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Catching cereal killers: a multi-omics approach to disentangle yeast-Fusarium interactions in the phyllosphere

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Chapter 8

General Discussion

General discussion

The **phyllosphere**, the aboveground part of a plant, hosts a diverse microbiome, including viruses, bacteria, filamentous fungi, and yeasts. **Yeasts** (see Glossary) are single-celled eukaryotic organisms, comprising approximately 1% of the fungal kingdom^{1,2}. Currently, over 180 yeast genera and 1,500 species have been identified, spanning unrelated lineages within both ascomycetes and basidiomycetes¹. Particularly species such as *Saccharomyces cerevisiae* and *Candida albicans* have received substantial scientific interest due to their industrial and medical importance, respectively. The comprehensive analyses and well-developed genetic tools for *S. cerevisiae* provide a strong foundation for investigating the largely untapped potential of environmental yeasts. The overall aims of my PhD thesis were to explore the taxonomic and functional diversity of yeasts that inhabit the wheat phyllosphere and their interactions with the mycotoxigenic pathogen *Fusarium graminearum*, the primary causal agent of Fusarium Head Blight (FHB) in wheat. For that, I coupled classical microbiology with different 'omics techniques including transcriptomics, metabolomics and genomics. Here, I discuss the key findings of my thesis in the broader context of existing knowledge, concluding with a discussion of knowledge gaps and future research directions.

Diversity and spatiotemporal distribution of yeasts in the wheat phyllosphere

Wheat is a critical crop for global food security, contributing to one-fifth of the world's total caloric intake. The wheat fungal community was one of the first phyllosphere communities studied using culture-dependent methods³. A meta-survey to determine the microbial diversity of the wheat phyllosphere identified 34 different genera, comprising 45 species (**Chapter 2**). Subsequent targeted isolation from wheat phyllosphere samples resulted in a collection of phenotypically and taxonomically diverse yeasts, representing 15 genera and 25 species (based on partial **ITS** and **D1/D2** sequences), that largely matched with the diversity observed in the culture-independent meta-survey (**Chapter 3**). Several studies have been conducted to examine the local variation in fungal communities, and which factors influence and shape the wheat phyllosphere microbiome diversity and functions. A study on wheat fungal communities across different growth stages and host genetic backgrounds (i.e. cultivars) found that yeast genera such as *Cryptococcus*, *Sporobolomyces*, *Dioszegia*, and the pathogen *Zymoseptoria tritici* were particularly dominant⁴. For the yeasts, the abundance of genera such as *Aureobasidium*, *Rhodotorula* and *Sporobolomyces* increased at the early stages of invasion by the pathogens *Puccinia striiformis* f.sp. *tritici* and *Blumeria graminis* f.sp. *tritici*⁵. These genera were also highly abundant in our yeast collection, representing 68% of the total collection (**Chapter 3**).

Most metabarcoding studies are based on (short) amplicon sequencing, which provides sufficient resolution for bacteria. However, amplicon sequencing gives limited resolution for filamentous fungi and yeast due to the high conservation of the ITS and D1/D2 domains (± 500 kb)⁶. Therefore, we tracked the distribution of two phyllosphere yeast genera, and the total fungal community, throughout the wheat growing season

with genus-specific qPCR primers. Preliminary **RT-qPCR** analysis revealed an increase in *Aureobasidium* and total fungal abundance over the growing season and across plant compartments (e.g. from lower to upper plant leaves) (Box 7.1). In contrast, no such pat-

Box 7.1 What is the spatiotemporal distribution of yeasts in the wheat phyllosphere?

