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Protein Biosynthesis and Carbon Catabolite Repression Are Transcriptionally Upregulated in *Saccharomyces cerevisiae* by Extracellular Fractions From Several Wine Yeast Species

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ABSTRACT

Non-*Saccharomyces* yeast species are increasingly used in winemaking in combination with *Saccharomyces cerevisiae* to modulate sensory attributes or as processing aids. Consequently, there is academic and practical interest in understanding how different yeast species interact with each other in grape must. Although interactions will depend on the metabolic capabilities of the strains involved, there are other possible interaction mechanisms between wine yeasts. In this work we used extracellular vesicle (EV)-enriched fractions from different non-*Saccharomyces* species to challenge *S. cerevisiae* inoculated in synthetic grape must. The results show that the previously described response to EVs of *Metschnikowia pulcherrima* was not an isolated phenomenon, but that *S. cerevisiae* responds in a general way to EVs of other yeast species. Meta-analysis of the results points to protein biosynthesis and carbon catabolite repression as general targets; both being stimulated by the interaction, beyond the acclimatisation to the synthetic juice experienced by the control cells. The intensity of the response showed differences between the four species; while the transcriptional response to *M. pulcherrima* EVs clearly diverges from that to EVs of the other yeast species, which show greater similarity to each other.

1 | Introduction

Saccharomyces cerevisiae is the yeast species that dominates most spontaneous wine fermentations and, until the beginning of this century, was the only yeast species used industrially as a starter culture for alcoholic fermentation (Gonzalez and Morales 2022). However, during the 2000s, several new species (non-*Saccharomyces*) have been added to the catalogues of wine yeast starters. Their use was originally intended to prevent the alleged sensory standardisation of wines fermented with *S. cerevisiae*; but a number of additional benefits have been proposed for non-*Saccharomyces* starters, including the management of total and volatile acidity, decreased ethanol content, colloids, or the control of spoilage microorganisms (Vejarano and Gil-Calderón 2021). Non-*Saccharomyces* starters are commonly used in combination with conventional ones, either in sequential or simultaneous inoculation, to ensure complete fermentation. This practice and others, such as local multi-species starters and controlled spontaneous fermentations (Mas and Portillo 2022), involve a relevant part of the fermentation process being carried out by a consortium of different yeast species. Under these conditions, yeast strains can engage in different types of interaction, such as competition for available resources, toxin production, or metabolite exchange. In addition, interspecific communication mechanisms, such as physical contact, volatile or soluble molecules, or extracellular vesicles, could also be involved (González

et al. 2018; Kemsawasd et al. 2015; Luyt et al. 2024; Mencher, Morales, Tronchoni, et al. 2021; Pérez-Torrado et al. 2017; Ramakrishnan et al. 2016).

From an academic point of view, wine fermentation constitutes an affordable and natural ecosystem for establishing operating rules that can be extrapolated to more complex environments (Conacher et al. 2021). There are indications that the most common yeast species found in wine fermentations worldwide have co-evolved in this ecosystem (Conacher et al. 2019). Previous work has revealed transcriptomic responses of *S. cerevisiae* to culture in the presence of different non-*Saccharomyces* strains shortly (2–3 h) after inoculation (Curiel et al. 2017; Mencher, Morales, Curiel, et al. 2021; Tronchoni et al. 2017). This was considered an indication of communication mechanisms beyond trophic interactions.

Extracellular vesicles (EVs) are produced by the cells of almost all living organisms studied so far and have been associated with several biological functions (Logan et al. 2024; Raposo and Stahl 2024; Stahl and Raposo 2019; Welsh et al. 2024). They have been extensively studied in pathogenic fungal species, where they play a role in infection and antimicrobial activity (Aor et al. 2024; Brown Harding et al. 2024; Karkowska-Kuleta et al. 2023; Kulig et al. 2024; Rodrigues and Janbon 2021). Recently, EVs have been explored as possible mediators of interspecific communication between wine yeasts or bacteria (Mencher et al. 2022, 2020; Morales et al. 2021). All wine microorganisms studied so far showed production of EVs. Proteomic analysis revealed they were associated with proteins related to both intracellular and extracellular functions in *S. cerevisiae*, *Torulaspora delbrueckii* and the lactic acid bacterium *Oenococcus oeni*. Interestingly, a wine strain of *S. cerevisiae* showed a similar transcriptomic response to live cells or to the EV-enriched fractions of one strain of *Metschnikowia pulcherrima* (Mejias-Ortiz et al. 2023). The transcriptomic signature of both treatments suggests that *S. cerevisiae* attempts a faster resumption of growth in response to these signals. It also suggests that EVs might play a role in the communication between wine yeast species.

The aim of the present work was to extend our knowledge on the role of communication mechanisms in wine fermentation and wine yeast physiology by exploring whether *S. cerevisiae* is able to respond to fractions enriched in EVs from other wine yeast species (*T. delbrueckii*, *Candida sake* and *Hanseniaspora uvarum*).

