

Development of yeasts for environmentally low-impact ammonia production

Arianna Montanari¹, Alex Pessina², Gaia Salvatori³, Luca Brambilla², Michele Maria Bianchi¹

1. Dipartimento di Biologia e Biotechnologie “Charles Darwin”, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italia.

2. Dipartimento di Chimica, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italia.

3. Dipartimento di Biotechnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Piazza Della Scienza 2, 20126 Milano, Italia.

Ammonia (NH₃) is a key chemical compound, essential for agricultural fertilizer production and with growing potential for low-carbon energy storage. Currently, industrial production of ammonia is mainly based on the Haber-Bosch process, an extremely energy-intensive method responsible for a significant share of global CO₂ emissions. However, the ability of certain microorganisms to convert atmospheric nitrogen into ammonia under moderate environmental conditions suggests the possibility of a less energy-intensive and lower-impact production method. We recently demonstrated that the yeast *Saccharomyces cerevisiae* can release significant amounts of ammonia from nitrogenated carbon sources when placed under the right culture conditions without any genome intervention¹. Elucidating the genes involved in this process could help us make this behaviour more predictable, controllable, and generalizable. To explore this phenomenon further, we extended the study to include other related yeasts in an attempt to improve the process.

We are currently interested in gene variants of *Kluyveromyces lactis* strains that exhibit the most promising ammonia production. *K. lactis* is a biotechnological yeast with a solid background of basic biological knowledge and a variety of industrial applications. Here we describe the use of wild type *K. lactis* strains and the parental strains mutated for carbon metabolic structural or regulatory genes for the production of ammonia from media containing enzymatic or acid hydrolyzed protein compounds as carbon and energy source. Powerful producer strains have been isolated by the selection of phenotypic revertant strains from deleted mutant strains.

Furthermore, through the genetic manipulation of yeast cells, we are exploring the potential for efficient ammonia production even in the presence of non-canonical nitrogenous substrates for yeast. Developing efficient microbial processes for ammonia production is a promising frontier for the circular bioeconomy in valorisation of carbon- and nitrogen-rich waste streams as fermentation substrates while mitigating the carbon footprint of the fertilizer industry.