Global view of metabolome changes between domesticated and wild yeasts

Background

Metabolism displays immense diversity across species. Traditionally, most of this diversity is thought to stem from differences in the enzyme content and pathway structure between species. However, **many crucial aspects of metabolic diversity remain hidden.**

We studied unicellular yeasts (*Saccharomyces* spp.), which are of great economic importance in agriculture and industry and are ideal for integrative evolutionary studies. *S. cerevisiae* is the most widely studied eukaryote in functional genomics, allowing us to capitalize on previous large-scale works. As such, the genomes of several wild species and hundreds of *S. cerevisiae* strains have been sequenced, including both wild and industrial ones. During the association of *S. cerevisiae* with human activity, ethanoic fermentation has been discovered several times, leading to multiple yeast domestication events. This resulted extensive genomic and phenotypic changes. Despite the importance of yeast's metabolic properties, **the influence of human activities on the evolution of metabolism is and its genomic background unexplored.**

Aims

In line with our original research plan we aimed to answer several questions regarding the metabolome divergence and its connection to domestication:

i; Does the metabolome evolve by a constant rate or its rate of evolution could change on evolutionary timescales?

ii; If there is a change in tempo of metabolome evolution in yeast, what is the genomic driving force behind it?

iii; How did the mode of metabolome evolution and metabolite levels changes upon adaptation to man-made environments in yeasts?

iv; What is the physiological importance of metabolite level changes in stress resistance?

v; What is the genomic and proteomic background of metabolome divergence?

vi; Can we generalize our findings on the tempo of metabolome evolution from unicellular yeasts to multicellular species also?

Results

To answer these questions we followed the project proposal but we have expanded the scope of our study at many points.

i; We examined strains from "1011 yeast" collection (Peter et al 2018). We probed the metabolome of 15 *S. cerevisiae* populations and 8 other yeast spanning ~90 million years of evolution with 70 isolates to get a higher resolution picture about the metabolome evolution of *Saccharomyces ssp*..

ii; We did not only utilized already available genomic data, we also took advantage of a newly available systematic protein abundance dataset covering ~1500 proteins from most strains what we have examined.

iii; We aimed to generalize our findings on the metabolome evolution of yeasts to multicellular species, such as to mammalian metabolome evolution.

Method developments and technical advancements made during the project period were as follows:

- **We developed metabolomics pipelines to make feasible a reliable interspecies comparative metabolomics study.** To overcome the potential biases in metabolite concentrations resulted by growth rate and sampling OD dependencies of metabolite levels, i; we cultivated our samples in 25°C to minimize the growth rate differences between species, ii; we used optical density data for linear regression based normalization to remove the OD-dependence of concentrations for each metabolites (Zampieri et al. 2018). For amino acid concentration determination we implemented an internal standard based absolute quantification.

- We optimized a rapid high throughput non-targeted metabolome fingerprinting method to determine ~ 100 putatively identified metabolites. This method was utilized

during the project to provide metabolome profile data about the non-amino acid metabolome.

-**I developed a comprehensive LC-MS based metabolomics pipeline,** which provides **semi-quantitative data from 110+ metabolites** with unambiguous identifications, and give global picture about the metabolome and provide pathway activity data also. (In the proposal this task was assigned to an analytical chemist but finally I was the one fulfilling the method development. This task delayed the other elements of the project). We will apply this method in a follow up project to find dysregulated pathways in domesticated lineages, and locate more precisely in the metabolic network where the most important domestication associated changes are. We will examine TCA cycle a detailed way.

We have applied this method to elucidate role of chloroplast ascorbate level in the metabolic regulation of *A. thaliana*. We have found that ~50% of relatively quantified metabolite levels are changed upon the perturbation of ascorbate metabolism in *A. thaliana*. We have uncovered the dysregulation of arginine and lysine metabolism, and hormone levels. These results suggest the metabolic regulatory role of ascorbate in plants. I had fundamental contribution in this project therefore the paper will be submitted soon to Plant physiology with my shared first authorship.

- Yeasts are forming aggregates under various stresses. We developed a stress resistance assay which is utilizing a cell repellent well plate and plate reader with well scan measurement mode with controllable scanning area size to provide representative optical density data at every data points during the cultivation. This method allowed us to compare growth rates of yeast strains with diverse stress behaviour under various stress conditions.

Results 1; - Variability of amino acid levels during the evolution of Saccharomyces ssp.

With analysing the amino acid data, we can conclude that, **there is substantial diversity in metabolite levels of yeast lineages.** Statistical analysis revealed that of the same species, *S. cerevisiae* 17 out of 19 amino acids vary significantly; therefore the observed differences are not restricted to few of the amino acids. (*Figure 1)*