2 | Results and Discussion

S. cerevisiae cells were inoculated in synthetic must and challenged with EV-enriched fractions from either *M. pulcherrima*, *T. delbrueckii*, *C. sake* and *H. uvarum* (Supporting Information S1). Transcriptomic analysis was performed 3 h after the challenge, in comparison to an untreated control, inoculated and incubated under the same conditions in synthetic must (Supporting Information S2). The preliminary differential expression analysis of each sample versus all other samples shows a few hundred differentially expressed genes (DEGs) in the response of *S. cerevisiae* to any of the fractions enriched in EVs, as compared to the control condition (Figure S3). This indicates that, in addition to *M. pulcherrima* (Mejias-Ortiz et al. 2023) EV-enriched fractions from *H. uvarum*, *T. delbrueckii* or *C. sake* also induce transcriptomic responses in *S. cerevisiae* under wine-like conditions. None (or very few) DEGs were identified for the pairwise comparisons between the responses to *T. delbrueckii*, *C. sake* and *H. uvarum* (Figure S3). This suggests that the response triggered by these three EV-enriched fractions is similar. In contrast, the response to *M. pulcherrima* follows a different pattern, with many DEGs compared to the other responses, almost as many as with the control condition (Figure S3).

2.1 | GO Categories Affected by EV-Enriched Fractions

The response of *S. cerevisiae* to EV-enriched fractions of all tested species compared to the control condition was then analysed by Gene Set Enrichment Analysis (GSEA). The results were first explored by means of a meta-analysis to identify features of the transcriptomic response that are shared among the different test conditions. The impact of the different EV-enriched fractions was further analysed individually (Figure 1A).

The three up-regulated GO-biological processes in the meta-analysis are related to ribosome biogenesis. This includes rRNA processing genes, but also genes involved in rRNA transcription, tRNA processing, or ribosome assembly (Supporting Information S4). This result points to protein synthesis as one pathway generally stimulated by the extracellular fractions, in line with the 'ribosome' category highlighted in the previous study with EVs from *M. pulcherrima* (Mejias-Ortiz et al. 2023). Taken together, the number of genes contributing to highlight these categories is relatively high (636 genes after accounting for overlapping). It should be noted that, due to the features of multiGSEA analysis, these numbers are higher than the number of DEGs according to the criteria used in Figure S3. Also, Xing et al. (2023) found that categories related to ribosome biogenesis are enriched in *S. cerevisiae* when cocultured with *T. delbrueckii*. On the other side, Fu et al. (2024) found that, in mixed cultures with *L. thermotolerans*, *S. cerevisiae* genes involved in translation were significantly up-regulated, while those related to aerobic respiration were down-regulated. It is worth noting that the similarity in the transcriptomic responses is observed despite studies by other laboratories using living yeast cells from other species to challenge *S. cerevisiae*, while the present study uses cell-free extracellular fractions enriched in vesicles. All this suggests that either the EVs or other metabolic products contained in these EV-enriched fractions can be perceived by *S. cerevisiae* as signals of the presence of competitors from other yeast species.

The two down-regulated GO categories in this analysis are 'disaccharide catabolic process' and 'hexose transmembrane transport' (Figure 1A). The first one includes genes related to the catabolism of non-preferred carbon sources for *S. cerevisiae* such us maltose, sucrose, isomaltose or trehalose (Supporting Information S4). Invertase and maltose utilisation genes are known to be repressed by glucose (Hu et al. 2000; Neigeborn and Carlson 1987); while expression of genes coding for acid or neutral trehalases (downregulated in the treated samples) also seem to be under glucose repression (Chen et al. 2024)

FIGURE 1 | Legend on next page.

FIGURE 1 | Enriched categories obtained the Gene Set Enrichment Analysis (GSEA), assigned through the Gene Ontology (GO) Biological Process database with the Non-Redundant Biological Process (GOBP) dataset. To enhance clarity, weighted set cover redundancy reduction was applied. (A) GOBP categories enriched in all the conditions through a meta-analysis of the individual lists. (B–E) GOBP categories for the response to EV-enriched fractions from (B) *Metschnikowia pulcherrima*, (C) *Torulaspora delbrueckii*, (D) *Candida sake* and (E) *Hanseniaspora uvarum*. Panel A shows a transformation of the statistical significance. Panels B–E show the Normalised Enrichment Score. The colour intensity of each bar represents the statistical significance.

among other regulatory signals. Finally, among the three isomaltase coding genes with positive scores in this analysis, *IMA2* was previously shown to be moderately repressed by glucose, while *IMA1* and *IMA5* were considered insensitive (Teste et al. 2010).

Almost all the genes coding for hexose transporters with known glucose uptake activity (Boles and Hollenberg 1997) show negative scores (i.e., are downregulated), including *HXT2-8* and *GAL2* (Supporting Information S4). The highest differential expression for this set of genes is observed against *H. uvarum* and the lowest against the extracellular fraction of *M. pulcherrima*. However, *HXT1*, the main glucose transporter induced by high glucose concentrations (Boles and Hollenberg 1997) and encoding a low-affinity glucose transporter, is the only hexose transporter gene that is not downregulated in treated versus control samples.

