S. K. Galiwango, E. G. Kızılyar, G. De Rouck, I. Van de Voorde and F. Weiland

The Barley Malting Microbiome: Influence on Malt Quality, Control and Characterization Methods

The conversion of raw grains to malt is a pivotal step in the beer production process. By germination, the grains produce enzymes necessary for the conversion of starch into sugars during the mashing process, but also cell wall constituents are broken down which aids in wort filtration. Further, storage proteins are converted into soluble peptides and amino acids important for yeast nutrition during fermentation. However, the performance of the malting process and the quality of malt produced is also significantly influenced by the active microbial communities that naturally colonise the barley grains. These diverse microbial communities have the potential to produce extracellular enzymes, phytohormones, mycotoxins, and exopolysaccharides, and also compete with the grains for oxygen. While microbes can contribute positively to the break-down of cell wall β-glucans and arabinoxylans, they can also negatively impact the final malt quality by production of gushing precursors, compounds leading to premature yeast flocculation (PYF) and mycotoxins. This review provides an overview of the current knowledge on the different microbial communities interacting with the barley grain on the field and during malt production, their influence on the quality of produced malt, and the brewing process. Further, current strategies to control the proliferation of detrimental microbial species during malting are presented, including a summary on recent techniques to identify, quantify and functionally characterise the barley malting microbiome.

Descriptors: *Hordeum vulgare*, microbial community profiling, microflora management, metagenomics, metatranscriptomics, metaproteomics

1 Introduction

Barley (*Hordeum vulgare*) is a widely grown cereal crop, ranking globally fourth in terms of production after maize (corn), wheat and rice [1], and is one of the key ingredients used in beer production. Before usage in the brewing process, barley generally undergoes a malting phase, which can be defined as the germination of a quiescent dry seed or grain under strictly controlled environmental conditions [2]. Malting represents an economically inexpensive source of enzymes needed for 1) breakdown of cell walls (cytolysis) and transformation of the grain into a friable state for efficient milling and extraction of starch during the mashing process, 2) providing enzymes to break down the starch during mashing, 3) conversion of storage protein into low molecular weight peptides and amino acids which are important for yeast metabolism (proteolysis). Most brewing operations employ well-modified malts with high mashing-in temperatures (between 62 to 65 °C) to shorten the mashing process [3], increasing brewhouse throughput. Well-modified malts of high quality need to exhibit a high degree of cytolysis, but only a limited degree of proteolysis, as high concentrations of amino acids can lead to high concentrations of their aldehyde degradation products via the yeast Ehrlich pathway or Strecker degradation via intermediates of the Maillard reaction which negatively impact the stability of beer flavour [4].

Production of high-quality malts that consistently meet all the requirements of the brewer is challenging. The maltster often relies on test maltings and/or experience to manipulate the environmental conditions to achieve a well-balanced degree of cytolysis, proteolysis, while also limiting amylolysis, the break-down of starch into sugars which are utilised by the growing plant embryo, to achieve a homogenous modification of the starchy endosperm [5, 6]. These three biological processes are mainly driven by de novo synthesised enzymes (e.g. alpha-amylase, and other, generally, endo acting enzymes) [7, 8] and also those activated inside the barley grain during germination (e.g. β-amylase and other exo-acting enzymes) [7]. However, the malting ecosystem does not only consist of the actively respiring growing barley grain but also the metabolically active microbial communities harbouring the surfaces and inner

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Authors

Steven Kasozi Galiwango (ORCID ID: 0000-0002-9859-9247, these authors contributed equally), Gert De Rouck (ORCID ID: 0000-0001-9092-8205), Ilse Van de Voorde (ORCID ID: 0000-0001-8706-7934), Florian Weiland (ORCID ID: 0000-0001-9702-9245), KU Leuven, Faculty of Engineering Technology, Department of Microbial and Molecular Systems (M2S), Laboratory of Enzyme, Fermentation and Brewing Technology, Ghent Campus, Ghent, Belgium; Leuven Institute for Beer Research (LIBR), KU Leuven, Belgium; Elif Göksu Kızılyar (ORCID ID: 0000-0002-5389-5405, these authors contributed equally), KU Leuven, Faculty of Engineering Technology, Department of Microbial and Molecular Systems (M2S), Laboratory of Enzyme, Fermentation and Brewing Technology, Ghent Campus, Gebroeders De Smetstraat 1, Ghent, Belgium; Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Bioengineering, Davutpasa Campus, Esenler, Istanbul, Turkiye; corresponding author: florian.weiland@kuleuven.be

Table 1 Microorganisms (genera) identified on/in raw barley using molecular techniques [16, 24–27, 36–43, 62, 63, 227]

Bacteria			Fungi		
Gram-positive	**Gram-negative**	**Gram-variable**	**Ascomycota**	**Basidiomycota**	**Mucoromycota**
Microbacterium	Sphingomonas	Saccharibacillus	Cladosporium	Sporobolomyces	Rhizopus
Arthrobacter	Enterobacter	Propionibacterium	Aureobasidium	Cryptococcus	
Paenibacillus	Sandarakinorhabdus	Truepera	Alternaria	Bullera	
Curtobacterium	Erwinia		Phaeosphaeria	Dioszegia	
Clavibacter	Xanthomonas		Aspergillus	Rhodotorula	
Rhodococcus	Kluyvera		Drechslera	Malassezia	
Salinibacterium	Pseudomonas		Bipolaris	Cystofilobasidium	
Lactococcus	Serratia		Pichia	Bulleromyces	
Propionibacterium	Aeromonas		Candida	Udeniomyces	
Sanguibacter	Pantoea		Exserohilum	Bensingtonia	
Leucobacter	Rhizobium		Pleospora	Exobasidium	
Pseudoclavibacter	Pedobacter		Microdochium	Mrakia	
Kocuria	Chryseobacterium		Fusarium	Tilletiopsis	
Frigoribacterium	Duganella		Septoriella		
Actinomyces	Stenotrophomonas		Pyrenophora		
Curtobacterium	Brevundimonas		Penicillium		
Plantibacter	Methylobacterium		Trichoderma		
Corynebacterium	Bacillus		Epicoccum		
	Phyllobacterium		Davidiella		
	Trabulsiella		Lecanicillium		
	Ralstonia		Zymoseptoria		
	Delftia		Stagonospora		
	Kosakonia		Lophiostoma		
	Epilithonimonas		Sclerotinia		
	Deinococcus		Sarocladium		
	Exiguobacterium		Leptosphaeria		
	Staphylococcus		Crocicreas		
	Enterococcus		Botrytis		
	Massilia		Neoascochyta		
	Wautersiella		Verticillium		
	Streptococcus				
	Prevotella				
	Morococcus				
	Cronobacter				
	Rahnella				
	Achromobacter				
	Alistipes				
	Ralstonia				
	Edaphobacter				
	Saccharibacillus				
	Blautia				
	Faecalibacterium				
	Candidatus				
	Epilithonimonas				
	Thermus				
	Pseudobutyrivibrio				
	Ruminiclostridium				
	Rubritepida				
	Sutterella				
	Rubritepida				
	Sutterella				

