



WETENSCHAPPEN &
BIO-INGENIEURSWETENSCHAPPEN



The Research Groups of
**Industrial Microbiology and Food Biotechnology (IMDO-VUB)
and Microbiology (LM-UGent)**

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

ir. David LAUREYS

to obtain the degrees of Doctor of Bioengineering Sciences (VUB) and
Doctor of Science: Biochemistry and Biotechnology (UGent)

**Microbial species diversity, community dynamics, substrate
consumption, and metabolite production during water kefir
fermentation**

Promotors:

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

Prof. Dr. Peter Vandamme (UGent)

The defense will take place on

Wednesday, January 25, 2017, at 17 h

in Auditorium D2.01 of 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. ir. G. DESMET (VUB, chairman)

Prof. Dr. A. CARLIER (UGent, secretary)

Prof. Dr. ir. L. DE VUYST (VUB, promotor)

Prof. Dr. P. VANDAMME (UGent, promotor)

Prof. Dr. T. COENYE (UGent)

Prof. Dr. J. WENDLAND (VUB)

Prof. Dr. ir. S. LEBEER (UA)

Prof. Dr. M. EHRMANN (TUM, München, Germany)

Curriculum vitae

David Laureys (°Dec 28, 1984, Lokeren, Belgium) graduated from the Sint-Lodewijkscollege Lokeren in 2003. He obtained his MSc. in Bioscience Engineering (Food Science and Nutrition) from Ghent University (Belgium) in 2011. In October 2011, he started his PhD at IMDO-VUB under the supervision of Prof. Dr. ir. L. De Vuyst. As part of a Joint PhD, he also carried out experimental work at LM-UGent under the supervision of Prof. Dr. P. Vandamme. He got a fellowship from the VUB. His research focused on water kefir fermentation. He is first author of three peer-reviewed papers published in international journals and co-author of a peer-reviewed book chapter. He obtained the best poster award at a national conference and was selected for the best oral paper presentation at an international conference.

Abstract of the PhD research

Water kefir is a relatively unknown traditionally fermented beverage made by inoculation of a mixture of water, sugar, and dried figs with water kefir grains. After anaerobic fermentation at room temperature for two to four days, the liquor is separated from the grains by sieving. The grains can be reused to start a next fermentation process through backslopping. The liquor is an acidic, sparkling, slightly sweet, and lightly alcoholic beverage with a fruity taste and aroma. At the start of the present study, the water kefir fermentation process was only poorly understood. Therefore, changes of the water kefir grain wet and dry mass, water kefir grain growth, pH evolution, microbial species diversity over time and/or community dynamics, substrate consumption profile, and metabolite production course during laboratory and industrial water kefir fermentation processes, performed under various process conditions, were examined. In some cases, the experimental data were described by mathematical models. Additionally, the water kefir grains were assessed visually, their microbial colonization was investigated by scanning electron microscopy, and their density and chemical composition were determined. Lactic acid bacteria (LAB) and yeasts were the main microorganisms during water kefir fermentation. As soon as a grain inoculum was added to the liquor, their viable counts in the liquors and on the grains plateaued at certain levels. There were always more LAB cells than yeast cells, both in the liquors and on the grains, but the yeasts (producers of ethanol and glycerol) were metabolically more active than the LAB (producers of lactic acid, acetic acid, and mannitol). The fermentation rate was mainly influenced by the incubation temperature and the viable counts on the grain inoculum. Also, the amount of grain inoculum added to the fermentation had an impact on this rate, as the majority of the viable counts and metabolic activity were always associated with the grains. Nevertheless, substantial metabolic activity occurred also in the liquors. Hence, a water kefir fermentation process could be started with a liquor inoculum too, although it proceeded only at half the rate of a similar process inoculated with grains. The key microorganisms were *Lactobacillus paracasei*, *Lactobacillus nagelii*, *Lactobacillus hilgardii*, and *Saccharomyces cerevisiae*, but other microorganisms could be present, depending on the inoculum and process conditions. Acetic acid bacteria (AAB) proliferated only under aerobic fermentation conditions, resulting in high acetic acid concentrations. *Bifidobacterium aquikefiri*, described as a novel species during the present study, was isolated and characterized for the first time. Its presence was determined by the inoculum. The microbial species diversity of water kefir was influenced by the nutrient concentration, type of fruit, backslopping time, rinsing of the grains during backslopping, and incubation temperature during fermentation. The water kefir grain wet mass usually increased upon fermentation. The water kefir grain growth was determined by the grain inoculum, but decreased under high acidic stress, insufficient calcium concentrations, and high nutrient concentrations. The water kefir grain growth could be controlled by partially substituting sucrose with glucose and/or fructose, whereby glucose was consumed faster than fructose. The aroma compounds that could be of impact were higher esters, but their concentrations decreased when AAB proliferated.