HXK1 and *GLK1*, coding for hexose/glucose kinases involved in glucose uptake, are also downregulated in treated samples. Both genes have been shown to be repressed in high glucose or fructose media (Herrero et al. 1995). In contrast, *HXK2*, known to be induced in the presence of glucose or fructose (Herrero et al. 1995) shows an inconsistent expression pattern among the samples. Negative scores were also obtained for some genes involved in carbon catabolite repression, like *SNF3* and *MTH1* (Supporting Information S4). Snf3 is required for induction of *HXT2* and *HXT4* expression but not for induction of *HXT1*, while transcription of *SNF3* is repressed by high levels of glucose (Ozcan et al. 1996). Degradation of Mth1, induced by glucose, has been related to promotion of *HXT1* transcription (Polish et al. 2005).

As a whole, these few GO categories are reminiscent of the processes necessary for the transition from starvation conditions to a high concentration of nutrients, mainly glucose and fructose, including reinforced carbon catabolite repression. Since the control cells are under the same culture conditions, the simple explanation is that the treated cells are (transcriptionally) switching to high-glucose growth conditions faster than the control cells. Alonso-del-Real et al. (2019) found transcriptomic indications that *S. cerevisiae* wine yeasts accelerate nutrient uptake when co-inoculated with *Saccharomyces kudriavzevii*. Conacher et al. (2022) found a strong transcriptional response of *S. cerevisiae* after 7 h of coculture with *T. delbrueckii*, also involving activation of glucose metabolism among many other adaptations. More recently, Contreras-Ruiz et al. (2025) showed that *S. cerevisiae* strains showing the best competitive performance against *S. kudriavzevii* activate transcription of genes coding for the glycolytic, ribosomal, and DNA synthesis machinery faster than poor competitors. In general, this view is reinforced by the individual analysis of the responses to extracellular fractions from the different non-*Saccharomyces* yeast species (Figure 1). But there are differences between them that suggest they are not equivalent. Categories related to ribosome biosynthesis are highlighted with positive scores for the responses to *M. pulcherrima* (Figure 1B), while they appear as less significative and flanked by other categories for *C. sake* and *H. uvarum*, and are not revealed at all for *T. delbrueckii*, despite most of the genes involved showing similar expression patterns in the responses to all species (Supporting Information S4). Among the GO categories not appearing in the meta-analysis, those related to sulphate assimilation and amino acid metabolism show up for just *T. delbrueckii* (Figure 1C), although the genes involved show similar expression patterns for the response to *C. sake* or *H. uvarum*. On the other hand, there is almost no transcriptional response (or even negative scores) for these genes in response to *M. pulcherrima* (Supporting Information S4). Sterol metabolism is revealed, with positive scores, for all the species but *M. pulcherrima* (Figure 1). Indeed, although most genes in this category show a similar expression pattern in the response to *M. pulcherrima* extracellular fractions, this is not true in all instances (Supporting Information S4).

Categories highlighted with negative scores in the pairwise comparisons with the control quite consistently reproduce what is observed for the meta-analysis. The categories 'disaccharide catabolic process' and 'hexose transmembrane transport' or variants thereof appear in all instances (Figure 1). The genes involved show similar expression patterns for all treatments and are essentially the same found for the meta-analysis (Supporting Information S4).

The analysis of GO-cellular component categories reinforces the identification of ribosome biogenesis as a common feature of the response of *S. cerevisiae* to non-*Saccharomyces* extracellular fractions enriched in vesicles, with terms like 'nucleolus', 'ribosome', 'sno(s)RNA-containing ribonucleoprotein complex', or 'preribosome' (Figure S5).

2.2 | KEGG Pathways Affected by EV-Enriched Fractions

GSEA enrichment analysis of KEGG pathways provides a complementary view of the transcriptional response to non-*Saccharomyces* EV-enriched fractions (Figure 2). In the meta- analysis the positive scores include, for example, 'ribosome' or 'ribosome biogenesis in eukaryotes' (Supporting Information S4). Enrichment in these terms is supported by positive scores of many ribosomal proteins, both from the large and small subunits and both cytoplasmic and mitochondrial. The category 'RNA polymerase' reflects positive scores for subunits of RNApol I, II, and III. There is an intriguing exception,