The following phyla and genera were known formerly as: For bacteria; Phyllum Bacillota as Firmicutes; Phyllum Pseudomonadota as Proteobacteria; Genus *Candidatus Spencerbrownia* as Brownia; Genus *Massilia as Naxibacter*; For fungi: Genus *Bipolaris* as *Cochlibolus*; Genus *Exserohilum* as *Setosphaeria*; Genus *Fusarium* as *Gibberella*; Genus *Septoriella* as *Vagicola*; and Genus *Mrakia* as *Mrakiella*.

tissue layers of the barley grain [9]. Interactions of these microbial communities with the barley grains significantly influence the extent of cytolysis, proteolysis, and amylolysis via the production of enzymes, stimulating plant hormones, and metabolites required for nutrient utilisation, host colonisation, and virulence [10]. The resulting impact of these interactions can be either beneficial or detrimental to the malting process and the final malt produced [11–15].

Adequate control of the malting process, together with an understanding of the interactions between the barley grain and its associated microflora, and how these interactions affect the malting performance and malt quality is essential. Thus, the main motivation of this review is to give an update on the current knowledge of the microflora associated with the barley grain through the malting process and the impact of the barley-microbiome interactions on the overall quality of malt. To describe the microflora associated with the barley grain, and those derived from green malt and the final malt after kilning, emphasis was laid on findings or studies based on recently introduced molecular techniques. Within this manuscript, also an overview of these molecular techniques used to study the plant microbiome including their strengths and limitations is discussed. Lastly, a couple of strategies are discussed that could be applied during malting to suppress the growth of exopolysaccharide-producing bacteria that negatively affect the lauter performance of malt and toxigenic fungal species to improve malt characteristics and to ensure the safety of the malt and resulting beer produced thereafter.

1.1 Barley

The barley grain is a complex and non-uniform substrate for microorganisms and is composed largely of around 80 % carbohydrates (such as starch, cellulose, β-glucans), 10 % protein, and (in decreasing order) of inorganic matter, fat, and other substances, including polyphenols and vitamins [16]. Field barley for the use of malting has a microbiome originating from the environment, insects, and animals. Weather and other conditions affect the microbial community growing on barley, where wet years promote microbial growth and pathogenesis [17]. After the harvest, microbes continue to grow and interact with the grain during its storage prior to malting. Microbial growth has to be suppressed as it has a great impact on the quality of the final malt [12, 18, 19] and subsequent wort and beer quality [13, 20–22], thus storage has to keep conditions of low water activity ($a_w < 0.7$ which is approximately 14 % moisture content) and low temperature (15 – 25 °C) [23].

1.2 Overview of the composition of the barley micro flora through the malting process

The microbial communities associated with barley during the malting process are diverse and vary greatly in abundance across the different stages of the malting process [9, 24–26]. This variation in diversity and abundance can be explained in large part by the changing conditions applied through the malting process, effects of barley genotypes, initial microbial diversity and load on the raw barley grains [9, 27, 28]. At a taxonomy level, many more plant genera are restricted to a particular continent or provenance than plant families [29]. The peculiar climatic conditions (especially rainfall, and temperature) and soil types of these ecological zones shape the microbiome composition of the plant genotypes.

Furthermore, differences in the sample preparation and the subsequent analytical techniques used thereafter to catalogue the microbial species during malting can also have a significant impact on the documented differences in species richness (or diversity) of the barley-associated microflora during malting. For example, some microbiome studies combined both epiphytes (i.e., the microflora that colonises the surface of the grains that may or may not become internalised with grain tissues) and endophytes (i.e., the microbial community that resides within the internal tissues (embryo and endosperm) of the barley grain and mainly inherited from the mother plant) [30], while others selectively focused on either one of the two. The differences in the sensitivity and detection limit of the various methods applied is also another source of variation. Regardless of the plant genotype, geographical location of the field and methodology used, there is a general pattern that the barley microbial diversity increases from the raw grain through steeping until the end of germination and then reduces in the final malt due to the high temperatures applied during drying and kilning.

1.2.1 Barley associated microbiota

The microbial community of raw barley grains is mainly composed of bacteria (10^6 – 10^8 CFU/g [14, 16, 31–33]), yeasts (10^3 – 10^5 CFU/g [14, 16, 31, 34]) and filamentous fungi (10^2 CFU/g [31]), whose species richness is influenced by the genotype, the environmental conditions in which the grain and its seedling develop, agronomical practices during harvest, as well as the postharvest conditions during transportation and storage [9, 12, 27, 35]. In addition, the relative abundance of the microbial communities on raw barley grains can also be significantly influenced by the crop/harvest year [26]. Comparing the relative abundance of microbial species of barley variety Sebastian between two harvest years, 2010 and 2011, [26] reported a significantly higher relative abundance of genus *Streptococcus* on barley grains from harvest year 2011 in comparison with harvest year 2010.

The bacterial community of raw barley is commonly dominated by Gram-negative bacteria from phylum Pseudomonadota (formerly known as Proteobacteria). Other highly abundant phyla include; Actinobacter, Bacillota (formerly known as Firmicutes), Bacteroidetes, Acidobacteria, Cyanobacteria, and Patescibacteria [26, 36–41]. For the fungal community, Basidiomycota [24, 27, 39, 42] and Ascomycota [43] are the only two phyla that have been reported to be dominant in raw barley grains. In comparison to the bacterial community, the fungal composition of raw barley grains is more influenced by the geographical location or place of harvest of the grain [39, 44]. This could be because bacteria have a more cosmopolitan distribution than fungi in terrestrial plant systems [44]. An overview of the most important microbial genera found on/in raw barley grains using current molecular techniques is presented in table 1 and a taxonomic chart is provided in Suplementary figures 1 and 2.