To investigate the spatial and temporal differences in yeast colonization in the different phyllosphere compartments, we performed RT-qPCR sequencing as a proxy to quantify total fungal biomass, *Aureobasidium* and *Metschnikowia*. Total fungal biomass and *Aureobasidium* colonization both significantly correlated with increasing leaf age (For total biomass, $R2L2 = 0.78$, $p < 0.001$, $R2L3 = 0.29$, $p = 0.02$, and $R2L4 = 0.53$, $p < 0.001$, for *Aureobasidium* $R2L2 = 0.37$, $p = 0.02$, $R2L3 = 0.56$, $p < 0.001$, and $R2L4 = 0.29$, $p = 0.02$), while no correlation was observed for *Metschnikowia*

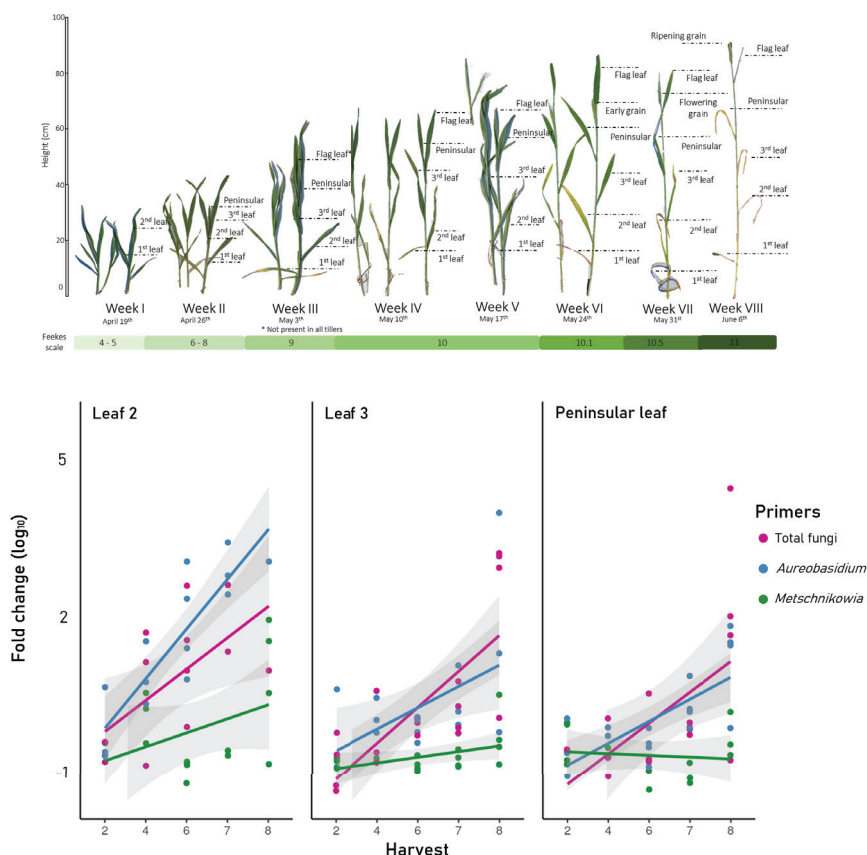


Figure 1 Spatiotemporal colonization of phyllosphere yeasts. Correlation between total fungal biomass, and *Aureobasidium* with increasing harvest date for all leaves (leaf 2 (L2), 3 (L3) and peninsular leaf (L4)). Points indicate average of three technical RT-qPCR replicates for one leaf. Linear regression analysis was used to calculate correlation efficient. Total fungal biomass shown in purple, *Aureobasidium* biomass in blue, and *Metschnikowia* biomass in green.

tern was observed for *Metschnikowia*. These findings indicate genus-specific variations in colonization efficiency, likely driven by nutrient availability. To further explore these dynamics, future studies will require the incorporation of long read amplicon sequencing (1,500 - 3,000 kb) coupled with metabolomics to examine shifts in the yeast community and nutrient availability throughout the growing season and across plant compartments.