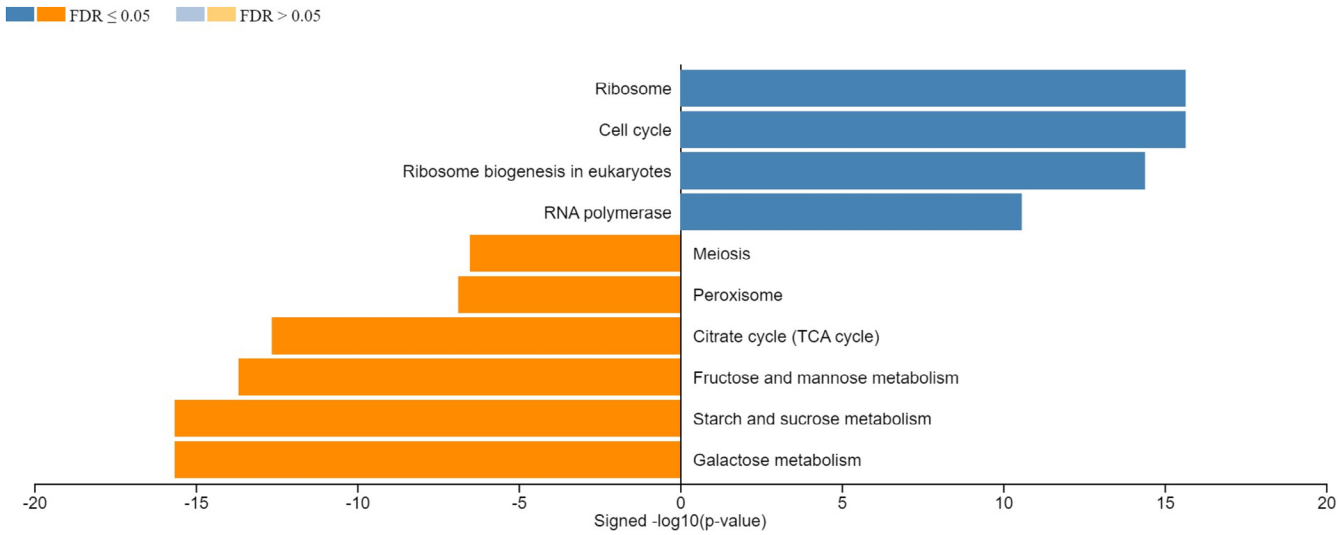

FIGURE 2 | Enriched categories obtained the Gene Set Enrichment Analysis (GSEA), assigned through the KEGG database. Weighted set cover redundancy reduction was applied. Categories identified as common to all conditions through a meta-analysis of the individual datasets are shown.

the highest score in absolute value in this gene list is negative and correspond to *RET1*, coding for the second-largest subunit of RNA polymerase III. The reverse behaviour of *RET1* with respect to other RNA polymerase III subunits in this experiment might be due to a different induction kinetics but would require further investigation. Finally, the category 'cell cycle', although globally positive, also includes many genes with negative scores (Supporting Information S4), probably reflecting the complex interplay of gene expression and posttranslational control of protein activities in cell cycle progression.

Three of the KEGG terms showing negative scores, 'fructose and mannose metabolism', 'starch and sucrose metabolism' and 'galactose metabolism', involve mostly genes already discussed under the GO-term 'disaccharide catabolic process', but additionally highlights downregulation for some genes involved in glycerol catabolism or in gluconeogenesis (Supporting Information S4). The category 'TCA cycle' involves, in addition, genes coding for proteins in mitochondrial respiratory chain complex, some of which are also highlighted in this analysis. Repression of aerobic respiration was previously observed in *S. cerevisiae* in response to cells of *M. pulcherrima* (Mencher, Morales, Curiel, et al. 2021). The category 'meiosis' groups downregulated genes coding for hexose permeases, filamentous growth, or stress responsive factors, together with genes involved in cell cycle progression (Supporting Information S4). Downregulation of 'meiosis' in *S. cerevisiae* was also previously observed in response to *M. pulcherrima* cells or EVs (Mejias-Ortiz et al. 2023). This is also probably related to the exit from stationary phase, since the genes involved are some of the previously discussed hexose permeases, as well as some stress factors. With different nuances, the observations of this work are in line with previous work on the short-term response of *S. cerevisiae* to co-culture with other yeast species or to extracellular fractions rich in *M. pulcherrima* vesicles (Curiel et al. 2017; Mejias-Ortiz et al. 2023; Mencher, Morales, Curiel, et al. 2021; Tronchoni et al. 2017). Finally, the 'peroxisome' category involves a high number of genes required for peroxisomal biogenesis and metabolic activity.

In summary, the analysis of KEGG pathways provides new arguments to consider the transcriptional response of *S. cerevisiae* to the treatments reflects a faster transition to nutrient (mostly sugar) rich conditions and active growth, as compared to the control condition. This can also be stated as a shortening of the lag phase or as a faster exit from the stationary phase.

2.3 | Strength of Response Differs Between Species

The meta-analysis highlights features of the transcriptomic response that are shared between the responses to the different non-*Saccharomyces* yeast species. However, as mentioned above, there are particularities in the response triggered by each EV-enriched fraction. Also, the strength of the response is different in each case. A heat map was drawn from the GO-biological process analysis as representative of the analyses carried out above (Figure 3). For the genes included in the globally downregulated categories 'disaccharide catabolic process' and 'hexose transmembrane transport', the strength of the response is higher for the extracellular fraction from *H. uvarum*, with some genes showing LogFoldChange (LFC) values between −3 and −4, followed by *C. sake* and *T. delbrueckii*; and the weakest response to *M. pulcherrima*. Genes in the categories globally up-regulated (all three related to ribosome biogenesis) show a weaker response, with LFC values below 1 in general. In this case the strongest response is for *M. pulcherrima* together with *H. uvarum*. The response to the extracellular fraction of *T. delbrueckii* is the weakest for these three categories. Species and strain-specific interactions have also been reported by other authors during wine fermentation (Wang et al. 2015), or in co-flocculation experiments (Rossouw et al. 2018, 2015). This is also reminiscent of previous studies of short-term contact with whole non-*Saccharomyces* cells, inducing both common and specific transcriptional responses (Curiel et al. 2017). The most appealing explanation to the observed specificities in the responses to different yeast species, or their extracellular fractions, would be that *S. cerevisiae* has evolved dedicated transcription patterns to respond to the challenges posed by each non-*Saccharomyces*

