

The Research Group of

Industrial Microbiology and Food Biotechnology (IMDO)

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

MSc. Cristian Díaz Muñoz

to obtain the degree of Doctor of Bioengineering Sciences

Omics approaches to monitor starter culture-initiated cocoa fermentation processes

Promotors:

Prof. Dr. ir. Luc De Vuyst

Prof. Dr. Stefan Weckx

The defence will take place on

Friday April 14, 2023, at 17.00 h

in auditorium D.2.01 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. Wim VERSÉES (VUB, chairman)

Prof. Dr. ir. Geert ANGENON (VUB, secretary)

Prof. Dr. Kim ROELANTS (VUB)

Prof. Dr. Peter VANDAMME (UGent)

Dr. Zoi PAPALEXANDRATOU (ZOTO bv)

Dr. Cécile NEUVÉGLISE (INRAE, Montpellier, France)

Curriculum vitae

Cristian Díaz Muñoz was born in Vilanova i la Geltrú, Spain, in 1993. He obtained his Bachelor degree in Biochemistry at the Universidad de Córdoba (Córdoba, Spain) in 2015 and his Master degree in Health Biotechnology at the Universidad Pablo de Olavide (Sevilla, Spain) in 2017. In 2018, he started his PhD in the Research Group of Industrial Microbiology and Food Biotechnology of the Vrije Universiteit Brussel (Brussels, Belgium). His research dealt with the investigation of candidate yeast starter culture strains to assess their effect on cocoa fermentation processes through (meta) genomics, combined with high-throughput metabolomics. He is author of four first-author scientific papers published in peer-reviewed international journals, has supervised three MSc students, attended ten international scientific conferences (seven virtual and three in person), and gave three oral presentations at (inter)national conferences and symposia.

Abstract of the PhD research

The quality of chocolate is determined by a number of factors, including the cocoa variety, the post-harvest methods, and the chocolate manufacturing process. Chocolatiers have optimized and standardized the latter and they increased their control on the origin of the raw cocoa beans. However, the post-harvest methods or, in other words, the curing of the cocoa beans, is still a traditional process that follows local practices. Starter culture mixtures have been extensively applied during the last decades to investigate their capacity to steer the microbial community dynamics during fermentation and their influence on the final quality of the end-products.

Two field experiments were carried out in Costa Rica to investigate the effect of selected candidate yeast starter culture strains on the curing of cocoa beans and the characteristics of the chocolates produced thereof. The yeast strain *Saccharomyces cerevisiae* IMDO 050523, used as a positive control, corroborated previous results by accelerating the fermentation process and producing well-fermented cocoa beans in a reproducible manner. Besides that, two non-*Saccharomyces* cocoa yeast strains were tested, resulting in varying degrees of quality and different flavour profiles associated with the cured cocoa beans. In particular, the combination of *Pichia kudriavzevii* strains with *S. cerevisiae* IMDO 050523 offered a suitable balance between a fast fermentation process and the production of a wide variety of volatile organic compounds. In contrast, none of the *Hanseniaspora opuntiae* strains brought the fermentation process to a successful output.

During these field experiments, the ability of the starter culture strains examined to steer cocoa fermentation processes was first monitored by means of an amplicon-based high-throughput sequencing approach, and afterward by the application of a metagenomic approach. This way, a fine-scale monitoring of the starter cultures used was achieved by gathering evidence that differentiated the microbial communities of starter culture-initiated and spontaneous cocoa fermentation processes. Finally, the whole-genome sequence of the promising cocoa yeast starter culture strains was deciphered. The yeast strain used as positive control was compared to other domesticated and wild *S. cerevisiae* yeast strains, revealing signs of adaptation to the fermenting cocoa pulp-bean mass and a geographical distribution of the *S. cerevisiae* strains of cocoa origin. The telomere-to-telomere genome assemblies allowed the reconstruction of the genomic architecture of less known yeasts, such as *P. kudriavzevii* and, especially, *H. opuntiae*. A link between phenotypic data and the genetic content of these yeast strains was accomplished, revealing differences in their metabolic capabilities that helped explaining their performance during cocoa fermentation processes.