Key traits for yeast survival and colonization of the phyllosphere

The phyllosphere is considered a harsh environment where the microbial community is exposed to different (a)biotic stresses, including UV radiation, temperature and humidity oscillations, as well as toxic compounds^{7,8}. Phyllosphere yeasts require specific adaptive traits to colonize this dynamic environment including a broad substrate utilization spectrum, biofilm formation, and growth at a wide range of temperatures^{9,10}. Characterization of various of these adaptive traits required for colonization of the dynamic phyllosphere environment, indicated significant variability between wheat phyllosphere yeasts (**Chapter 3**). Two highly abundant yeast genera in the phyllosphere, *Vishniacozyma* and *Aureobasidium*, displayed the most diverse substrate utilization profiles, whereas all yeast isolates were able to grow at different temperatures ranging from 4°C to 25°C. Flexible carbon utilization and growth at different temperatures are crucial to survive in the phyllosphere where these microorganisms face oscillating temperatures (day and night, winter and spring) and dynamic nutrient availability. Comparative genomic studies have begun to uncover adaptations and features that facilitate successful colonization of plant environments by bacteria^{11–13}. For example, an overrepresentation of genes involved in carbohydrate metabolism could be observed in plant-associated bacteria. In this thesis, we began to unravel the plant-adaptive genomic features in yeasts (**Chapter 4**).

Genomic and metabolic potential of phyllosphere yeasts

Current insights into yeast genomics stems primarily from research on *Saccharomyces cerevisiae*, well-known for its role in fermentation (e.g. beer, wine, bread). The growing availability of yeast genomes due to accessible genome-sequencing techniques has made comparative genomics a powerful tool to perform in-depth characterization of genetic variations among species and strains, providing valuable insights into yeast ecology and evolution¹⁴. This approach has been extensively applied to wine yeast strains to improve winemaking through targeted selection and the creation of new strains^{15,16}. Different strains influence various aspects of winemaking, such as flavor profiles. By leveraging these genomic approaches, novel and improved strains can be developed to meet public demand¹⁶. Previous comparative genomic studies have started to unveil the genomic diversity in *Saccharomyces cerevisiae* from natural environments^{17,18}. However, the number of whole genome sequences from environmental yeasts not 'eligible' for industrial applications and medical importance remains limited.

To explore the genomic potential of phyllosphere yeasts, we selected 96 taxonomically diverse yeasts for whole genome sequencing (**Chapter 4**). I investigated genomic features predicted to play a role in nutrient acquisition and colonization in scarce environments¹⁹, including carbohydrate-active enzymes (CAZymes) and secondary metabolites

involved in defense and signaling²⁰. The genomes of *Aureobasidium* isolates displayed the broadest CAZymes diversity (based on glycoside hydrolases), with high number of cellulose-degrading enzymes and xylanases, likely contributing to their high abundance in a wide variety of different environments (e.g. phyllosphere, soil, water, human, and extreme conditions)²¹.

Yeasts are valuable resources of secondary metabolites that are exploited in various biotechnological applications. One of the most significant biotechnological processes is the production of bioethanol, where *Saccharomyces cerevisiae* converts sugars into ethanol and carbon dioxide, providing a renewable energy source. Yeasts are also used to produce 2-phenylethanol, a fragrant alcohol with a rose-like scent, widely applied in perfumes, creams, and as a flavoring agent in baked goods^{22–24}. A total of 824 biosynthetic gene clusters (BGCs) were identified in our 96 yeast genomes. However, the number of BGCs is likely underestimated by the antiSMASH (fungiSMASH) computational tool. Its reference database is more comprehensive for pathways in bacteria and well-characterized strains, leading to potential underrepresentation of fungal/yeast-specific BGCs. Since antiSMASH was initially designed for bacteria, the relatively smaller number of BGCs from fungal genomes limits its application on fungal secondary metabolites²⁵. For example, fungiSMASH failed to annotate the BGC for pulcherrimic acid in *Metschnikowia* isolates, despite the compound's clear visual presence as a red halo on plates. This suggests that the genomic potential of phyllosphere yeasts is much larger than currently predicted by the available software tools.

Eleven secondary metabolites could be predicted, including clavric acid (a terpene), the anticancer compounds chaetoglobosin A/C and burnettramycin A. Chaetoglobosin A/C is a secondary metabolite produced by the fungus *Chaetomium*, and was identified as a potent anticancer compound in human colorectal cancer²⁶, while it was also described as potential biopesticide²⁷. It possesses a broad range of activity against various plant pathogens, including *Colletotrichum gloeosporioides*, *Fusarium sporotrichioides*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*²⁷. In contrast, burnettramycin A, first described for *Aspergillus burnettii*, inhibits the growth of the yeasts *S. cerevisiae* and *C. albicans*²⁸. Other BGCs shared lower levels of similarity with the antibacterial compound monascorubrin (12%), and the phytohormone abscisic acid (35%). Further bioinformatic analyses will be crucial to fully characterize these BGCs involved in the biosynthesis of potential novel compounds with a functional role in the lifestyle of phyllosphere yeasts.