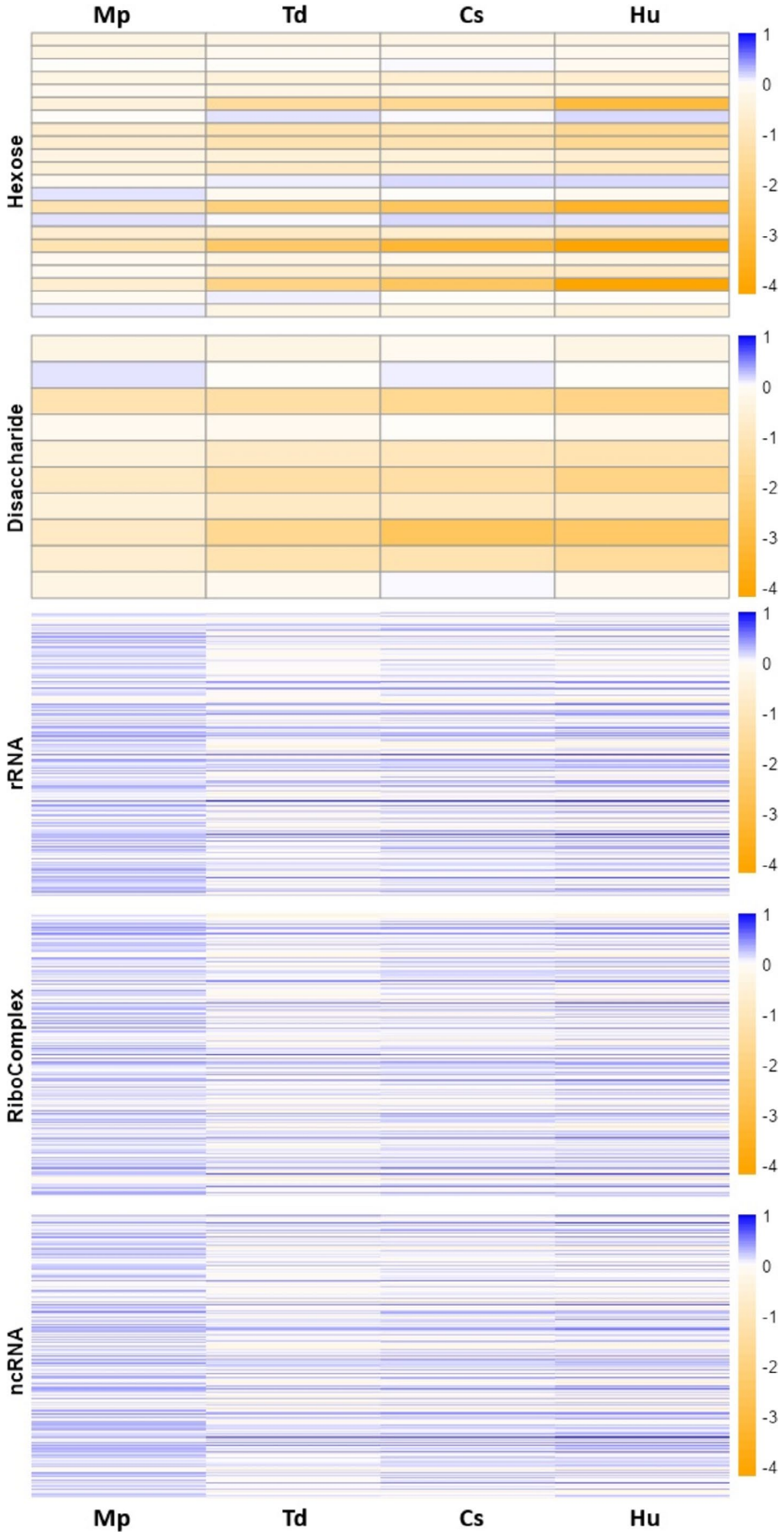

FIGURE 3 | Legend on next page.

FIGURE 3 | Heatmap of genes involved in the GOBP categories from the meta-analysis in Figure 1A. Each column corresponds to a condition (*Metschnikowia pulcherrima* [Mp], *Torulaspora delbrueckii* [Td], *Candida sake* [Cs] or *Hanseniaspora uvarum* [Hu]). Each row represents one gene, and the intensity of the colour represents the log2-fold change of each gene compared to the control condition.

species. Indeed, a certain degree of specificity cannot be ruled out. However, just as monocultures are not the real environment in which yeast evolution has taken place, dual cultures are still far from representing natural conditions. Other explanations to these differences might be related with the strength of the signals perceived in different conditions. This would eventually affect the intensity or the time course of the transcriptomic response. Indeed, the RNAseq samples were taken in a single time point. In these conditions, differences in the timing of the response cannot be distinguished from differences in the actual up- and downregulated genes or pathways. Future work will focus on the timing of the transcriptomic responses of *S. cerevisiae* to cells or extracellular fractions from other yeast species.