1.2.2 The barley malt microbiota

The malting process is divided into three stages; steeping, germination, and kilning. The temperature and humidity during steeping and germination not only induce barley germination but also activate dormant bacterial and fungal spores and trigger the growth of the vegetative cells of the microflora naturally harbouring the barley grain. The diversity and abundance of these different microbial communities vary along the malting process and are majorly influenced by the initial microbial load or species diversity on the raw barley grain, process conditions/applied malting regime, microbial quality of the water and air used during steeping and germination, use of additives, and the interaction between the different fungal and bacterial species [12, 25]. Bacterial-fungal interactions (BFIs) have been studied in humans, plant roots, soil, and some fermented foods like wine, beer, and cheese but only to a limited extent in barley malting [24, 45–47]. These BFIs influence the biology (e.g., growth and reproduction) and ecology (e.g., stress resistance, nutrition and pathogenicity) of the fungal and bacterial patterns which in turn has a profound influence on the temporal diversity and abundance levels of these microbial species [48]. Concerning these BFIs during the barley malting process, it has been documented that yeasts (such as *Candida* and *Pichia*), and lactic acid bacteria (e.g., *Lactobacillales*) show a positive correlation with *Arthrobacte*r spp., and an antagonistic behaviour towards mould growth [24]. Given the paucity of information concerning these BFIs at various stages of the barley malting process, more research into this topic is required to explore the various bacterial-fungal interactions and how they affect negatively or positively the malting process and quality of malt. Furthermore, different malting houses harbour specific microflora which further adds to the heterogeneity of the microbial communities observed during the malting process.

1.2.3 Field and Storage Origin of Microbiota

Barley grain carries a numerous, variable and complex microbial population consisting of bacteria, yeast, filamentous fungi, slime moulds and protozoa that can be partially detected and quantified using microscopic and molecular techniques [35, 49]. The grain community composition provided by these microorganisms changes greatly during post-harvest and storage periods [16]. With the first barley ear, the colonisation of the grain with microorganisms, originating from soil, vegetation, air, rain, insects, bird droppings, etc. begins. In the rich microbiological environment during growth, conditions are more favourable, especially for microorganisms that are trapped between the caryopsis and bracteoles in contrast to those on the outer surface of barley [35]. Variability is observed in the mycobiota and microbiota of barley depending on the impact of environmental conditions during the malting process. [35] divided these environmental conditions into three ecological systems: 1) The duration of the barley grain in the field, 2) its harvest and storage in a moisture-free environment before malting, and 3) its germination during malting. It is possible to understand the microbial metabolic activity in the malt production process by examining these steps. Changes occurring in the barley grain affect malt quality by including the secretion of enzymes, hormones, toxins, and acids that affect physiological processes [35]. Potential microbial contamination is an inherent feature of all food processing systems, with beer quality being particularly sensitive to microbiological risk [16]. After harvest, barley is stored for 2 months to 1 year to allow dormancy to be disrupted before malting. Time conditions are of paramount importance, as under the favourable combination of environmental factors, a selection process occurs among the dominant microorganisms. Some may become inactive and their numbers decrease during storage [16], although moulds generally remain dominant [39].

These filamentous fungi can be generally divided in two groups: Field fungi associated with the microflora of the soil, vegetation and air that occur on or in the grains until harvest time, and storage fungi that infect the grains after harvest [35]. In this context, the role of temperature and moisture content (climatic conditions) in determining the type and abundance of microflora cannot be denied, as they play an important role in the accumulation of microorganisms on barley grains and have a profound effect on colonisation. While field fungi can adapt to rapidly changing weather conditions, storage fungi (mostly xerophilic in nature) are more adapted to such stable conditions and generally low water activities [39, 50]. The water activity/humidity is of paramount importance for fungal growth. While field fungi do not grow below a water activity (a_w) of 0.90 (20 – 25 % moisture) [39], storage fungi usually grow at an a_w of 0.80 (18 % moisture) or even 0.68 (14 % moisture) [35]. The most frequently isolated fungi in barley are *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., [16, 39]. *Aspergillus* and *Penicillium* species are commonly found in environmental dust and air; and can be associated with agricultural machinery and malting equipment, including mills [16]. Of particular concern, is the infection of barley grains with *Fusarium* species which leads to the production of mycotoxins, Class II hydrophobins (which induce gushing in beer), and various hydrolytic enzymes (such as proteinases, endo-xylanases, and β-glucanases) whose effects can increase the soluble nitrogen content of malt and also produce premature yeast flocculation factors [51–54]. Hence, appropriate storage and malting conditions ought to be selected to restrict the development of natural contaminants in the barley grain [35].

1.3 Microbial Diversity: From Grain to Wort

The microbial community on the barley grain is conditioned by the environment endured prior to the start of the malting process, and thus the germination process in malting is largely affected by this accumulated microbiome [35]. To date, several studies have been conducted to investigate the barley malting microbiome (see below). Interestingly, these studies identified different dominant microbial taxa, confirming that the barley microbiome is strongly influenced by pre-malting conditions (e.g. field, storage). During steeping, the microbial cells start to multiply rapidly on the grains and also in the steep water (due to the steep washing effect) due to dissolved nutrients, moisture, temperature, and aeration. For reduction of the inhibitory effects of the microbiome on malt quality, reducing dissolved nutrients, and controlling the steep temperature to suppress microbial growth was recommended [17].

Studies of various bacteria and fungi in barley have also suggested that these microorganisms produce low amounts of gibberellic acid and abscisic acid, potentially affecting germination and enzyme production [17]. Successful malting involves maintaining the appropriate balance between the plant hormones gibberellic acid (GA3), abscisic acid (ABA) and indole-3-acetic acid (IAA) (part of the auxins group) of the germinating barley [35]. GA3 plays a role in promoting germination and enzymatic activity, while ABA maintains the grain dormancy and prevents endosperm degradation and IAA is considered as the primary shoot signal hormone in seed germination, limiting or stimulating endosperm degradation [55, 56].