Chemical and molecular basis of antagonistic activity of phyllosphere yeasts

Previous studies have described the antagonistic activity of yeasts against several pathogenic bacteria and filamentous fungi. These activities are conferred via different mechanisms, based on competition for nutrients and space, the secretion of enzymes or (killer) toxins, and the production of secondary metabolites (volatile and diffusible)²⁹. I found that the production of diffusible antimicrobial compounds was not widespread among phyllosphere yeast isolates, and only found for two *Papiliotrema* and a *Metschnikowia* isolate under *in vitro* conditions (**Chapter 3**). This is in line with other studies that indicate

the competition for nutrients and space as the primary mechanism of biocontrol yeasts²⁹. Functional characterization of the SNF2 gene in *Metschnikowia pulcherrima*, involved in the production of pulcherrimic acid, has shown the involvement of this iron-chelating compound in antifungal activity against various pathogens³⁰, while *Papiliotrema* has been described to inhibit *F. graminearum* in planta^{31,32}. However, whether the inhibition by *Papiliotrema* is based on the production of secondary metabolites or competition for nutrients and space remains unknown.

In contrast to the antifungal activity mediated by diffusible compounds, we observed that volatile-mediated activities against *Fusarium graminearum* were more frequent among the phyllosphere yeasts, and included all *Aureobasidium* and *Metschnikowia* isolates as well as some *Papiliotrema*, *Sporobolomyces*, and *Vishniacozyma* isolates (**Chapter 3**). Microorganisms use volatiles as a means of long-distance communication which can be inter- and intra-kingdom interactions^{33–36}. Microbial **volatile organic compounds** (VOCs) play diverse ecological roles, functioning as signaling molecules that activate plant defense mechanisms, or as antimicrobial agents thereby shaping the composition and dynamics of microbial communities³³. Due to their physico-chemical properties, VOCs can facilitate long-distance communication and competition between microorganisms by diffusing through air or air-filled soil pores^{36–38}. Growth inhibition of fungal pathogens by microbial VOCs has been widely reported for plant-associated bacteria and filamentous fungi^{36,39–42}. VOC profiling of 40 phyllosphere yeast isolates, representing 8 different genera, showed that most VOCs were phyla or genera specific, whereas only a small number of VOCs were considered as the ‘soft core’ volatilome, for example 3-methyl-1-butanol, 3-methyl-3-buten-1-ol, and 1,2,4-trithiolane (**Chapter 6**). The production of alcohol containing VOCs, such as 1-butanol, 2-phenylethanol and 2-heptanol was specific for the ascomycetes *Aureobasidium* and *Metschnikowia*, while sulfurous compounds such as dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide, were commonly found among the basidiomycetes *Holtermanniella*, *Papiliotrema*, *Sporobolomyces*, and *Vishniacozyma*.

Biocontrol microorganisms that produce VOCs have become a focal point of research, as their effectiveness does not require direct contact with pathogens³⁵. Furthermore, the lack of residual compounds left in the environment (e.g. unlike chemical application) makes the use of VOCs a promising and sustainable solution for agriculture⁴³. In this thesis, I further explored the potential role of yeast VOCs in suppressing *F. graminearum* (**Chapter 3 and 6**). Ascomycetes, including *Aureobasidium* and *Metschnikowia*, were the most effective inhibitors, followed by several yeast isolates from other genera, including *Papiliotrema*, *Sporobolomyces*, and *Vishniacozyma*. The identified antifungal VOCs produced by ascomycetes included alcohols such as 2-phenylethanol, isobutanol, and ethyl esters such as ethyl propionate, ethyl heptanoate and ethyl hexanoate. Basidiomycetes emitted sulfur-containing compounds, including dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide. While most of these VOCs showed moderate suppressive effects on growth of *F. graminearum*^{44,45}, the precursor of 2-phenylethanol, phenylacetaldehyde, significantly inhibited hyphal growth, even at low concentrations. Phenylacetaldehyde is a flavor and fragrance agent that is used as insect attractant⁴⁶. Since this is the first study to identify phenylacetaldehyde as a potential antifungal VOC, further research

is needed to elucidate its mode of action, and determine whether it is produced *in planta* at concentrations sufficient to suppress FHB.