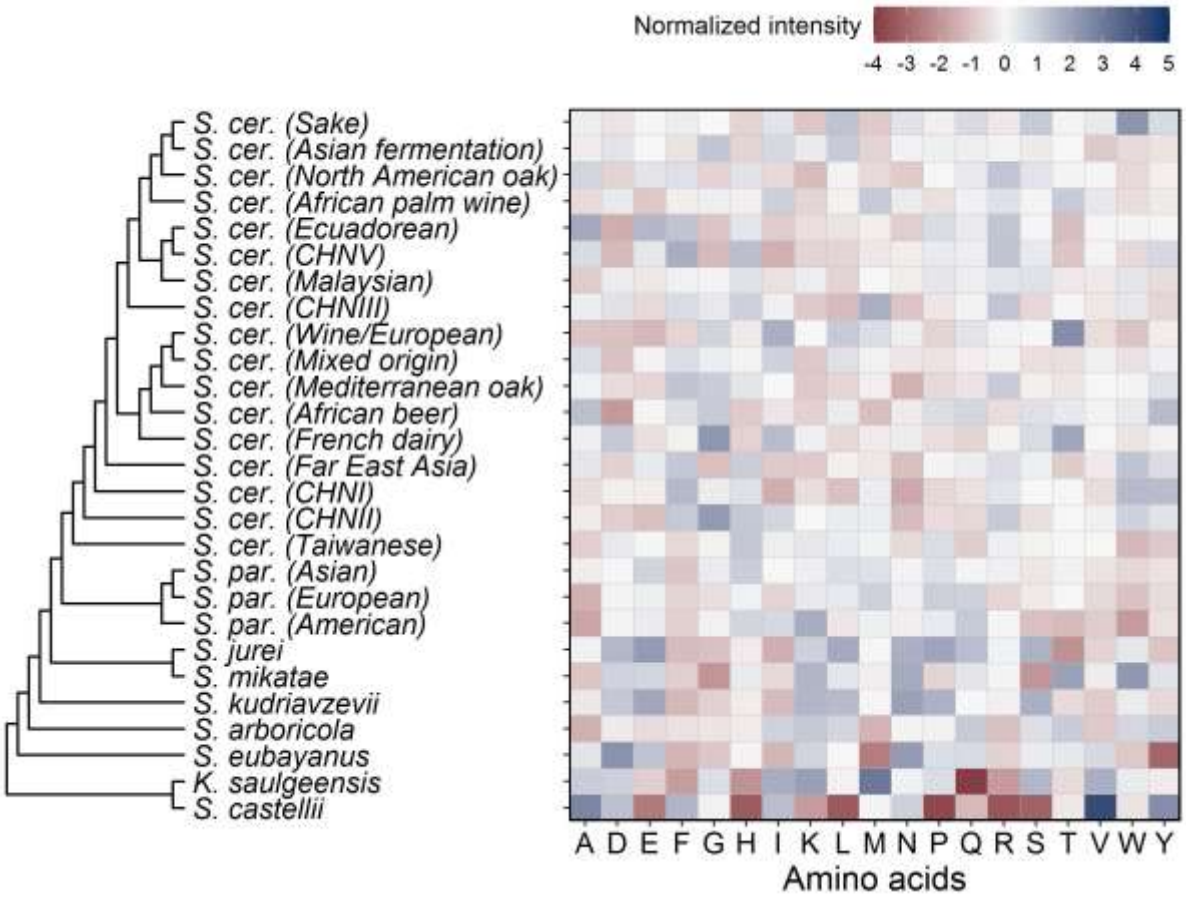

Figure 1: Heatmap showing amino acid levels of 27 yeast populations and species at 25 °C. Values are average amino acid levels across strains that belong to the same population or species. Metabolite intensities are OD calibrated and studentized for each metabolite. Cladogram shows the branching pattern of the 27 yeast clades.

We next interrogated the temporal dynamics of metabolome divergence. We found that metabolome evolution is not a constant in tempo, it could vary over time. **We identified two acceleration events in the metabolome evolution of *Saccharomyces ssp*.** in short evolutionary timescales: i; *S. cerevisiae* shows an accelerated metabolome evolution compared to evolutionary rate between *Saccharomyces ssp*. ii) The more recent acceleration of metabolome evolution is associated with the domestication of *S. cerevisiae* (Figure 2).

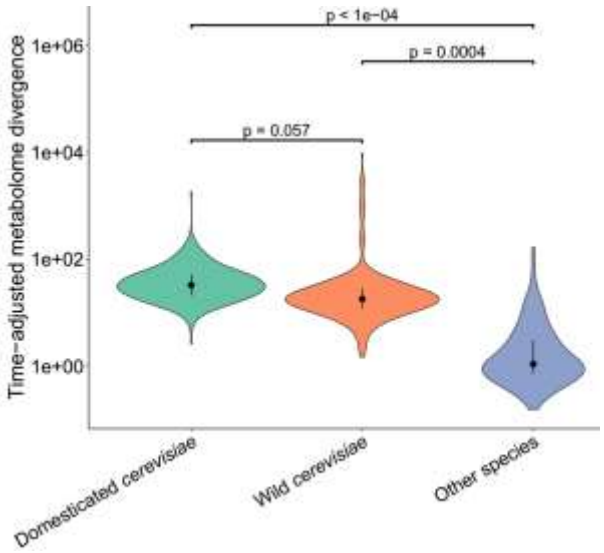

Figure 2: Metabolome divergence between lineage pairs of domesticated and wild S. cerevisiae, and pairs of lineages of non-cerevisiae yeast species. Metabolome divergence is calculated from the quantitative amino acid metabolome dataset and is adjusted for phylogenetic distance.

We found that the above conclusions can be generalized to metabolites beyond amino acids. For this we have utilized our recently developed rapid non-targeted metabolomics platform (Figure 3.).