In the study of [24], by examining microbial community changes during malting, they suggested that most, if not all, of the microbes found in malt originated from barley grains. This stands in contrast to the study by [16] where it was also concluded that malt generally has higher biodiversity than barley, suggesting that conditions in malting promote the growth of new and different microbial communities [16].

Studies also observed different dominant microbial taxa during malting, with one study finding that the microflora be especially numerically dominated by Gram-negative bacteria, and the most common and abundant bacterial species to be *Erwinia herbicola* (now *Pantoea agglomerans*)[35]. Recent studies found *Microbacteriaceae* to be the dominant family in the barley grain microbiome, as well as in the barley root microbiomes, which was ascribed to a microbial transition from roots to seeds [24]. Further, *Candida* and *Arthrobacter* which are regarded as beneficial microbes as they help break down barley components like complex carbohydrates into simpler sugars were identified during malting [24].

Staphylococcus was identified as the most common bacterial species in barley and malt but interestingly found that bacteria did not significantly contribute to beer production, likely due to their habitat preference for warmer temperatures and higher oxygen levels [16]. It has been observed that lactic acid bacteria constitute a small minority of the total number of bacteria in the bacterial population of barley before malting. The Gram-negative bacteria including *Serratia*, *Erwinia*, *Enterobacter*, *Pseudomonas*, and *Aeromonas* were found to be dominating over the lactic acid bacteria in the study of [39]. Among lactic acid bacteria, *Leuconostoc* spp. was found to be predominating during the malting process, but other LABs were identified, including *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Enterobacter* and *Pseudomonas* [39, 49]. It was also found that fungal communities are significantly affected by where barley is grown, in comparison to the bacteria communities [39]. In addition, *Rhizobium*, *Pantoea*, and *Sphingomonas* were observed among the commonly identified bacterial genera [39]. *Pantoea agglomerans*, a regularly reported bacteria in barley, produces growth hormones that encourage root growth as a common microorganism in soil [49].

1.4 Microflora during steeping

Steeping is the first stage of the malting process during which the quiescent dry barley grain is submerged under water (wet steep) to elevate its moisture content to trigger germination [57]. This is followed by the removal of the steep water to prevent detrimental effects of oxygen deprivation to the barley kernel (dry steep) and further wet steep/dry steep stages to bring the final moisture content of the grains to the desired concentration, commonly between 42 – 46 % [58]. The steep water temperature is controlled between 10 – 20 °C [9, 59]. Higher temperatures favour a faster uptake of water into the grain [59]. The steeping water is also aerated to provide oxygen to the respiring seed and facilitate a uniform temperature distribution throughout the steeping water.

Strong microbial growth occurs during the first wet steep, induced by the rapid hydration of the barley grain, warm environment, and leaching of nutrients into the steeping water [24, 39]. Aeration of the steeping water also enhances the proliferation of the Microbial communities, although a large number of microorganisms adhering to the surfaces of the barley kernels are washed away along with the steeping water [25]. As microorganisms in and on the barley begin to respire during soaking, they may compete with the grain tissue for oxygen. This competition is believed to be at least partially responsible for grain dormancy, as well as environmental conditions during germination and endogenous factors within the grain [60, 61].

The accumulation of barley microbes in steeping water also has a secondary effect. The steeping water acts as a medium of transfer of microbes between barley kernels. This is of concern in case a barley batch contains a small percentage of kernels infected with exopolysaccharide and biofilm-forming microbes [15]. In such a scenario, the whole malt batch might become contaminated, yielding wort filtration problems [15]. The variation of the oxygen content in the steeping water can also influence the composition of the microbiome. The limited amount of dissolved oxygen in the steeping water has been shown to favour the growth of lactic acid bacteria (LAB, *Lactobacillales*), which are normally not detected in raw barley grains (0 – 10^2 CFU/g [10, 33]) or only detected at low abundance levels [24, 62]. Some of the highly abundant LAB species identified in steeped barley include *Leuconostoc*, *Lactococcus*, *Streptococcus*, and *Lactobacillus* [24, 62]. Apart from LAB, other bacterial genera reported to be highly abundant during the steeping stage include; *Sphingomonas* and *Enterobacter* [25], *Erwinia* and *Pantoea* [62].

There is also a shift in the composition of the fungal community during steeping. In the case of epiphytic barley microbiota, the abundance of Ascomycota increased during steeping, while Basidiomycota showed a significant decline in steeped barley in comparison to raw barley [24]. *Cladosporium*, *Sporobolomyces*, *Cryptococcus* and *Bullera* were most abundant in the steeped grain samples. These species were also detected as abundant in the ungerminated barley samples. Factors like storage conditions, harvest practices, and even the pre-harvest environment (soil, roots, etc.) can significantly influence the barley's microbiota. This inherent complexity often leads to seemingly contradictory research findings, highlighting the need for further exploration.

1.5 Microflora during germination

The barley germination step is characterised by an increase in the compositional structure heterogeneity of both the bacterial and fungal communities [24]. Favourable conditions (temperatures around 16 – 20 °C, relative air humidity > 95 %, aeration) to enhance grain germination also stimulate the proliferation of the psychrotrophic and mesophilic bacteria activated during the steeping phase. Given that the germination stage is the longest among the three stages of malting, the dominant species during this timeframe have the greatest influence on the quality of the final malt [25].

Bacteria are commonly the predominant species during germination and some of the highly abundant bacterial genera identified include

Enterobacter, *Erwinia* [25], *Rhizobium*, *Sphingomonas*, *Chryseobacterium*, *Acinetobacter* [39], and *Pantoea* [63]. During germination, humidified air is blown through the germination bed to remove carbon dioxide, provide oxygen to the respiring grains, and maintain a uniform temperature within the germination bed. Aeration dramatically increases the abundance of the bacterial population owing to the enhanced growth of aerobic bacterial species [25]. The abundance of LAB also further increases during the germination process with a reported strong increase in the genera *Lactobacillus*, *Leuconostoc*, and *Lactococcus* [39, 62]. Among the reported LAB, *Leuconostoc* were observed overall as the most abundant genus [24, 63], possibly because of their ability to produce bacteriocins against other LAB, phytopathogens, or spoilage organisms [16, 24]. Towards the end of the germination process, the abundance of the bacterial population tends to decrease. This is evidenced by the disappearance of some bacterial species which are identified within the early days of germination [25], and also a reduction in the bacteria species richness in the last days of germination [62].

