

The Research Groups of
Industrial Microbiology and Food Biotechnology (IMDO-VUB)
and **Applied Microbiology and Biotechnology (UA)**

have the honor to invite you to the public defense of the Joint PhD of

ir. Sander WUYTS

to obtain the degrees of Doctor of Bioengineering Sciences (VUB) and
Doctor of Bioscience Engineering (UA)

**A genome-based view on lactobacilli and their role in the
carrot juice fermentation process**

Promotors

Prof. Dr. ir. Luc De Vuyst (VUB)

Prof. Dr. ir. Sarah Lebeer (UA)

Co-promotors

Prof. Dr. Stefan Weckx (VUB)

Prof. Dr. ir. Bart Lievens (KU Leuven)

The defense will take place on

Monday, February 4, 2019, at 17:00 h
in the promotion room (Q0.02) of the Campus
Drie Eiken of the University of Antwerp,
Universiteitsplein 1, 2610 Antwerp/Wilrijk,
and will be followed by a reception.

Members of the jury

Prof. Dr. ir. Roeland Samson (UA, chairman)

Prof. Dr. Jürgen Wendland (VUB, secretary)

Prof. Dr. Kris Laukens (UA)

Prof. Dr. Peter Vandamme (UGent)

**Prof. Dr. Giovanna Felis (University of Verona,
Italy)**

Curriculum vitae

Sander Wuyts (°08/05/1991, Reet) graduated from the Sint-Ritacollege in Kontich in 2009. He obtained his BSc. and MSc. in Bioscience Engineering (Cell and Gene Technology) from the University of Antwerp (UA, 2012) and the University of Leuven (KU Leuven, 2014), respectively. He started his PhD in the Laboratory for Applied Microbiology and Biotechnology of the UA under supervision of Prof. Dr. ir. S. Lebeer. As part of a Joint PhD, he also carried out experimental work at IMDO-VUB under the supervision of Prof. Dr. ir. L. De Vuyst and Prof. Dr. S. Weckx. Also Prof. Dr. ir. Bart Lievens (KU Leuven) supervised his PhD. He got a fellowship from the IWT-Vlaanderen. He spent one month in the Laboratory for Computational Metagenomics of the University of Trento (Italy). His research dealt with the microbial ecology of the carrot juice fermentation process, with a focus on bioinformatics. He is first author of 2 and co-author of 8 peer-reviewed papers in international journals. He participated at 6 international conferences and gave one oral presentation.

Abstract of the PhD research

Spontaneous vegetable fermentations, with their rich flavors and postulated health benefits, are regaining popularity. Their usage by professional chefs is steadily growing worldwide, whereas interest has also increased at household level. In addition, fermented vegetable juices, such as fermented carrot juice, show a great potential as an alternative for the more commonly used dairy-based probiotic carriers. However, in general, their microbiology is still poorly understood, and hence raising concerns about food safety.

In this PhD thesis, the spontaneous fermentation process of carrot juice was studied. To this end, samples from 38 fermentations were collected through a citizen science initiative, termed *Ferme Pokes*, in addition to three well-controlled laboratory fermentations. An initial high relative abundance of unwanted *Enterobacteriaceae* was found, which shifted to a high relative abundance of *Leuconostoc* and *Lactobacillus* afterwards. Furthermore, lactic acid and mannitol were produced in high concentrations, as well as the biogenic amine cadaverine. These results showed that to avoid high numbers of *Enterobacteriaceae* and high biogenic amine concentrations, this fermented food product could benefit from the development of starter cultures. Therefore, the genomes of 54 lactic acid bacterial strains, isolated from these fermentations, were sequenced for further analysis. Phylogenetic analysis and comparison with the high number of publicly available *Lactobacillus* (*Lb.*) genome sequences showed that these strains belonged to eight different species. For two of these species, putative *Lb. plajomi* and *Lb. mudanjiangensis*, this PhD thesis was the first to describe their genome sequences. Several of the fermented carrot juice strains showed the genetic potential to produce either homo- or heteropolysaccharides. In addition, this study showed that, due to the presence of genes related to biogenic amine production, the usage of *Lb. brevis* strains as starter culture should be avoided. In contrast, the strains classified as *Lb. plantarum*, *Lb. plajomi*, and *Lb. mudanjiangensis* appeared to be good candidates in the light of starter culture development.

Since its first description, no other study provided additional characterization or reported the isolation of other strains of the *Lb. mudanjiangensis* species. However, four strains isolated from three different spontaneous carrot juice fermentations were members of this species. This species harbored one of the largest genomes and highest gene counts of the *Lb. plantarum* group. The species showed a broad repertoire of glycosyl hydrolases and a predicted capability to degrade cellulose, a fiber present in carrots. Furthermore, three of the four strains studied showed the presence of pili on scanning electron microscopy images, which were linked to conjugative gene regions.

Finally, the computational comparative genomics pipeline developed for the analysis of *Lb. mudanjiangensis* was applied to the economically important *Lb. casei* group. Classification of strains belonging to this phylogenetic group has previously been troublesome. The phylogenetic analysis showed that many *Lb. casei* strains were wrongly annotated in the National Center for Biotechnology Information Assembly database and should be reclassified as *Lb. paracasei*. Reclassification led to the discovery of at least one catalase gene in all of the members of *Lb. casei*, making it the first described catalase-positive species of the *Lactobacillus* genus. Finally, the use of average nucleotide identity values was proposed as a computational cheap way to distinguish the species *Lb. casei* and *Lb. paracasei*.