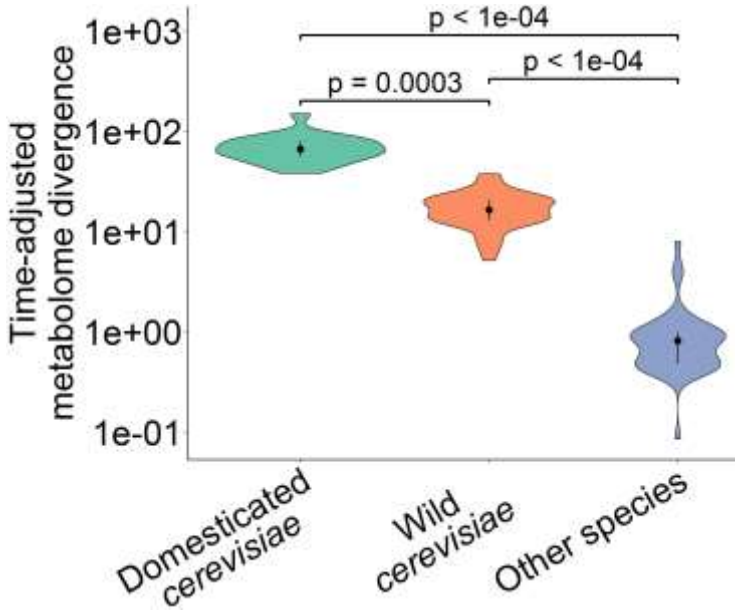

Figure 3: Metabolome divergence between lineage pairs of domesticated and wild S. cerevisiae and pairs of lineages of non-cerevisiae yeast species. Metabolome divergence is calculated from the untargeted metabolome fingerprinting dataset and is adjusted for phylogenetic distance.

Results 2; - Genome dynamics background of accelerated metabolome evolution

- First, we hypothesized that the acceleration of metabolome evolution in wild *S. cerevisiae* is the result of accumulation of deleterious mutations in short phylogenetic distances. Indeed, we observed a larger time adjusted metabolome divergence in case of shorter phylogenetic time (Figure 4.). Therefore, we can conclude that **in short phylogenetic distances metabolome divergence is accelerating, independently from the examined yeast's its lifestyle.**

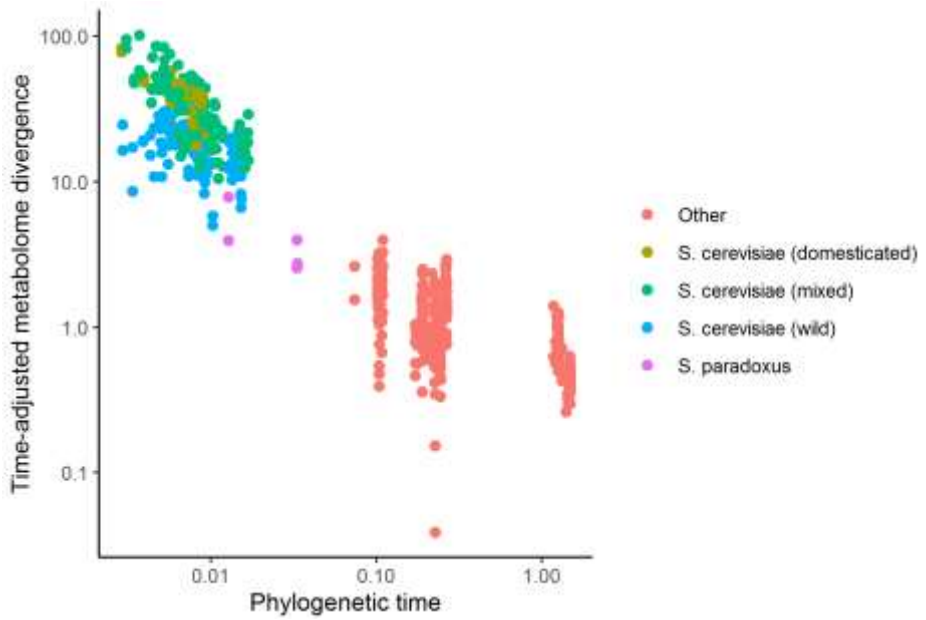

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Figure 4: Time adjusted metabolome divergence plotted against the phylogenetic time between lineage pairs of Saccharomyces ssp.. Metabolome divergence is calculated from the OD adjusted amino acid metabolome dataset and it is adjusted for phylogenetic distance.

We have tested the possible importance of larger genomic changings on the acceleration of metabolome evolution in domesticated *S. cerevisiae* lineages. We have found a remarkable difference in gene count divergence (number of CNV-s and presence absence differences normalized to evolutionary time) between wild and domesticated *S. cerevisiae* population pairs (Figure 5).

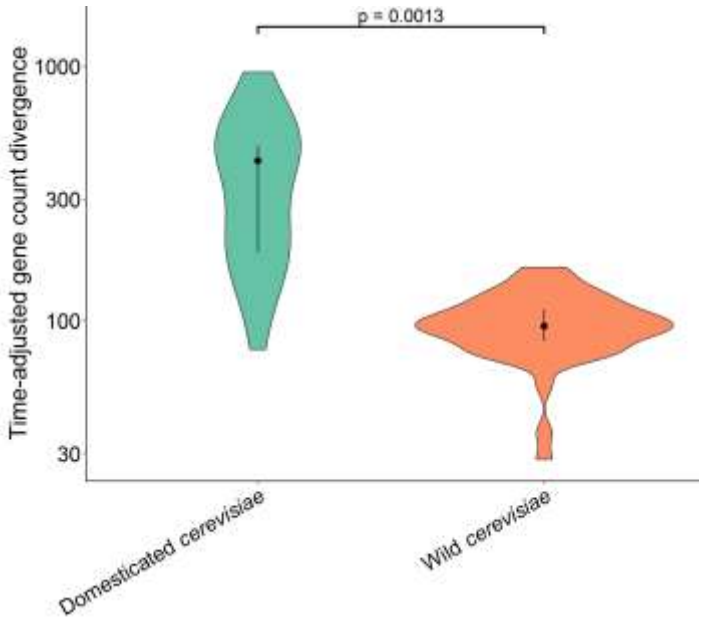

Figure 5: Time adjusted gene count divergence (Jacquard distance calculated from number of CNV-s and presence absence differences normalized to evolutionary time) *between pairs of domesticated and wild S. cerevisiae lineages.*

This suggests the **primary importance of larger genomic changes in domestication associated increasing of metabolome's evolutionary rate.**

Among the above mentioned genomic changes CNV-s seems to be more important factor of metabolome evolution acceleration. This finding is in line with previous studies on genomic background of phenotypic diversity in *S. cerevisiae*.