Concerning the trend of the fungal community diversity during germination, significant differences in germinated barley grains originating from different geographical regions were detected [39]. However, conflicting observations are seen in the literature regarding the fungal diversity during germination. Using a high-throughput sequencing approach, no difference between the fungal diversity of germinated and ungerminated barley samples was observed by [39]. This stands in contrast to the study of [62], where the fungal diversity (species richness) increased during germination. Further, *Alternaria* was detected to decrease during germination in the study of [64], while evidence from studies of [39] and [27] show that *Alternaria* significantly increases during germination.

1.6 Microflora during kilning

Once the desired level of modification of the barley endosperm is achieved, the green malt is dried to halt any ongoing metabolic processes and afterwards kilned to stabilise the malt and ensure good storability of the dried product. The drying and kilning programme applied strongly influences the microbial composition of malt. Most bacterial species are destroyed by the temperatures applied during the kilning process. In comparison to steeped and germinated barley, malt generally shows a lower bacterial flora diversity but still greater than that of the raw grain [25], e.g. LAB have been reported to be highly abundant in kilned malts [16, 24]. In comparison to the bacterial community, some fungi seem to survive and grow under the high temperatures applied during kilning [27, 62]. *Fusarium* has been frequently documented to increase significantly in malt after the kilning process [24, 27]. However, species from the fungal phylum Basidiomycota are in general more negatively affected by kilning temperatures than Ascomycota, which is the most prevalent fungal phyla in finished malt [24, 27]. An overview of the most important microbial genera found on/in barley malt using current molecular techniques is presented in table 2 and a taxonomic chart is provided in Supplementary figures 3 and 4.

Table 2 Microorganisms (genera) identified on/in barley malt using molecular techniques [16, 24, 24, 25, 27, 42, 44, 62]

Bacteria			**Fungi**		
Gram-negative	**Gram-positive**	**Gram-variable**	**Ascomycota**	**Basidiomycota**	**Mucoromycota**
Enterobacter	Arthrobacter	Saccharibacillus	Candida	Cryptococcus	Rhizopus
Erwinia	Corynebacterium	Propionibacterium	Pichia	Sporobolomyces	
Klebsiella	Leuconostoc		Fusarium	Bullera	
Sphingobacterium	Lactococcus		Alternaria	Filobasidium	
Wautersiella	Microbacterium		Aureobasidium	Holtermanniella	
Kluyvera	Sanguibacter		Wickerhamomyces	Sporobolomyces	
Pseudomonas	Streptococcus		Claviceps	Trichosporon	
Rhizobium	Paenibacillus		Pyrenophora	Rhodotorula	
Massilia	Lactobacillus		Pleospora	Cystofilobasidium	
Duganella	Brachybacterium		Geotrichum	Udeniomyces	
Chryseobacterium	Curtobacterium		Hanseniaspora	Bulleromyces	
Epilithonimonas	Curtobacterium		Pichia	Dioszegia	
Pantoea	Kineococcus		Saccharomyces	Bensingtonia	
Stenotrophomonas	Staphylococcus		Aspergillus		
Acinetobacter	Bacillus		Penicillium		
Xanthomonas	Kocuria		Trichoderma		
	Enterococcus		Stagonospora		
	Weissella		Sclerotinia		
	Frigoribacterium		Zymoseptoria		
	Exiguobacterium		Sarocladium		
			Zymoseptoria		
			Sarocladium		

Genera Pichia was formerly known as *Issatchenkia* or *Hansenula*

1.7 Impact of the barley-associated microflora on the quality of malt

As described above, the barley grain is in close association with a great diversity of microbial communities that actively interact with the grain during storage and further into the malting process, influencing the grain physiology, microbial safety, and technological properties of the finished malt. Diverse microbial communities colonise the inside tissue layers (termed as endophytes) and outer surfaces of the barley grain (termed as epiphytes) [30]. Although epiphytes generally colonise the outer surfaces of the grain, they can become internalised in case the grain surfaces are damaged creating avenues of ingress for the entry of these surface microbes into the internal tissue layers of the grain. Endophytes are believed to be only non-deleterious microbes that have established symbiotic relationships with their host plant and thus primarily confer beneficial effects to the plant or seed [30, 36]. On the other hand, the epiphytic microbiota can consist of synergistic, commensal, and pathogenic microbes [65] and thus can confer both beneficial and deleterious effects to the barley grain. In sum, the microbial consortium colonising either the inside or outer surfaces of the barley grain can positively or negatively influence the quality of the final malt.

1.7.1 Malt quality: Favourable effects of microbiota

Barley-associated microbes have been reported to stimulate grain germination by directly providing phytohormones such as IAA and cytokinins. For example, isolates of *Curtobacterium*, *Paenibacillus*, *Pantoea*, *Erwinia*, and *Kosakonia* were reported to produce IAA [37]. Furthermore, some strains of *Paenibacillus*, one of the dominant bacterial genera isolated from barley grains [24, 40], have been reported to synthesise cytokinins [66], that play a significant role in the regulation of seed germination [67]. Barley germination can also be stimulated indirectly by suppressing the growth of pathogenic microorganisms which is mediated via the production of siderophores [68, 69]. The siderophores chelate iron from the environment with the effect of additionally making it unavailable to pathogenic microbes, especially effective in iron-limiting environments [69, 70]. In this way, the growth of phytopathogens can be retarded since iron is an essential element needed for microbial growth [70]. Some of the barley-associated bacterial genera, reported to produce siderophores include; isolates of *Pantoea*, *Erwinia*, and *Kosakonia* [37]. Inoculation of plants with *Pantoea* significantly enhanced their growth rate in comparison to uninoculated plants, especially in sterilised nutrient-poor soil [40]. Alleviation of stress is another mechanism used by beneficial microbes to promote seed germination and seedling growth [68]. This is achieved via the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme that catalyses the conversion of ACC (the immediate precursor of ethylene) into α-ketobutyrate and ammonia [68, 71]. This results in the reduction of the levels of the stress hormone ethylene. Some of the barley-associated microbes reported to produce ACC deaminase include isolates of *Sanguibacter* and *Saccharibacillus* [37].