In this thesis, I also characterized the volatile-mediated interaction between *F. graminearum*, and *Metschnikowia* sp. F159, which displayed the highest and reproducible fungal inhibition. A total of 34 VOCs were identified, while a substantial overlap between the volatilome of *Metschnikowia* in single culture and in co-culture with *F. graminearum* was observed. Only three VOCs were detected for *F. graminearum* in single and co-culture with the yeast isolate: these included junenol, butanal and 4-methyl-heptane. Most studies have only focused on the unidirectional volatile interactions neglecting the importance of a VOC-mediated dialogue between microbe-microbe, or microbe-plants³⁴. Both micro-organisms can respond to one-another volatilome, which could result in diverse transcriptional changes, including alterations in the expression of genes involved in growth, cellular metabolism, or the biosynthesis of virulence factors. To further explore this bi-directional volatile-mediated interaction at the transcriptional level, I examined the impact of the volatilome of *Metschnikowia* sp. F159 and *Aureobasidium* sp. F57 on *F. graminearum*, and vice versa by RNAseq (**Chapter 7**). While minimal transcriptional changes were induced in the yeasts, major transcriptional changes were induced in *F. graminearum*, including genes involved in both primary and secondary metabolism. Significant down-regulation of **mycotoxin** biosynthetic and regulatory genes, particularly deoxynivalenol (DON), and upregulation of genes associated with the degradation of specific alcohols in *F. graminearum*. Transcriptional analysis of biosynthetic gene clusters revealed upregulation of alcohol biosynthetic genes in *Metschnikowia* sp. F159, while *Aureobasidium* sp. F57 showed high expression of several terpenoid biosynthetic genes as well as genes involved in methionine and alcohol biosynthesis. Several yeasts, including *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida albicans* have been previously shown to produce the floral compound 2-phenylethanol^{22,47,48}, which disrupts organelles (such as the nucleus and mitochondria), enzyme induction, membrane fluidity and potentially the synthesis of mRNA^{24,49,50}. Additionally, this compound was also detected in the volatilome of both yeasts and was suppressive against *F. graminearum* (**Chapter 6**). Analysis of the biosynthetic pathway from phenylalanine to phenylacetaldehyde and 2-phenylethanol revealed constitutive expression of these genes across all treatments (**Chapter 7**). These genes represent promising targets for mutagenesis to further investigate their role in *Fusarium*-inhibition. First attempts of site-directed mutagenesis were performed on *Aureobasidium* and *Metschnikowia* strains, based on protoplast-mediated and PEG transformation, and by the use of CRISPR/Cas9. However, these attempts have been unsuccessful so far and will be explored and optimized in future studies.

Degradation and inhibition of mycotoxin biosynthesis by phyllosphere yeasts

The primary concern regarding FHB lies in the accumulation of mycotoxins, especially DON, the most prevalent, and economically important toxin^{51–53}. Strikingly, more than 60–80% of all grains are contaminated with at least one mycotoxin, surpassing EU legal food safety limits in 20% of cases⁵³. Mycotoxins, such as DON, have important ecological roles,

