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Rational starter culture design using GEMs toward an upcycled mushroom umami sauce

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Introduction

One-third of all food produced for human consumption, ~1.3B tons annually, is wasted, despite the substantial ecological resources invested in its production. Transitioning from the linear "take–make–use–dispose" model to a circular food economy presents a promising path forward, and fermentation stands out as a powerful tool in food upcycling initiatives.

In collaboration with a Dutch canned mushroom producer, this project develops a fermentation process to upcycle rejected mushrooms (*Agaricus bisporus*) into a flavorful, safe, and umami-rich cooking and seasoning sauce, a soy-free alternative to traditional soy sauce.

To accelerate starter selection and demonstrate a broader methodology, Genome-scale Metabolic Modeling (GEM) is applied at the community level to identify microbial consortia with high predicted umami production potential.

Research design

Individual GEMs were constructed for 10 Lactic Acid Bacteria (LAB) isolated from fermented food products. All possible communities, ranging from 1 to 6 members (Fig. 1), were studied for their ability to enrich the medium with umami-related compounds using three complementary approaches (see sections 1-3 below).

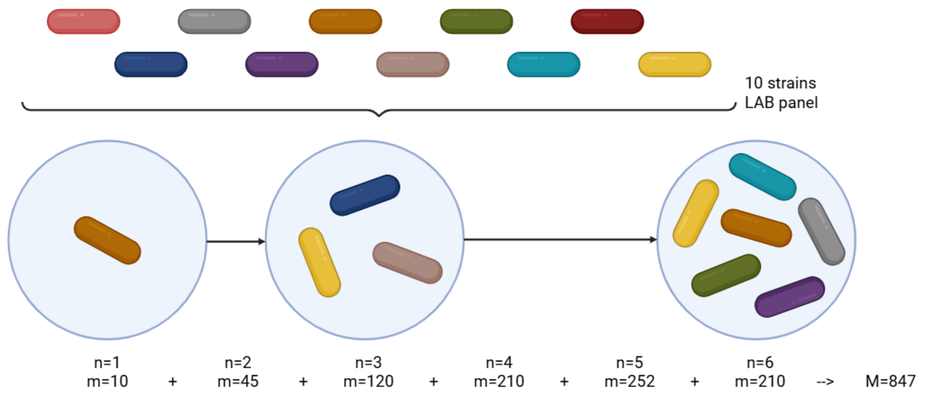

Fig 1. Illustration of all community compositions analyzed in this study. m is the total possible combinations per specific community size (n), and M is the total possible combinations in this study.

1 Direct umami production

The secretion fluxes of the umami amino acids and the 5'-nucleotides were obtained by Flux Balance Analysis (FBA) and used to predict the community Equivalent Umami (EU) flux (Fig. 2).

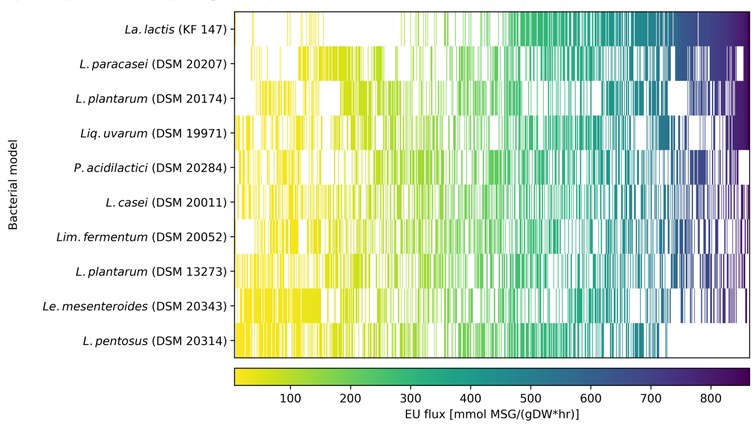

Fig 2. Community EU fluxes (color-bar) per community composition (highlighted rows per column). Rows ordered per averaged individual EU fluxes.

EU trends remain robust under relaxed growth fraction FBA (Fig. 3), indicating the absence of artificial "overflow" pathways.

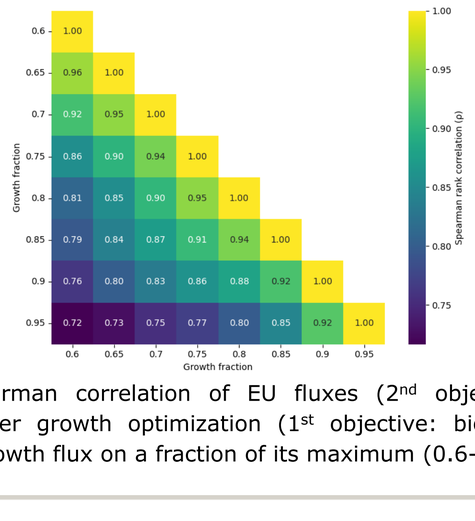

Fig 3. Spearman correlation of EU fluxes (2nd objective: EU), obtained after growth optimization (1st objective: biomass) and fixing the growth flux on a fraction of its maximum (0.6-0.95).

2 Extracellular protease secretion

In general, GEMs do not account for enzyme production or extracellular activity. Thus, we curated all bacterial GEMs to include synthesis, transport, and proteolysis reactions of the representative extracellular protease S1C (Fig. 4), and later studied its secretion flux using FBA (Fig. 5).