Table 3 **Microorganisms (genera) isolated during the barley malting process with hydrolytic enzyme production potential [34, 228]**

Bacteria	Fungi/yeast
Arthrobacter	Cryptococcus
Brachybacterium	Sporobolomyces
Cellulomonas	Auerobasidium
Clavibacter	Bullera
Paenibacillus	Candida
Pseudomonas	Cladosporium
Sphingobacterium	Geotrichum
	Exophiala
	Filobasidium
	Rhodotorula

The barley-associated microbial consortium is also a source of hydrolytic enzymes such as cellulases, β-glucanases, xylanases, amylases, and peptidases (see Table 3 for a list of the bacterial and fungal genera with hydrolytic enzyme production potential) [34, 37, 72]. These microbial enzymes complement the endogenous enzymes produced by the barley aleurone layer during germination for the degradation of important biomolecules such as cell wall polysaccharides, starch, and proteins. Among these hydrolytic enzymes, special attention has been paid to cell-wall degrading enzymes because of their central role in degrading the endosperm cell walls (composed of 70 % β-glucans and 20 % arabinoxylans), which is a prerequisite to grant other hydrolytic enzymes, especially alpha-amylases produced in the aleurone layer, access to the food reserves in the endosperm [6, 73]. In earlier years, much attention was paid to the degradation of β-glucans as they were seen as the main component that caused most of the brewhouse wort separation issues [74]. However, it is nowadays recognized that arabinoxylans also significantly influence the viscosity of the final wort and thus the lauter performance [75]. Due to the heterogeneous composition of arabinoxylans [76], their degradation requires the concerted action of three main enzymes [77] namely; endo-(1,4)-β-xylanases (commonly referred to as xylanases; EC 3.2.1.8) that cleave non-terminal glycosidic linkages of the xylan backbone [78]; (ii) exo-(1,4)-β-xylosidases (EC 3.2.1.37) which catalyse the release of terminal-end (xylp) residues from the xylanase-derived oligosaccharides [79, 80]; and (iii) arabinoxylan arabinofuranohydrolases (AXAHs, EC 3.2.1.55) which catalyse the release of arabinosyl substituents from the xylan backbone facilitating access of xylanases to the xylan backbone [79, 81]. Among this battery of enzymes, fungi are commonly associated with xylanase and arabinofuranosidase production [76, 82]. It is generally reported that arabinoxylans are only degraded to a lesser extent during the malting process [83]. This could be attributed to the late release of endo-(1,4)-β-xylanases by programmed aleurone layer cell death during/after germination [84]. Also, the barley aleurone layer cells synthesise and accumulate endo-(1,4)-β-xylanases as inactive precursors, and thus barley endo-xylanases contribute only minor to the degradation of these heteroxylans [84]. Unsurprisingly, the overwhelming portion of the xylanase activity measured in barley is of microbial origin [12, 72]. Similar results were also reported in mature wheat grains [85]. This implies that microbes have an important impact on the cell wall degradation process during malting.

1.7.2 Malt quality: Unfavourable effects of microbiota

A. Effect of biofilm-forming microbes on the wort filtration performance of malt

The presence of microbes on the barley seed surface can significantly affect the malting process. Due to the poor nutrient status of the husk layers of barley an increase in the population of biofilm-forming microbial cells triggers biofilm formation and intensive production of extracellular polymeric substances (EPS) that provide the architectural form of the biofilms [86, 87]. Ninety percent of the surface of tissue layers between the testa and the outer epidermis of steeped barley kernels were reported to be covered with an extended matrix of EPS [14].

Generally, the EPS matrix is composed of a complex mixture of biopolymers, such as exopolysaccharides, fibrous and globular proteins (including extracellular enzymes), lipids, and extracellular DNA (eDNA) [88, 89]. The architecture of the EPS matrix enables it to provide mechanical stability and scaffolds that allow biofilm cells to enhance nutrient sorption given the poor nutrient state of the barley husk layers, and also protect against stressful conditions such as extreme values of pH and temperature [86, 89, 90]. EPSs are quite stable compounds persisting even long after the microbial mother has disappeared [90] and it was postulated that these exopolysaccharides adhere to the husk of malt and are dissolved during the mash production but are not hydrolyzed to a large extent [49]. This means that even when the microbial population is reduced (for example as an effect of kilning), these polymeric substances might still be present and subsequently cause issues with wort filtration.

The exopolysaccharides (i.e.,gellan, xanthan, and dextran) produced by biofilm-forming microbial communities (i.e., *Flavobacterium*, *Pseudomonas*, *Leuconostoc* [15]) that naturally colonise the barley grain have a negative effect on wort filterability [14, 15, 49, 63]. A concentration of 5 mg/L of these polysaccharides added to wort was reported to decrease the filterability of wort from good to critical with 10 – 15 mg/L stopping filtration [21]. Further, the addition of 12.5 µg/mL of exopolysaccharides produced by *Pantoea agglomerans* during wort production led to a 50 % decrease in the wort filtration speed [49]. The formation of these exopolymeric substances is more intensified in the husk layers of split barley kernels than in sound barley kernels [14, 63]. In two different studies, the application of a *Lactobacillus plantarum* E76 starter culture during steeping suppressed growth of EPS-forming bacteria and had a positive effect on the wort filtration rate [14, 15]. This starter culture also restored wort filtration performance in intentionally husk-damaged barley kernels to the same rate as that of the barley samples where no treatment was applied [15]. This suggests that besides non-starch polysaccharides (β-glucans, and arabinoxylans), exopolysaccharides of microbial origin can also significantly impact the lauter performance of malt.

B. Premature Yeast Flocculation factors from malting barley

Premature yeast flocculation (PYF) is defined as the phenomenon in which flocculation-competent yeast settles out of the fermenting wort abnormally early or heavily during primary fermentation before full attenuation has been achieved [91]. PYF occurs sporadically and leads to undesirable effects during primary beer fermentation, such as; (i) incomplete fermentations, resulting in poorly attenuated worts, and final products with lower alcohol by volume level than the brewer's target value expected from the extract value of the malt; (ii) accumulation of undesirable flavour compounds especially diacetyl, and (iii) increased susceptibility of beer to microbial spoilage [91–94]. .