including a role in virulence and in competitiveness, and has been shown to be toxic to eukaryotes and to repress chitinase and glucanase gene expression in *Trichoderma atroviride*^{54,55}. We showed a significant reduction in FHB symptoms for wheat heads inoculated with the yeast isolates, with the majority of the heads remaining healthy. Additionally, we observed a prominent reduction in DON content of (detached) wheat heads for both yeast isolates (up to 95%), when individually and co-inoculated (**Chapter 5**). *In vitro* experiments further showed that the yeast volatiles caused downregulation of the complete deoxynivalenol cluster (**Chapter 7**). Volatile-mediated mycotoxin repression has been described for other plant pathogenic fungi. For example, exposure of *Aspergillus flavus* to the yeast volatile 2-phenylethyl alcohol (2-PE) repressed aflatoxin production, while volatiles (likely 2-PE) from low- and non-fermenting yeasts inhibited ochratoxin A production in *A. carbonarius* and *A. ochraceus*^{23,47,56}. Fungi can produce a wide variety of chemically complex secondary metabolites, which arise from only a limited number of primary metabolism precursors⁵⁷. It is plausible that the precursors needed for DON production are unavailable due to the significant shift in primary metabolism observed after exposure to the yeast volatiles. Additionally, we showed that *Aureobasidium* sp. F57 and *Metschnikowia* sp. F159 are able to grow in minimal medium supplemented with DON as the sole carbon source. DON only slightly affected the growth of *Metschnikowia* sp. F159, while no effect on *Aureobasidium* sp. F57 was observed (**Chapter 5**). However, how both yeast isolates metabolize DON and which degradation products they produce, is not yet known. In future studies, metabolomics analysis will be employed to identify the degradation products, while transcriptomics will be used to identify DON-degrading genes, based on existing literature, for mutagenesis and over-expression studies.

Leveraging phyllosphere yeasts for sustainable agricultural practices

Biological control is defined as the use of microbial antagonists or their traits to prevent or suppress pathogen infections. This approach can be adopted at different developmental stages of the host plant. Namely, pre-harvest, in-field application to limit contamination, and post-harvest application of micro-organisms or enzymes for detoxification. The use of microbial-based pesticides is anticipated to rise globally as part of efforts to adopt more sustainable agricultural practices⁵⁸. Yeasts have shown the ability to suppress fungal growth via different mechanisms²⁹. Besides the strong antifungal activities, yeasts are phyllosphere competent¹⁸, regarded as environmentally safe and are widely employed in the food and feed industry. They can be grown in bulk with fermenters for biotechnological applications and are tolerant to various fungicides used during post-harvest pathogen control^{58,59}. Also, the volatiles produced by the yeasts provide a promising alternative to synthetic pesticides to suppress pathogens from a distance without requiring direct contact. Additionally, the high volatility also allows for broad and uniform distribution of the active VOCs, a key challenge in open-field agriculture⁴³. Several studies have described the application of yeast VOCs to suppress fungal pathogens, including *Alternaria alternata* and *Botrytis cinerea* by ethanol and 2-phenylethanol from *A. pullulans*^{60,61}, or the inhibition of *Pestalotiopsis vismiae* by 3-methyl-1-butanol, 2-phenylethanol and 1-hexanol from *Met-*

*schnikowia pulcherrima*⁶². While application of VOCs to control post-harvest pathogens has proven to be promising, VOC application in the field has been more challenging⁴³, but several successful examples are available. Application of the green leaf volatiles 3-pentanol and 2-butanone in cucumber fields has demonstrated effectiveness against *Pseudomonas syringae* pv. *lachrymans*, the causal agent of bacterial angular leaf spot⁶³. Additionally, field application of 3-pentanol from *Bacillus amyloliquefaciens* resulted in antagonistic effects against *Xanthomonas axonopodis* pv. *vesicatoria* on pepper leaves by priming jasmonic acid and salicylic acid signaling, inducing resistance⁶⁴, and application of 2-phenylethanol on wheat heads inhibited growth and DON production by *F. graminearum*⁴⁵. These promising studies highlight the potential of microbial VOCs in sustainable agricultural practices. Whether the precursor of 2-phenylethanol, phenylacetaldehyde, discovered in my work would also be suitable for VOC-mediated suppression of *F. graminearum* under field conditions, will be investigated in future studies.