3 | Conclusions

This study explores the transcriptional responses of *S. cerevisiae* to EV-enriched fractions from non-*Saccharomyces* yeast species under wine-like fermentation conditions. It is shown that EV-enriched fractions from *M. pulcherrima*, *T. delbrueckii*, *C. sake*, and *H. uvarum* elicit distinct transcriptomic changes in *S. cerevisiae*, suggesting a conserved communication mechanism. These responses commonly stimulate ribosome biogenesis while enhancing carbon catabolite repression. Since both treated and control cells were freshly inoculated in synthetic juice, differential expression suggests a faster transition to nutrient-rich conditions. In addition, they show particularities in both the strength of the response and the transcription profile, depending on the non-*Saccharomyces* yeast species involved. Given that these transcriptomic responses are triggered by cell-free fractions, the results support the hypothesis that EV-enriched fractions carry potential signals for interspecific communication. Further research is necessary to elucidate the mechanisms underlying these interactions.

Author Contributions

Miguel Mejias-Ortiz: methodology, data curation, visualization, writing – original draft. **Pilar Morales:** conceptualization, supervision, funding acquisition, project administration, writing – original draft, writing – review and editing. **Guillermo Juárez:** methodology. **Ramon Gonzalez:** conceptualization, supervision, funding acquisition, project administration, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in European Nucleotide Archive at https://www.ebi.ac.uk/ena/browser/home, reference number PRJEB65853.

References

Alonso-del-Real, J., R. Pérez-Torrado, A. Querol, and E. Barrio. 2019. "Dominance of Wine *Saccharomyces cerevisiae* Strains Over S. Kudriavzevii in Industrial Fermentation Competitions Is Related to an Acceleration of Nutrient Uptake and Utilization." *Environmental Microbiology* 21: 1627–1644. https://doi.org/10.1111/1462-2920.14536.

Aor, A. C., L. S. Sangenito, T. P. Mello, et al. 2024. "Extracellular Vesicles From Scedosporium Apiospermum Mycelial Cells: Implication for Fungal-Host Interplays." *Journal of Fungi* 10, no. 4: 277. https://doi.org/10.3390/jof10040277.

Boles, E., and C. P. Hollenberg. 1997. "The Molecular Genetics of Hexose Transport in Yeasts." *FEMS Microbiology Reviews* 21: 85–111. https://doi.org/10.1111/j.1574-6976.1997.tb00346.x.

Brown Harding, H., G. N. Kwaku, C. M. Reardon, et al. 2024. "*Candida albicans* Extracellular Vesicles Trigger Type I IFN Signalling via cGAS and STING." *Nature Microbiology* 9: 95–107. https://doi.org/10.1038/s41564-023-01546-0.

Chen, A., S. E. Stadulis, K. DeLeuze, and P. A. Gibney. 2024. "Evaluating Cellular Roles and Phenotypes Associated With Trehalose Degradation Genes in *Saccharomyces cerevisiae*." *G3: Genes, Genomes, Genetics* 14: 215. https://doi.org/10.1093/g3journal/jkae215.

Conacher, C., N. Luyt, R. Naidoo-Blassoples, D. Rossouw, M. Setati, and F. Bauer. 2021. "The Ecology of Wine Fermentation: A Model for the Study of Complex Microbial Ecosystems." *Applied Microbiology and Biotechnology* 105: 3027–3043. https://doi.org/10.1007/s00253-021-11270-6.

Conacher, C., D. Rossouw, and F. Bauer. 2019. "Peer Pressure: Evolutionary Responses to Biotic Pressures in Wine Yeasts." *FEMS Yeast Research* 19, no. 7: foz072. https://doi.org/10.1093/femsyr/foz072.

Conacher, C. G., R. K. Naidoo-Blassoples, D. Rossouw, and F. F. Bauer. 2022. "A Transcriptomic Analysis of Higher-Order Ecological Interactions in a Eukaryotic Model Microbial Ecosystem." *mSphere* 7, no. 6: e00436-22. https://doi.org/10.1128/msphere.00436-22.

Contreras-Ruiz, A., J. Alonso-del-Real, J. C. Torrat-Noves, E. Barrio, and A. Querol. 2025. "Transcriptional Response of *Saccharomyces cerevisiae* During Competition With S. Kudriavzevii: Genetic Expression Variability and Accelerated Activation." *International Journal of Food*

Microbiology 430: 111053. https://doi.org/10.1016/j.ijfoodmicro.2024.111053.

Curiel, J. A., P. Morales, R. Gonzalez, and J. Tronchoni. 2017. "Different Non-Saccharomyces Yeast Species Stimulate Nutrient Consumption in *S. cerevisiae* Mixed Cultures." *Frontiers in Microbiology* 8: 2121. https://doi.org/10.3389/fmicb.2017.02121.

Fu, X., L. Guo, Y. Li, X. Chen, Y. Song, and S. Li. 2024. "Transcriptional Analysis of Mixed-Culture Fermentation of Lachancea Thermotolerans and *Saccharomyces cerevisiae* for Natural Fruity Sour Beer." *Fermentation* 10: 180. https://doi.org/10.3390/fermentation10040180.