The existing body of knowledge shows that premature yeast flocculation in lager yeast fermentations is induced by a particular compound originating from malt or barley infected with fungi either during malting or in the field [91, 95–97]. However, to date the identity of the PYF factor is unknown; polysaccharides derived from the breakdown of barley husk (predominantly composed of arabinoxylan and cellulose) by fungal enzymes or antimicrobial peptides produced by the living barley plant as a defence strategy against fungal infection have been suggested [93, 94, 97–99]. Some of the examples of fungal strains associated with PYF, that have been isolated from barley grains, and PYF-positive malts include: *Aureobasidium pullulans*, *Aspergillus flavus*, *Fusarium graminearum*, *Alternaria tenuissima* [100]; *Gibberella baccata* [96] and *Cochliobolus sativus* [93]. Fungi cause the barley husk during steeping and germination to be degraded producing high-molecular-weight acidic arabinoxylan products rich in arabinose and xylose that have been documented as the most probable causative compounds in malt for premature yeast flocculation [94, 97, 100]. It was further shown that removal of the husks from PYF-positive malts could reduce the severity of PYF, while PYF-negative malt husk treated with fungal extracts and commercial fungal xylanase [94] or arabinoxylanases [101] induced PYF. However, the knowledge regarding the formation of PYF factors is still limited. One study profiled the enzymes from the secretome of *F. graminearum* MH1 involved in the degradation of the barley husk [97]. Among the identified metabolic proteins, arabinoxylan degrading hemicellulases were the most abundant, among cellulose and pectin degrading enzymes. It was postulated that *F. graminearum* thus could produce arabinoxylan-based factors that might be later released into the brewing process, hence causing premature yeast flocculation. Findings from this study also revealed that the PYF factor was related to an acidic polysaccharide with a high molecular weight, most likely acidic arabinoxylan. However, it remains unknown whether this specific postulated PYF factor is also produced by other fungal species associated with PYF [97]. It is worth noting that the susceptibility of yeast strains to PYF differs. Flocculent lager yeast strains are sensitive to PYF while non-flocculent lager yeast strains and ale yeast strains (whether flocculent or non-flocculent) are insensitive to PYF [102, 103]. This explains why PYF-positive malts can give rise either to "normal" or PYF worts. Among flocculent lager yeast strains, the degree of sensitivity to PYF also varies with the more flocculent strains exhibiting a higher degree of sensitivity than the less-flocculent strains [103]. Furthermore, the PYF factor is highly resistant to mashing and boiling temperatures during brewing and does not decline during malt storage [104]. Thus brewers ought to pay attention to PYF-positive malts although their propensity to cause PYF can be reduced by blending with PYF-negative malts or by selecting yeast strains that are insensitive to PYF.

C. Impact of (toxigenic) fungal infection of brewing grains

Although different toxigenic fungal species have been detected on barley grain [35, 105], only a few of these have a notable relevance to malt- and beer quality. One of the most notable and prevalent plant-pathogenic fungi known to infect barley, and other brewing cereals, such as wheat and sorghum, is *Fusarium*. This genus contains several species with the main species colonising wheat, barley, sorghum being; *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae*, *Fusarium sporotrichioides*, *Fusarium langsethiae*, and *Fusarium tricinctum* [58, 106]. *Fusarium* infection of barley and other brewing grains, is mainly associated with Fusarium Head Blight (FHB, also known as scab), one of the most economically devastating plant diseases [106, 107]. FHB has been reported to cause economically damaging effects such as a reduction in grain yield, a reduction in the grade quality of the grains due to discoloration and shrivelling of the grains; a reduction in end-use quality due to reduction in grain vigour and contamination with mycotoxins [108]. An impact of the economic loss pertaining to FHB infection of wheat and barley in the United States can be found in [109]. Results of this survey estimated crop yield losses due to FHB infection of $ 1.176 billion for wheat and $ 293 million for malting barley between 2015 and 2016.

Furthermore, contamination of barley grains with *Fusarium* species (mainly *F. culmorum*, *F. graminearum*, and *F. tricinctum*) increases the propensity for beer gushing [51, 110]. Beer gushing is a phenomenon caused by class II hydrophobic fungal peptides (commonly referred to as hydrophobins), mainly produced by *Fusarium* and other filamentous fungal genera such as *Nigrospora* and *Trichoderma* [111, 112]. These hydrophobins serve as nucleation sites for carbon dioxide bubbles in beer, resulting in the spontaneous release of gas and violent over-foaming of beer when a bottle is opened [17, 51]. Hydrophobins are small proteins, (surface-active and amphiphilic proteins in nature) that are secreted by most filamentous fungi to protect the growing hyphal tip, to facilitate their growth at the liquid-air interface [113]. Secretion of hydrophobins can begin as early as three weeks after seeding and can continue throughout the development period of barley while in the field [52]. Although a substantial amount of hydrophobins can be produced in the field, intensive production occurs during steeping and germination as a result of activation of fungal spores and growth of fungal mycelial [52, 111]. Nevertheless, most of the hydrophobins produced during malting are found in rootlets and their removal after kilning substantially reduces hydrophobin concentration in the final malt [114]. The concentration of hydrophobins needed to cause gushing depends on the fungal species [111], with concentrations as low as 3 µg/L, secreted by *Trichoderma harzianum*, being sufficient to induce gushing [115]. The consequences of beer gushing are economically damaging as this defect is irreversible and the final product unmarketable.

Infection of barley, and other small brewing grains, with toxigenic fungi especially of *Fusarium* genus, poses a serious health issue as infection leads to the production of mycotoxins (by-products of fungal metabolism), that undermine the health of humans and animals [116, 117]. A number of these secondary metabolites (mycotoxins) have been detected in barley and malt, and include, deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, HT-2 toxin, zearalenone (ZEN), fumonisin (B1, B2, B3, B4) and diacetoxyscirpenol (DAS), moniliformin, enniatins (A, A1, B, B1) and their respectable derivatives [17, 118–121]. The consequences of ingestion of these mycotoxins are diverse and can vary from short-term to long-term adverse effects on human and animal health. Trichothecenes, like DON, NIV, T-2 toxin are potent inhibitors of protein, DNA and RNA synthesis in eukaryotic cells, resulting in decreased cell proliferation [122]. The structure of ZEN is similar to that of the oestrogen hormone and competitively binds to oestrogen receptors causing alterations in the reproductive tract of laboratory rats, guinea pigs and domestic animals [123]. ZEN is also reported to be genotoxic, immunotoxic and hepatotoxic and nephrotoxic and an enhancer of lipid oxidation [123]. Fumonisins, among which fumonisin B1 is the most toxic, are cancer promoters and have been associated with apoptosis, esophageal cancer and neural tube defects in humans [116, 122] while enniatins, ENNs, are enzyme inhibitors and have been shown to cause inhibition of enzymes, such as acyl coenzyme A, cholesterol acyl transferase, and 30,50-cyclo-nucleotide phosphodiesterase leading to mitochondrial dysfunction [122].