Successful application of biocontrol agents relies on several key factors including specificity against the pathogen, environmental suitability, application method and timing, persistence and colonization, and environmental and human safety⁶⁵. While *Aureobasidium* sp. F57 and *Metschnikowia* sp. F159 were initially isolated from flag leaves, successful colonization was also obtained on inoculated wheat heads (**Chapter 5**). An increase in population density was observed after 14 days, demonstrating their ability to colonize and proliferate on the wheat heads. Simultaneous inoculation of both yeast isolates resulted in colony counts comparable to individual inoculations, suggesting these two yeast genera can co-exist on the wheat heads, either because of different niche occupancy or their different carbon utilization spectrum⁶⁶. Furthermore, I showed that the order of arrival was critical for the antagonistic effects of *Aureobasidium* sp. F57 towards *F. graminearum* (**Chapter 5**). Significant pathogen inhibition was observed only when the yeast was inoculated at least 24 h prior to *F. graminearum*. This early arrival, also referred to as niche pre-emption, allows species to occupy space and utilizing resources before others establish⁶⁷. Additionally, early arriving species may already be producing secondary metabolites, thereby altering the chemical landscape on the leaf and impacting the growth of the latecomers. Extensive metabolomic identification of the nutrients present on the leaves and leaking from these leaves upon *Fusarium* infection will be needed to support this hypothesis. Collectively, these results highlight the potential of phyllosphere yeasts as a novel and largely unexplored microbial resource for managing FHB and minimizing mycotoxin levels in wheat. Identifying the underlying molecular mechanisms responsible for these (volatile-mediated) yeast-pathogen interactions is an important step to move from the lab into the field.

Concluding remarks

The research presented in this thesis explored the taxonomic and phenotypic diversity of yeasts in the wheat phyllosphere. By coupling classical microbiology with different 'omics approaches, we started to unravel the chemical and molecular basis of yeast-pathogen interactions in the wheat phyllosphere (Figure 2). Future studies will examine changes

in yeast population dynamics throughout the growing season and on different plant compartments using long-amplicon sequencing. Furthermore, the genomic analysis represents the first steps towards exploring the functional potential of phyllosphere yeasts and their plant-adaptive traits, whereas the transcriptomic analysis of the volatile-mediated interaction between yeasts and the fungal pathogen provided insights into the potential molecular mechanisms. Future research will investigate whether inhibition of *F. graminearum* and DON biosynthesis is linked to phenylacetaldehyde production by phyllosphere yeasts, and if this compound is produced on wheat heads in the field at concentrations sufficient for pathogen control. Mutagenesis, combined with transcriptomic and metabolomic analysis, will be needed to identify the regulatory pathways involved in volatile production in yeasts and their functional importance in FHB control. Additionally, field trials will be conducted to evaluate the effectiveness of these beneficial phyllosphere yeasts under real life agricultural conditions.

Glossary

Yeast: single-celled eukaryotes, spanning unrelated lineages within ascomycetes and basidiomycetes. Ascomycetes comprise the 'true yeasts' (e.g. *Saccharomyces cerevisiae*) and 'fission yeasts' (e.g. budding or binary fission), while basidiomycetes include dimorphic filamentous fungi with a yeast-like developmental stage^{1,2}.

Phyllosphere: the total aboveground surface of a plant, comprising both floral and vegetative parts.

ITS and D1/D2 domains: The Internal Transcribed Spacer (ITS) and part of the large subunit of rRNA (D1/D2, also known as LSU or 28S). These regions are widely used for yeast taxonomic characterization through amplicon sequencing

RT-qPCR: Quantitative reverse transcription polymerase chain reaction is used to quantify the concentration of RNA in real-time. This technique is widely used to quantify gene expression levels.

Mycotoxins: Secondary metabolites produced by fungi, such as *Fusarium graminearum*, which are harmful to human and animal health.

Comparative genomics: The comparison of complete genome sequences of different strains to identify overlap or differences within the genome.

Volatile organic compounds (VOCs): Small, low molecular weight compounds with high vapor pressure that are produced during primary and secondary metabolism. These compounds play a key role in multiple ecological processes such as long-distance communication and competition

Transcriptomics: The technique used to study the changes in gene expression based on RNA levels.