Results 3; Mode of metabolome evolution is S. cerevisiae

To test whether change in the rate of metabolome evolution is coupled with change in the mode of evolution, metabolome divergence and phylogenetic distances were correlated in our amino acid dataset In wild *S. cerevisiae* lineages show strong correlation between metabolome divergence and phylogenetic distance (r = 0.76, p = 0.003, phylogenetic Mantel test; Figure 6/A). However, domesticated *S. cerevisiae* lineages do not show this correlation ((r = 0.1, p = 0.15, phylogenetic Mantel test; Fig. 6/B)). These results suggest that **metabolome divergence is strongly affected by genetic drift only in wild *S. cerevisiae***, and **in domesticated ones it is mostly dominated by adaptive evolution.**

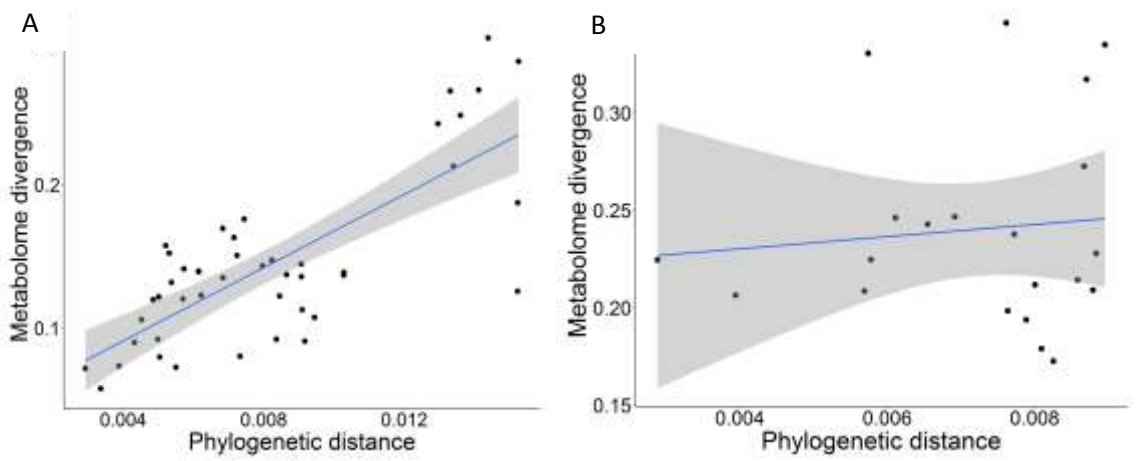

Figure 6: Association between metabolome divergence and phylogenetic distance across pairs of S. cerevisiae lineages. A) Correlation between metabolome divergence and phylogenetic distance in wild S. cerevisiae lineages. B) Correlation between metabolome divergence and phylogenetic distance in domesticated S. cerevisiae lineages. The blue line and the grey area show the linear regression line and its confidence interval, respectively.

To **reinforce the importance of adaptive evolution** in shaping the metabolome of domesticated *S. cerevisiae* we have applied phylogenetic comparative methods (phylogenetic ANOVA) to find metabolite levels associated with domestication. **We found 5 domestication associated metabolite level changes** in the amino acid dataset, namely; isoleucine, leucine, histidine, arginine, asparagine. (Figure 7)

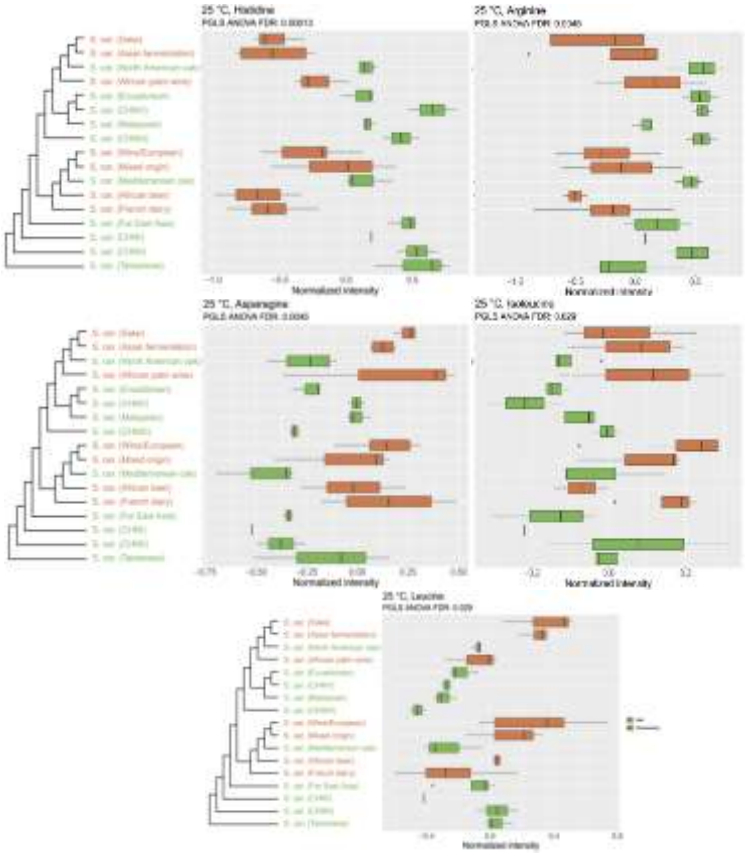

Figure 7: *Domesticated lifestyle-associated metabolite level changes plotted from OD adjusted and scaled metabolite concentrations. Significances were calculated with phylogenetic ANOVA to consider the phylogenetic relationship between populations*