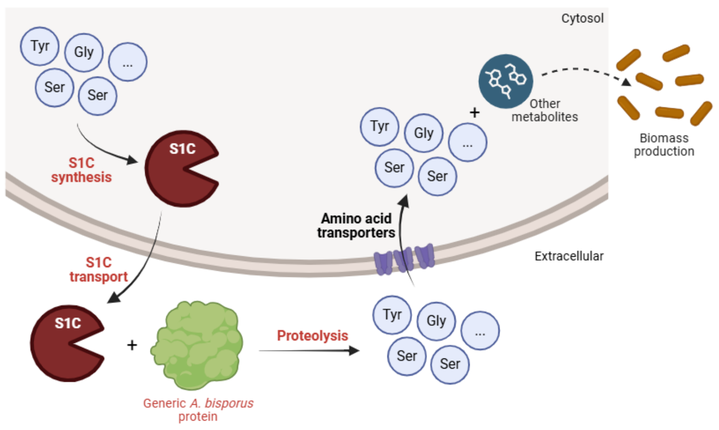

Fig 4. Illustration of the additional reactions and metabolites added to each GEM (labeled in red) to represent protease secretion.

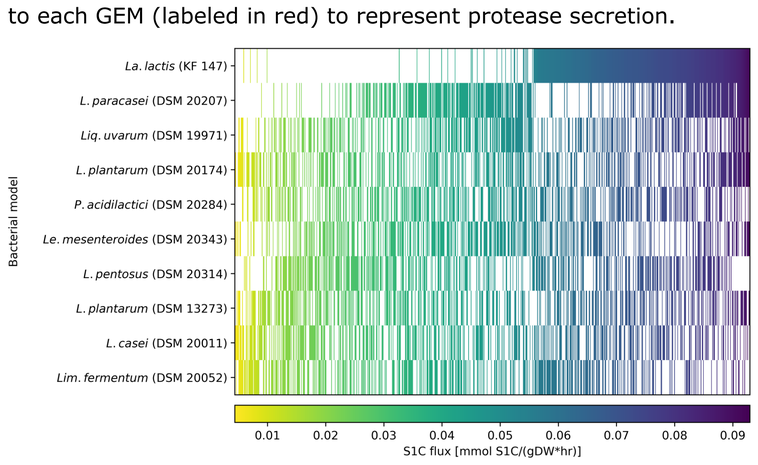

Fig 5. Community S1C fluxes (color-bar) per community composition (highlighted rows per column). Rows ordered per averaged individual S1C fluxes.

3 Metabolic & growth dependencies

The extent of resource competition (Fig. 6) and the individual growth improvement within each community (Fig. 7) were studied to identify communities with good potential for growth synergy, serving as intrinsic predictors for umami production.

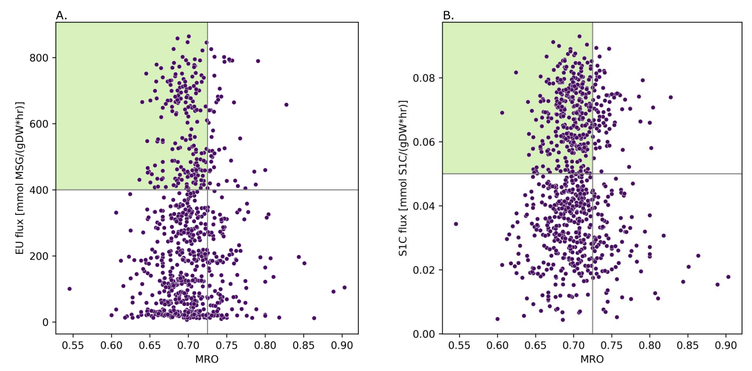

Fig 6. Metabolic Resource Overlap (MRO) scores calculated using SMETANA vs. EU (6.A) and S1C (6.B) community fluxes. The green area indicates the area of the highest umami-producing communities, with a relatively low MRO and High EU/S1C flux.

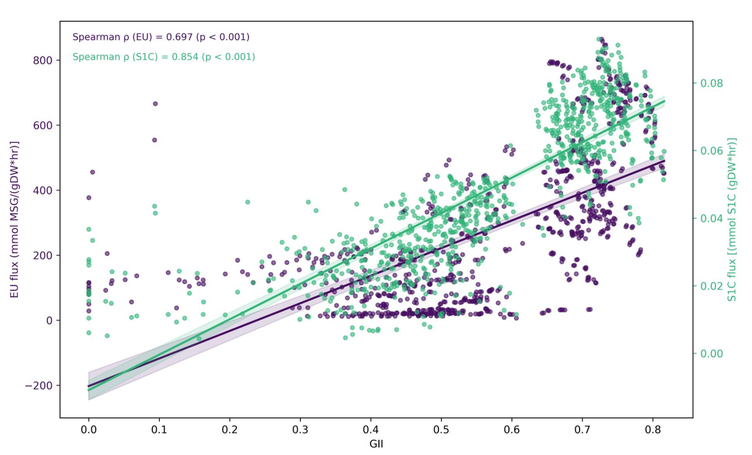

Fig 7. Community level Growth Improvement Index (GII) vs. EU and S1C fluxes. GII was calculated based on the individual growth rate in monoculture compared to the growth rate in a community.

Upcoming work

1. Analyze cross-feeding patterns and identify relationships to the umami production.
2. Cluster communities for high/low umami producers based on three prediction approaches.
3. Validate GEM-based predictions of umami fluxes in experimental mixed-cultures assay.

Conclusion

GEMs are powerful for community selection based on complex phenotypes, like umami production; yet, lab validation is necessary.

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