, nitrite- and/or nitrate-containing curing salts are still an integral part of recipe formulations, as their use incurs important technological properties such as colour formation and microbiological stability. However, the use of these chemical preservatives has come under pressure as they may potentially be linked with the formation of carcinogenic compounds and colorectal cancer. Therefore, a phasing-out urges itself within the industry, especially in view of current clean-label trends.

Therefore, the present PhD study explored the potential of Gram-positive catalase-positive cocci (GCC) to replace the antibacterial activity that is attributed to curing salt, thereby focusing on the inhibition of the food pathogen *Clostridium botulinum*. To facilitate the selection of a bacterial strain with anticlostridial activity, a large collection of GCC strains was screened. As a result, a GCC strain (*Mammaliicoccus sciuri* IMDO-S72) was selected based on its ability to inhibit *C. botulinum*. After sequencing and *de novo* assembly of the genome of *M. sciuri* IMDO-S72, genome mining revealed a plasmid-associated biosynthetic gene cluster (BGC) which was responsible for the antibacterial phenotype. The product of this BGC was structurally identified as micrococcin P1. Using comparative (patho)genomics, it could be concluded that *M. sciuri* IMDO-S72 has a low cytolytic potential and is free of virulence and antibiotic resistance-associated mobile genetic elements, except for a plasmid-encoded *ica* cluster, supporting its safety as a potential candidate starter.

To further understand the ecological functioning of this strain/species, an RNA-seq workflow was successfully set up and implemented. Genes involved in nitrate-based respiration, were marked by a strong upregulation under anaerobic conditions being under the regulatory control of the oxygen-sensing two-component system NreBC. In the presence of oxygen, acetic acid and gluconic acid were the major metabolites produced by *M. sciuri* IMDO-S72 whereas a rapid switch to lactic acid production was found in response to decreasing oxygen levels. Amino acids represented important alternative carbon sources, the degradation products of which most likely fuelled the tricarboxylic acid cycle under aerobic conditions.

Finally, application of *M. sciuri* IMDO-S72 as starter culture in nitrate/nitrite-free fermented sausages revealed a limited proliferation potential within this food matrix, especially under more pronounced acidification conditions. Using challenge tests with atoxigenic proteolytic *C. botulinum* strains, no additional anticlostridial effect by *M. sciuri* IMDO-S72 was found. These results further indicate that the anticlostridial role that is typically attributed to nitrate/nitrite in fermented meats may be overstated. More research is however needed to substantiate these results, given the seriousness of botulism, and to exclude the *in-situ* production of toxins by this pathogen.