With analysing the non-targeted metabolomics data, we found 19 putatively annotated metabolic traits (corresponding to 30 metabolites), which levels are associated with domestication at population level. These metabolites belong to various pathways including TCA cycle and amino acid metabolism. This implies altered TCA cycle in domesticated lineages. TCA cycle activity is linked to respiration as it fuels the electrons to it. Therefore –the previously described - domestication associated altered respiration seems to be important factor of shaping metabolite levels.

In the follow up of this project, we will more precisely asses the domestication associated metabolite level changes of TCA cycle and connected pathways. We will apply our recently developed LC-MS based metabolomics pipeline and this will allow us to uncover how central carbon metabolism is changed upon domestication.

Results 4; Physiological importance of domestication associated metabolite levels

To find the role of (domestication associated) metabolite levels, systematic exploration of links between metabolite levels and stress resistance was performed. With utilizing our recently developed stress resistance assay we found 12 significant positive strong correlations in the presence of 6 industrially or environmentally relevant stressors among 11 stressors tested. The strongest connections were associated with domestication, namely i; Asparagine - tartaric acid (wine must component), ii; NaCl and LiCl (salt and osmo-stress) - leucine and isoleucine, iii; ethanol - arginine.

To find casual stress resistance - metabolite level connections, we tested the strongest correlations but only the previously known arginine level - ethanol tolerance was verified. Generally we can tell that the **metabolite level stress resistance correlations are widespread, but causal connections seem to be rare.** Therefore it is unlikely that the adaptation to man-made environment-specific stressors directly drive the metabolite level evolution thought causal metabolite level – stress resistance connections.

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Results 5; Multiomic background of metabolome evolution in domesticated S. cerevisiae lineages

- Finally we have aimed to uncover the genomic and proteomic basis of metabolome evolution domesticated lineages. Changes in gene presence/absence and copy numbers have been rampant during yeast evolution (Yue et al. 2017). However the effect of this phenomenon to molecular phenotypes and the importance in domestication haven't been examined yet. To find the genomic and proteomic basis of metabolome evolution we utilized two datasets; i) gene count and gene presence absence differences for *S. cerevisiae* strains (Peter et. al. 2018), and a protein abundance dataset covering ~1500 proteins (unpublished data from Markus Ralser and his colleagues). These datasets were used to correlate metabolome distance with gene count distance and proteome distance specifically for domesticated *S. cerevisiae* lineages.

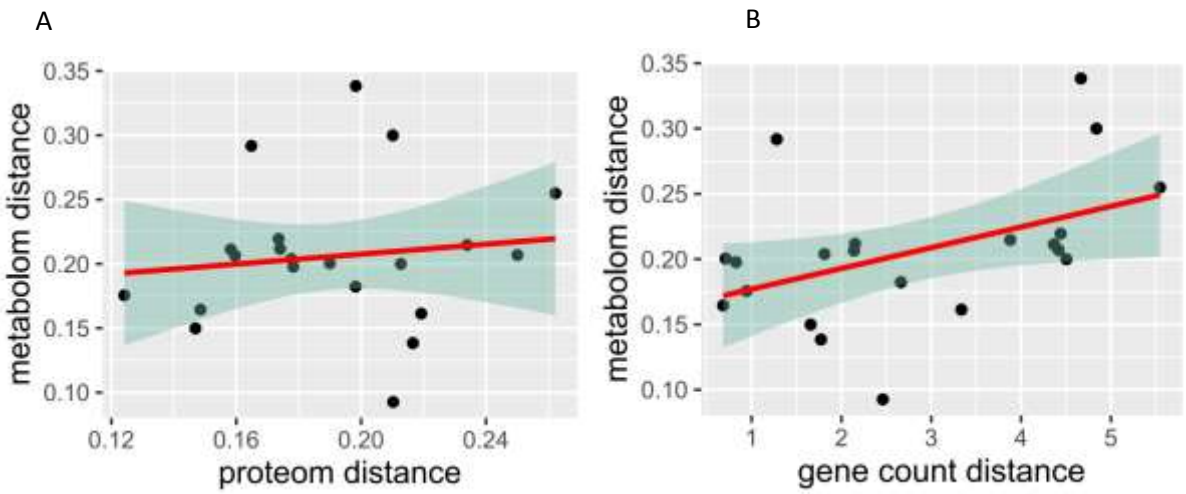

Figure. 8: Association between metabolome distances and A) proteome distance across pairs of domesticated S. cerevisiae lineages. (Spearman's rho = 0.11, p = 0.62) B) Gene count distance across pairs of domesticated S. cerevisiae lineages (Spearman's rho = 0.54, p = 0.011). Red line and the green area show the linear regression line and its confidence interval, respectively.