González, B., J. Vázquez, P. J. Cullen, A. Mas, G. Beltran, and M.-J. Torija. 2018. "Aromatic Amino Acid-Derived Compounds Induce Morphological Changes and Modulate the Cell Growth of Wine Yeast Species." *Frontiers in Microbiology* 9: 670. https://doi.org/10.3389/fmicb.2018.00670.

Gonzalez, R., and P. Morales. 2022. "Truth in Wine Yeast." *Microbial Biotechnology* 15: 1339–1356. https://doi.org/10.1111/1751-7915.13848.

Herrero, P., J. Galíndez, N. Ruiz, C. Martínez-Campa, and F. Moreno. 1995. "Transcriptional Regulation of the *Saccharomyces cerevisiae* HXK1, HXK2 and GLK1 Genes." *Yeast* 11: 137–144. https://doi.org/10.1002/yea.320110205.

Hu, Z., Y. Yue, H. Jiang, B. Zhang, P. W. Sherwood, and C. A. Michels. 2000. "Analysis of the Mechanism by Which Glucose Inhibits Maltose Induction of MAL Gene Expression in Saccharomyces." *Genetics* 154: 121–132. https://doi.org/10.1093/genetics/154.1.121.

Karkowska-Kuleta, J., K. Kulig, G. Bras, et al. 2023. "*Candida albicans* Biofilm-Derived Extracellular Vesicles Are Involved in the Tolerance to Caspofungin, Biofilm Detachment, and Fungal Proteolytic Activity." *Journal of Fungi* 9, no. 11: 1078. https://doi.org/10.3390/jof9111078.

Kemsawasd, V., P. Branco, M. G. Almeida, J. Caldeira, H. Albergaria, and N. Arneborg. 2015. "Cell-To-Cell Contact and Antimicrobial Peptides Play a Combined Role in the Death of Lachanchea Thermotolerans During Mixed-Culture Alcoholic Fermentation With *Saccharomyces cerevisiae*." *FEMS Microbiology Letters* 362: fnv103. https://doi.org/10.1093/femsle/fnv103.

Kulig, K., M. Rapala-Kozik, and J. Karkowska-Kuleta. 2024. "Extracellular Vesicle Production: A Bidirectional Effect in the Interplay Between Host and Candida Fungi." *Current Research in Microbial Sciences* 7: 100255. https://doi.org/10.1016/j.crmicr.2024.100255.

Logan, C. J., C. C. Staton, J. T. Oliver, et al. 2024. "Thermotolerance in *S. cerevisiae* as a Model to Study Extracellular Vesicle Biology." *Journal of Extracellular Vesicles* 13: e12431. https://doi.org/10.1002/jev2.12431.

Luyt, N. A., R. N. de Witt, B. Divol, et al. 2024. "Physical Cell-Cell Contact Elicits Specific Transcriptomic Responses in Wine Yeast Species." *Microbiology Spectrum* 12: e0057223. https://doi.org/10.1128/spectrum.00572-23.

Mas, A., and M. C. Portillo. 2022. "Strategies for Microbiological Control of the Alcoholic Fermentation in Wines by Exploiting the Microbial Terroir Complexity: A Mini-Review." *International Journal of Food Microbiology* 367: 109592. https://doi.org/10.1016/j.ijfoodmicro.2022.109592.

Mejias-Ortiz, M., A. Mencher, P. Morales, J. Tronchoni, and R. Gonzalez. 2023. "*Saccharomyces cerevisiae* Responds Similarly to Co-Culture or to a Fraction Enriched in Metschnikowia Pulcherrima Extracellular Vesicles." *Microbial Biotechnology* 16: 1027–1040. https://doi.org/10.1111/1751-7915.14240.

Mencher, A., M. Mejias-Ortiz, P. Morales, J. Tronchoni, and R. Gonzalez. 2022. "Protein Content of the *Oenococcus oeni* Extracellular Vesicles-Enriched Fraction." *Food Microbiology* 106: 104038. https://doi.org/10.1016/j.fm.2022.104038.

Mencher, A., P. Morales, J. A. Curiel, R. Gonzalez, and J. Tronchoni. 2021. "Metschnikowia Pulcherrima Represses Aerobic Respiration in *Saccharomyces cerevisiae* Suggesting a Direct Response to Co-Cultivation." *Food Microbiology* 94: 103670. https://doi.org/10.1016/j.fm.2020.103670.

Mencher, A., P. Morales, J. Tronchoni, and R. Gonzalez. 2021. "Mechanisms Involved in Interspecific Communication Between Wine Yeasts." *Food* 10: 1734. https://doi.org/10.3390/foods10081734.

Mencher, A., P. Morales, E. Valero, J. Tronchoni, K. R. Patil, and R. Gonzalez. 2020. "Proteomic Characterization of Extracellular Vesicles Produced by Several Wine Yeast Species." *Microbial Biotechnology* 13: 1581–1596. https://doi.org/10.1111/1751-7915.13614.

