Utilising a genetically encoded biosensor for in-line monitoring of H2O2 generation during methanol assimilation in the yeast Komagataella phaffii

caffeine dependent way. The anti-caffeine nanobody-based heterodimeric switches were incorporated into a split CAR, and, upon caffeine administration, our caffeine-dependent CAR (CaffCAR) achieved tumor lysis and cytokine production levels comparable to that of a traditional CD19 CAR while showing a total lack of background activity in the absence of caffeine. We also show that pausing caffeine administration could limit CaffCAR T cell exhaustion in vitro. Importantly, the caffeine concentration necessary to activate CaffCARs is in the range of caffeine plasma concentrations reached after 1-2 cups of coffee.

We expect that this molecular ON-switch has high potential for application in a wide range of cellular therapies.

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BIO-005

Utilising a genetically encoded biosensor for in-line monitoring of H2O2 generation during methanol assimilation in the yeast *Komagataella phaffii*

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The interest in the methylotrophic yeast Komagataella phaffii (syn Pichia pastoris) has been increasing significantly over the years. Presenting unique features, such as high secretory capabilities and ability to utilise methanol as carbon source, K. phaffii is a very interesting non-conventional yeast to be used in the contexts of recombinant protein production and methanol-based technologies.

Generated either during respiration, oxidative protein folding in the endoplasmic reticulum or during the initial steps of methanol assimilation via the alcohol oxidase enzymes, H_2O_2 can be significantly toxic to the cells, and at high levels cause not only the disruption of different cellular activities, but also damage membranes and organelles. Therefore, a better understanding and monitoring of this non-radical reactive oxygen species (ROS) is essential. Dye-based detection of H_2O_2 is often ambiguous and does not allow for real-time measurements.

Here, we present the first application of the ratiometric, pH-independent genetically encoded fluorescent H_2O_2-responsive biosensor, named HyPer7 (Pak et al., 2020), in K. phaffii. By cultivating cells in microbioreactors, we were able to have in-line monitoring of the biosensor oxidation and reduction. HyPer7 responsiveness was first tested by adding exogenous stressors.

H_2O_2 dynamics were measured in intact cells during growth on glucose or methanol. Cultivation of cells using methanol revealed a general increase in biosensor oxidation, with significant oxidation peaks shortly after the administration of methanol. Furthermore, strains with altered methanol metabolism pathways were created and showed decreased levels of sensor oxidation, thus confirming that H_2O_2 generation was derived from methanol assimilation.

Poster presentations
Poster presentations
BMC - Biotechnology to mitigate climate change
FBI – Frontiers of biotechnology I
FBII - Frontiers of biotechnology II
FF - Feeding the future
GB - General biotechnology
H – Health
IB - Industrial biotechnology

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Biotechnology to mitigate climate change
BMC-001

Conversion of food processing residues into sustainable soil improvers by using enzymatic hydrolysis

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Recovering food processing residues from food manufacturing represents a key aspect to achieve a more sustainable future for the global population. During the last few years, there is a growing interest to create different valuable solutions to upcycle food waste biomasses by minimizing their loss. In this context, the European-founded project Waste4Soil (Project: 101112708 — Waste4Soil — HORIZON-MISS-2022-SOIL-01) focuses on the development of methodological and technological solutions to convert food processing residues into local and circular soil improvers. To achieve this aim, living labs located in different EU regions were created. Three different food processing residues from animal and plant sources were selected in the Italian living lab: poultry carcasses, residues from biodigester and okara. They were characterized for their proximate composition and in particular for their nitrogen and phosphorus content. They were then hydrolysed by using different experimental conditions (enzyme, temperature, reaction time). Protein hydrolysates obtained were characterized (e.g., nitrogen, amino acid composition) in order to select the optimized conditions for the process scaling up. The hydrolysates are being tested in field on typical Italian crops (e.g., tomatoes, wheat), as soil improvers. To evaluate the efficacy of the treatment, the results obtained with the produced innovative soil improvers will be compared to a standard treatment and an untreated control.

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BMC-002

A novel experimental method to determine substrate uptake kinetics of gas-fermenting microorganisms applied to carbon monoxide-fermenting *Clostridium autoethanogenum*

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Syngas fermentation has gained momentum over the last decades. The cost-efficient design of industrial-scale bioprocesses is highly dependent on quantitative microbial growth data. Kinetic and stoichiometric models for syngas-converting microbes exist, but accurate experimental validation of the derived parameters is