We found no association between proteome distance and metabolome distance (Figure 8/A) therefore protein level changes in general don't explain metabolome evolution in domesticated *S. cerevisiae*. However we found a remarkable correlation between gene count distance (copy number variation + gene presence absence) and metabolome distance (Figure 8/B). This supports that previously presented finding (Figure 5) that **the evolution of metabolome in domesticated S*. cerevisiae* strains was mainly shaped by gene losses and CNV-s.**

Although, these findings don't exclude that possibility of specific protein level changes being important in shaping the levels of domestication associated metabolites. To find phylogenetic correlation (coevolution) between amino acid levels and protein levels, we performed phylogenetic least square analysis in all the examined *S. cerevisiae* lineages. 113 significant (pgls $p < 0.05$ after false discovery rate correction) associations were found. Among them we found 61 protein metabolite pairs which both the metabolite and the protein showed association with domestication. This highlights that, in domesticated lineages, one fraction of proteome seems to evolve in parallel with metabolome.

Among these ELP2 (Subunit of elongator complex) and AIM29 (Putative protein of unknown function) protein levels negatively correlate with arginine level (Figure. 8), and we have found an analogous connection in the amino acid level dataset of yeast single gene KO collection. In two single gene KO strains, – namely Δ*elp2* and Δ*aim29* - showing increased intracellular arginine level. This partly validates our finding with on the phylogenetic connection between ELP2 and AIM29 protein levels and arginine level.

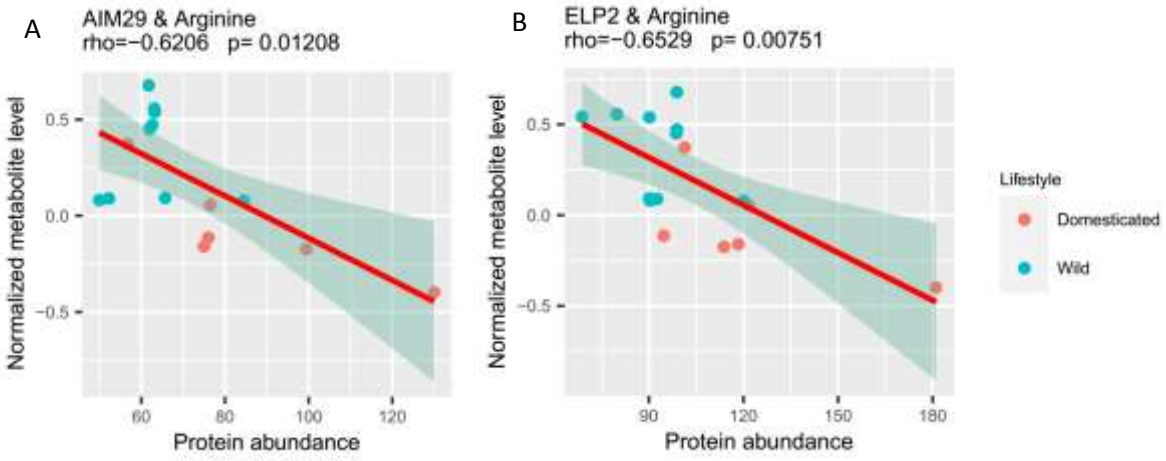

Figure 9: Associations between Normalized Metabolite (Arginine) level and A) protein abundance of AIM29 in S. cerevisiae lineages. B) Protein abundance of ELP2 in S. cerevisiae lineages. The red line and the green area show the linear regression line and its confidence interval, respectively. Dots were coloured according to the population lifestyles.

We next investigated the genomic basis of metabolome divergence by focusing on gene content changes. To identify specific gene content variants underlying metabolome divergence;

i; We performed a genome-wide association study. We have found putatively causative connection between gene copy number variation of S-adenosyl methyltransferase (SAM3-4) genes and leucine. Indeed, the copy number of *sam3-4* is higher in domesticated strains.

ii; We compared the copy number of each gene between wild and domesticated strains. The copy number variation of lithium transporter (*ena1;2;5*) seems to be especially interesting because domesticated strains show higher lithium resistance and lithium resistance is strongly correlated (rho ~ 0.7) with intracellular leucine level. This is suggests that the

increased copy number of *ena* genes might increase intracellular leucine level as a side effect.

We aim to validate these putative causative connections from genomic and proteomic analysis experimentally. For this, we increased the copy number of *sam*3-4, *ena*1, *epl*2, *aim*29 genes in a wild strains utilizing a low copy number plasmid vector. We will compare the intracellular leucine and arginine levels of strains with normal and increased gene copy numbers, with utilizing the recently developed LC-MS based metabolomics method.

Results 6; Outlook from Saccharomyces ssp. - Metabolome evolution variability in mammals

As it was shown in case of *Saccharomyces ssp*. we have found a non-uniform evolutionary rate of metabolite levels. However it was unknown whether the observed variation is something unique or variability is a general property of metabolome evolution rate. To answer this question we focused on mammals according to the availability of a comprehensive multi-species metabolomics dataset, which offers relative concentrations of approx. 150 metabolites in 26 species (Ma et al., 2015). Evolutionary rates calculated on two independent clades of the tree — rodents, rabbit and primates in one clade and the rest of species, in the other clade – show a strong correlation. Thus, metabolites evolving slowly in a particular clade also evolve slowly in the rest of mammals, therefore metabolite concentrations evolve at roughly constant rates throughout the mammalian phylogeny (Figure 9).