Morales, P., A. Mencher, J. Tronchoni, and R. Gonzalez. 2021. "Extracellular Vesicles in Food Biotechnology." *Microbial Biotechnology* 14: 8–11. https://doi.org/10.1111/1751-7915.13657.

Neigeborn, L., and M. Carlson. 1987. "Mutations Causing Constitutive Invertase Synthesis in Yeast: Genetic Interactions With Snf Mutations." *Genetics* 115: 247–253. https://doi.org/10.1093/genetics/115.2.247.

Ozcan, S., J. Dover, A. G. Rosenwald, S. Wölfl, and M. Johnston. 1996. "Two Glucose Transporters in *Saccharomyces cerevisiae* Are Glucose Sensors That Generate a Signal for Induction of Gene Expression." *Proceedings of the National Academy of Sciences* 93: 12428–12432. https://doi.org/10.1073/pnas.93.22.12428.

Pérez-Torrado, R., K. Rantsiou, B. Perrone, E. Navarro-Tapia, A. Querol, and L. Cocolin. 2017. "Ecological Interactions Among *Saccharomyces cerevisiae* Strains: Insight Into the Dominance Phenomenon." *Scientific Reports* 7: 43603. https://doi.org/10.1038/srep43603.

Polish, J. A., J.-H. Kim, and M. Johnston. 2005. "How the Rgt1 Transcription Factor of *Saccharomyces cerevisiae* Is Regulated by Glucose." *Genetics* 169: 583–594. https://doi.org/10.1534/genetics.104.034512.

Ramakrishnan, V., G. A. Walker, Q. Fan, et al. 2016. "Inter-Kingdom Modification of Metabolic Behavior: [GAR+] Prion Induction in *Saccharomyces cerevisiae* Mediated by Wine Ecosystem Bacteria." *Frontiers in Ecology and Evolution* 4: 137. https://doi.org/10.3389/fevo.2016.00137.

Raposo, G., and P. D. Stahl. 2024. "Extracellular Vesicles, Genetic Programmers." *Nature Cell Biology* 26: 22–23. https://doi.org/10.1038/s41556-023-01277-8.

Rodrigues, M., and G. Janbon, eds. 2021. "Current Topics in Microbiology and Immunology." In *Fungal Extracellular Vesicles*, 1st ed. Springer International Publishing. https://doi.org/10.1007/978-3-030-83391-6.

Rossouw, D., B. Bagheri, M. E. Setati, and F. F. Bauer. 2015. "Co-Flocculation of Yeast Species, a New Mechanism to Govern Population Dynamics in Microbial Ecosystems." *PLoS One* 10: e0136249. https://doi.org/10.1371/journal.pone.0136249.

Rossouw, D., S. P. Meiring, and F. F. Bauer. 2018. "Modifying *Saccharomyces cerevisiae* Adhesion Properties Regulates Yeast Ecosystem Dynamics." *mSphere* 3: e00383-18. https://doi.org/10.1128/mSphere.00383-18.

Stahl, P. D., and G. Raposo. 2019. "Extracellular Vesicles: Exosomes and Microvesicles, Integrators of Homeostasis." *Physiology (Bethesda, Md.)* 34: 169–177. https://doi.org/10.1152/physiol.00045.2018.

Teste, M.-A., J. M. François, and J.-L. Parrou. 2010. "Characterization of a New Multigene Family Encoding Isomaltases in the Yeast *Saccharomyces cerevisiae*, the IMA Family." *Journal of Biological Chemistry* 285: 26815–26824. https://doi.org/10.1074/jbc.M110.145946.

Tronchoni, J., J. A. Curiel, P. Morales, R. Torres-Pérez, and R. Gonzalez. 2017. "Early Transcriptional Response to Biotic Stress in Mixed Starter Fermentations Involving Saccharomyces Cerevisiae and Torulaspora Delbrueckii." *International Journal of Food Microbiology* 241: 60–68. https://doi.org/10.1016/j.ijfoodmicro.2016.10.017.

Vejarano, R., and A. Gil-Calderón. 2021. "Commercially Available Non-Saccharomyces Yeasts for Winemaking: Current Market, Advantages Over Saccharomyces, Biocompatibility, and Safety." *Fermentation* 7: 171. https://doi.org/10.3390/fermentation7030171.

Wang, C., A. Mas, and B. Esteve-Zarzoso. 2015. "Interaction Between Hanseniaspora Uvarum and *Saccharomyces cerevisiae* During Alcoholic Fermentation." *International Journal of Food Microbiology* 206: 67–74. https://doi.org/10.1016/j.ijfoodmicro.2015.04.022.

Welsh, J. A., D. C. I. Goberdhan, L. O'Driscoll, et al. 2024. "Minimal Information for Studies of Extracellular Vesicles (MISEV2023): From Basic to Advanced Approaches." *Journal of Extracellular Vesicles* 13: e12404. https://doi.org/10.1002/jev2.12404.

Xing, X., Q. Chu, C. Li, et al. 2023. "Early Transcriptional Response of *Saccharomyces cerevisiae* in Mixed Culture With Torulaspora Delbrueckii in Cherry Wine." *LWT* 181: 114749. https://doi.org/10.1016/j.lwt.2023.114749.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.