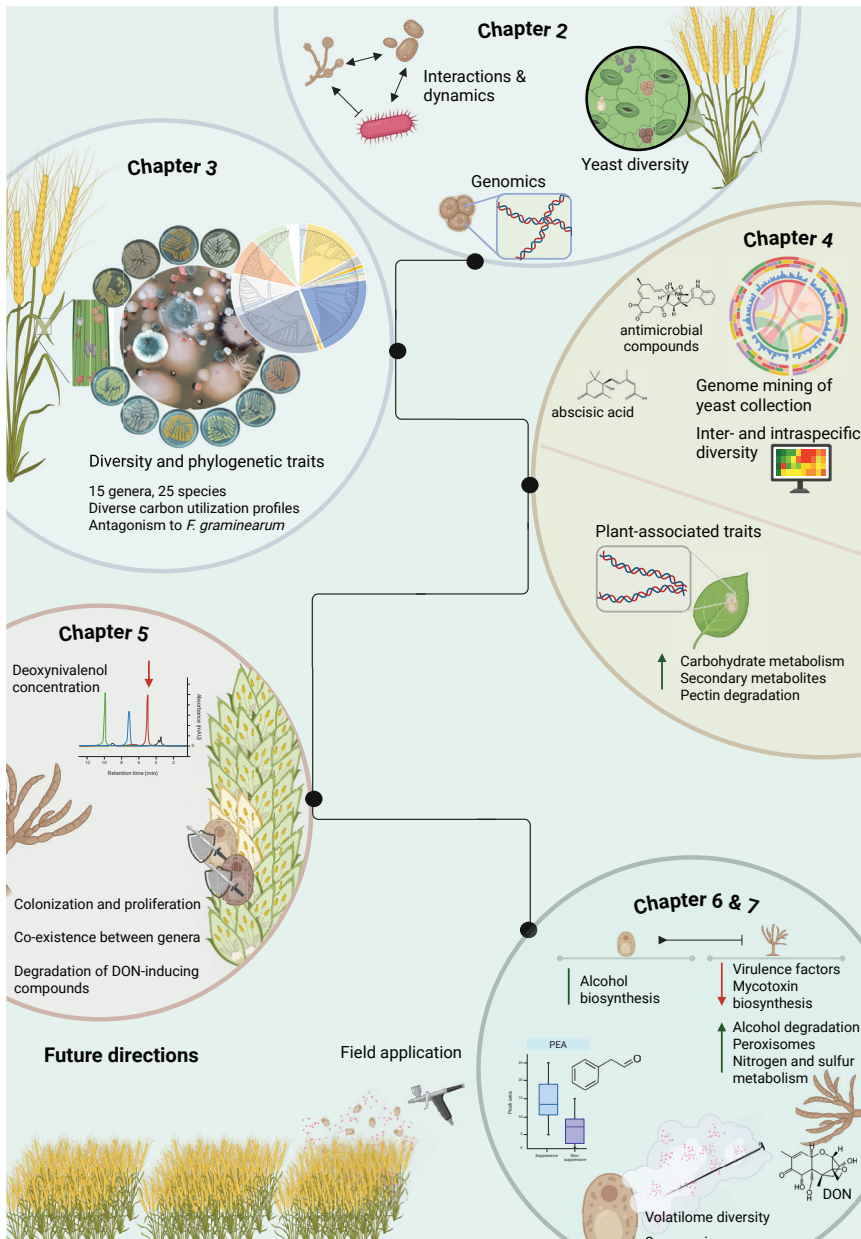


Figure 2 Schematic overview of key findings from each chapter of this thesis. First, I explored the diversity, dynamics, interactions and genetics of phyllosphere yeasts, identifying the current knowledge gaps (Chapter 2). I determined the diversity of (culturable) yeast in the wheat phyllosphere and found variation in functional traits across genera (Chapter 3). Next, I performed genome mining to dive deeper into the functional potential of these yeasts, and used comparative genomics to identify plant-associated traits (Chapter 4). I validated the results in planta and identified two yeasts as promising biocontrol agents (Chapter 5). In follow-up experiments, I investigated the volatile-mediated interaction between yeast-pathogen based on volatilome diversity and transcriptional changes (Chapter 6 and 7). These yeasts will be used during field trial experiments (Future directions).

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