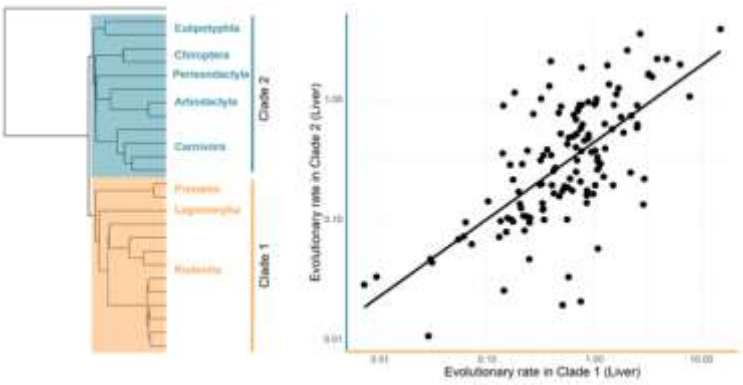

Figure 9: Evolutionary rates calculated on two independent clades of the mammalian tree are well correlated in liver (Pearson's r = 0.67, p = 1.1e-18). Line depicts the fitted linear regression. Phylogenetic tree on the left shows the two independent clades of mammals for which evolutionary rates were inferred, with the names of the constituent taxonomical orders.

Conclusion

During the project period we have accomplished all the scientific goals presented in the project proposal. Moreover we have developed two metabolomics methods and fundamentally improved one to answer our questions. These method developments and the applied phylogenetic framework, were allow us to get a higher resolution picture about the metabolome evolution of *Saccharomyces ssp*. These results have wider implications than the description of metabolome evolution is *Saccharomyces ssp*. Here I will summarize these findings and their importance:

Metabolites are building blocks, signals, fuels and energy transfer molecules of living systems. Despite their importance understanding their changes during evolution lagged behind genome and transcriptome evolution studies. Our research highlighted a previously hidden diversity and evolutionary dynamics of metabolite levels in *Saccharomyces ssp.* In shorter phylogenetic timescales the strength of purifying selection not sufficient to purge out weakly deleterious genetic variants, which could increase the genetic diversity of recently diverged lineages. Metabolite levels are thought to be under strict bioenergetics control. However metabolite level's rapid evolution and rapid change in their evolutionary rate suggest the virtually negligible importance of bioenergetics control in short term

evolution. Moreover our results are suggesting that microbial metabolome might be evolving under different constraints than for example mammalian one.

Mode of metabolome evolution not changed upon species formation of *S. cerevisiae* and seems to be nearly neutral. In contrast to wild *S. cerevisiae,* the missing connection between phylogenetic distance and metabolome divergence in domesticated *S. cerevisiae* suggests the possible importance of adaptive evolution in shaping metabolite levels. This finding is supported by the 5 amino acid levels and 19 other metabolic traits which are domestication associated. Among the putatively identified domestication associated metabolites 4 are involved in the TCA cycle, which imply altered TCA cycle in domesticated lineages. TCA cycle activity is linked to respiration as it fuels the electrons to it. Therefore – the previously described - domestication associated altered respiration seems to be important factor of shaping metabolite levels.

Moreover domestication not only triggered a change in the mode, but increased further the tempo of metabolome evolution also. This could be connected with the changed lifestyle of *S. cerevisiae*. Natural isolates often face with starvation or exhaustion of nutrients. To cope with these challenges they are forming spores and going through meiotic recombination, which is a strong selection force against for example chromosomal rearrangements. However domesticated strains are only seasonally or almost never meet with nutrient limitation therefore they have altered or missing sexual cycle. This decreasing the ability of domesticated S. cerevisiae to going through meiotic recombination, which therefore could affect the subsistence of weakly deleterious mutations beside the niche specific adaptive ones. These weakly deleterious mutations and their combinations could increase the variability of metabolite levels lead to novel metabolic states.

We have analysed the association between metabolite level changes and genomic / proteomic changes. We found that gene losses and copy number changes are more important in shaping metabolite levels of domesticated yeasts. However two putative protein level - domestication associated metabolite level connections were also identified. Moreover two putative metabolite - CNV- associations were uncovered, which possibly underlies one fraction of domestication associated metabolite level changes. ENA (P-type ATP-ase sodium pump) have an importance is stress (sodium and lithium) resistance and

lithium stress resistance correlates with leucine level. As domesticated lineages are showing higher sodium and lithium stress resistance and higher leucine level also, ENA could link stress resistance evolution to domestication associated metabolome evolution is yeasts.

However our findings raise important questions on how universal the variability of metabolome evolutionary rate or this is just a unique example. Our knowledge on the genomic determinants of specific metabolite levels is still very limited and needed to be expanded to modify industrially important metabolite levels and identify the physiological importance of some metabolites.

Despite the successful scientific efforts, the above mentioned LC-MS metabolomics method developments and Covid-19 pandemic together delayed the publications. However our results will be published in 3 Q1-D1 journals soon. Two of them will be published with my first authorship and one with my co-authorship. (Manuscripts are attached to report via google drive and a Biorxiv links)

Citations:

Peter, Jackson, et al. "Genome evolution across 1,011 Saccharomyces cerevisiae isolates." *Nature* 556.7701 (2018): 339-344.

Zampieri, Mattia, et al. "High-throughput metabolomic analysis predicts mode of action of uncharacterized antimicrobial compounds." *Science Translational Medicine* 10.429 (2018): eaal3973.

Yue, Jia-Xing, et al. "Contrasting evolutionary genome dynamics between domesticated and wild yeasts." *Nature genetics* 49.6 (2017): 913-924.

Ma, Siming, et al. "Organization of the mammalian metabolome according to organ function, lineage specialization, and longevity." *Cell metabolism* 22.2 (2015): 332-343.

Other projects I involved during the project period and I have utilized knowledge or method developed in connection with the reported NKFIH project.

- Underground metabolism as a rich reservoir for pathway engineering: In this project I have utilized my expertise I gained during in project period in understanding the variability of metabolite networks.
- Stellaria media tea protects against diabetes-induced cardiac dysfunction in rats without affecting glucose tolerance: In this project I have utilized my metabolomics experience gained during the presented metabolomics method developments, especially is metabolite identification.
- Bicarbonate Evokes Reciprocal Changes in Intracellular Cyclic di-GMP and Cyclic AMP Levels in Pseudomonas aeruginosa: In this project I have utilized my experience gained during the stress resistance assay in cultivation of microbes with diverse behaviour.

Cell-cell metabolite exchange creates a pro-survival metabolic environment that extends lifespan -

In this project I have utilized my experience gained during the project period in cultivation of diverse yeast strains in high throughput manner for metabolomics experiments.

Publication and presentation activity:

Papers to be published soon:

Tempo and genomic basis of metabolome evolution in yeasts
Roland Tengölics*; Balázs Szappanos*; Gábor Grézal; Dorottya Kalapis; Dóra Spekhárdt;Balázs Bálint, Laszló G. Nagy, Michael Mülleder; Enrica Calvani; Markus Ralser; Balázs Papp
https://drive.google.com/file/d/18YrNYK6D9CNXYddWNWW0YN6Sbnqun556/view?usp=sharing

Chloroplastic ascorbate content is a modulator of the plant metabolome
Dávid Tóth*, Roland Tengölics*, André Vidal-Meireles, László Kovács, Soujanya Kuntam, Alisdair R. Fernie, Balázs Papp, and Szilvia Z. Tóth
https://drive.google.com/file/d/1RvIkYJKlQGq7SLA2stdwmTW3DNWcUo4R/view?usp=sharing

Principles of metabolome conservation in animals
Orsolya Liska*, Gábor Boross*, Charles Rocabert*, Balázs Szappanos, Roland Tengölics, Balázs Papp
https://drive.google.com/file/d/17kQlyYLXWcYXSr41f0GV1eeuSQ5u188a/view?usp=sharing

Not directly connected to this work:
Cell-cell metabolite exchange creates a pro-survival metabolic environment that extends lifespan
Clara Correia-Melo, Stephan Kamrad, Christoph B. Messner, Roland Tengölics, Lucía Herrera-Dominguez, St John Townsend, Mohammad Tauqeer Alam, Anja Freiwald, Kate Campbell, Simran Aulakh, Lukasz Szyrwiel, Jason S. L. Yu, Aleksej Zelezniak, Vadim Demichev, Michael Muelleder, Balázs Papp, and Markus Ralser
https://www.biorxiv.org/content/10.1101/2022.03.07.483228v1.full.pdf

Published papers:
Stellaria media tea protects against diabetes-induced cardiac dysfunction in rats without affecting glucose tolerance
Virág Demján, Andrea Sója, Tivadar Kiss, Alexandra Fejes, Flóra Diána Gausz, Gergő Szűcs, Andrea Siska, Imre Földesi, Roland Tengölics, Zsuzsanna Darula, Dezső Csupor, Márton Pipicz, Tamás Csont, JOURNAL OF TRADITIONAL AND COMPLEMENTARY MEDICINE 12 : 3 pp. 250-259. , 10 p. (2022)

Underground metabolism as a rich reservoir for pathway engineering
Kovács Szabolcs Cselgő; Szappanos Balázs; Tengölics Roland; Notebaart Richard A; Papp Balázs - BIOINFORMATICS 38 : 11 pp. 3070-3077. , 8 p. (2022)

Bicarbonate Evokes Reciprocal Changes in Intracellular Cyclic di-GMP and Cyclic AMP Levels in Pseudomonas aeruginosa
Kasidid Ruksakiet, Balázs Stercz, Gergő Tóth, Pongsiri Jaikumpun, Ilona Gróf, Roland Tengölics, Zsolt M Lohinai, Péter Horváth, Mária A Deli, Martin C Steward, Orsolya Dobay, Ákos Zsembery - BIOLOGY-BASEL 10 : 6 Paper: 519 , 12 p. (2021)

Most important conference lectures and posters presented during the project period:

Accelerated metabolome evolution in *Saccharomyces cerevisiae* driven by gene content changes
Roland Tengölics; Balázs Szappanos; Gábor Grézal; Michael Mülleder; Enrica Calvani; Dorottya Kalapis; Krisztina Ambrus; Markus Ralser; Balázs Papp - Molecular Mechanisms in Evolution and Ecology 2020

Rapid non-targeted metabolite profiling of yeast for systems and evolutionary biology applications
Roland Tengölics, João B. Mokochinski, Balázs Szappanos, Dorottya Kalapis, Krisztina Ambrus, Balázs Papp - MOVISS Metabolomics conference 2018

Rapid non-targeted metabolite profiling of yeast
Roland Tengölics, João B. Mokochinski, Balázs Szappanos, Dorottya Kalapis, Stefánia Erdei, Szilvia Z. Tóth, Balázs Papp - The 29th International Conference on Yeast Genetics and Molecular Biology (ICYGMB) 2019

Accelerated metabolome evolution and its phenotypic impact in *Saccharomyces cerevisiae*
Roland Tengölics; Balázs Szappanos; Michael Mülleder; Enrica Calvani, Dorottya Kalapis; Joao Mokochinski; Csaba Pál; Markus Ralser; Balázs Papp - Experimental approaches to evolution and ecology using yeast and other model systems 2018

Accelerated metabolome evolution of *S. cerevisiae*
Darwin days conference 11-13 February 2020.