Among the *Fusarium* toxins, DON, (reported as the most prevalent mycotoxin in barley grains globally [124]) is of particular concern to the malting and brewing industries because of its thermal stability (i.e., DON is heat stable under temperature intervals between 170 °C and 135 °C [125])) and water solubility that renders it capable of being transferred from raw materials, barley, and/or malt into the final beer product [126, 127]. The transfer of these mycotoxins from the malt into boiled wort was investigated by [128]. The authors observed that the concentration of DON found in wort samples was twice the initial amount contained in the malt grist meaning that DON was released from the malt matrix into the wort during the mashing process. Although a reduction of DON was observed during boiling, DON levels at approximately 29 – 59.6 % remained in wort after boiling. Apart from posing a threat to human and animal health, the transfer of mycotoxins from malt into the brewing process also poses technological challenges. It has been reported that DON inhibits both top (ale) and bottom (lager) fermenting yeasts [129].

It is also worth noting that, even though some of the *Fusarium* mycotoxins, especially ENNs, might be in low concentrations in beer, they can accumulate to high concentrations in the spent grains during brewing [130]. It was observed that ENNs from malt accumulated in spent grains during the brewing process with low concentrations to below the detection limit registered in collected wort samples [130–132]. This represents a feed safety concern since spent grains are mostly used for animal feed, as a valorisation approach. To guarantee feed safety and protect livestock health, close monitoring of ENNs and other mycotoxins in brewer’s spent grains is warranted.

Besides *Fusarium* mycotoxins, toxins are also produced by other fungal genera such as *Alternaria*, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Claviceps* [133–135]. Of these genera, more attention has been given to mycotoxins originating from *Alternaria* given its relatively high incidence in malting barley [11, 42, 132, 136, 137]. The most commonly detected *Alternaria* mycotoxins are alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, III (ATX-I, II, III) and tenuazonic acid (TeA). The European Food

Safety Authority has raised concern for these mycotoxins because of their genotoxic, mutagenic, and carcinogenic properties [135]; altertoxins I,II,III are mutagenic while tenuazonic acid (TeA) is associated with hematological disorders in humans [136]. *Aspergillus* and *Penicillium* species often produce ochratoxin A (OTA). Exposure to OTA is reported to cause nephrotoxicity in animals [116]. *Claviceps* species are known to biosynthesize ergot alkaloids (EAs), although these mycotoxins appear to be less relevant from a food safety point of view based on published assessments [116].

Fusarium infection of barley has been reported to alter the proteolytic enzyme activity during malting and thus the normal barley grain proteolysis pattern yielding detrimental effects on the quality of the final malt and beer [22, 53]. *Fusarium culmorum* infection of barley resulted in a two-fold increase in the proteolytic activity in comparison to the control sample [53]. The enhanced proteolysis (reflected in terms of increased wort soluble protein and free amino nitrogen) darkens the beer colour and is detrimental to flavour stability as such malts deliver an increased concentration of staling precursors and aldehydes into the brewing process. A higher concentration of acetaldehyde (98 mg/L in comparison to the 7 mg/L in the control beer sample) and Strecker aldehydes were reported for beer produced from in vitro *Fusarium culmorum* infected malt [13]

In sum, it is therefore of importance to control the incidence of *Fusarium* infection and other toxigenic fungal species given their strongly detrimental effects caused to the mother plant and along the value addition chain of the grains.

2 Control of microflora

Suppression of the growth of harmful microorganisms is possible by performing microbial inoculation. Mycotoxin-producing moulds can be inhibited by controlling microbial activity. As high grain moisture, moderate temperatures or high relative air humidity provides the ideal environment for *Fusarium* growth during germination, if alterations are made in accordance with these conditions, it is possible to regulate the microbial activity [138]. The control of the microbial activity might also be done through chemical control where antimicrobials are introduced, i.e., by application of a bacteriocin produced by LAB to reduce the contamination caused by Gram-positive bacteria in the brewing industry [139]. Utilising helpful microbes as biocontrol agents aids in limiting the development of undesirable microorganisms, such as the addition of *Wickerhamomyces anomalus* during steeping to prevent the growth of *Fusarium* on the malt, leading to the reduction in hydrophobin production of *Fusarium*, preventing beer gushing [17]. Starter cultures serve a similar purpose and are used to suppress the growth of undesirable microbes when applied during malting, where several LAB strains have been confirmed to show mould-suppressing effects [140]. Suppression of *Fusarium* growth in barley by field application of lactic acid bacteria (LAB) promoters has been used successfully to avoid such negative consequences [17].

2.1 Control of biofilm-forming microbial species during malting

One of the issues posing for the malting process is the growth of EPS-producing bacteria during the process. In order to prevent EPS formation that negatively affect the filtration of the wort [21], natural starter cultures were proposed [14, 15, 33]. One of the most exploited biological control means for retarding the growth of microbial biofilm-formers during malting, is the addition of lactic acid bacteria (LAB) starter cultures to the steeping water to suppress EPS-producing bacteria. The antagonistic effect of LAB is based both on competition for nutrients and space and the production of various antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins, and low molecular weight antimicrobial compounds [33]. A *L. plantarum* E76 starter culture significantly improved wort filtering performance [15], while the addition of LAB suppressed the formation of exopolysaccharides [33]. Logarithmic reductions of 3 – 4 units were reported for *Pseudomonas* species (a well-known EPS producer) being attributed to the effect of organic acid and pH reduction of the steeping water [33].

Further, there are studies showing that suppression of bacterial communities by LABs promote yeast growth and increase microbial β-glucanase and xylanase production during steeping, which can improve wort filtration behaviour [15]. Lactic acid, acetic acid and phenyl lactic acid, the main metabolites of LAB, also possess an inhibitory effect on filamentous fungi, reducing mycotoxin accumulation [49]. The use of lactic acid bacteria as a starter culture during malting can also reduce the pH value of malt, increase wort buffer capacity, increase amylase activity, promote the hydrolysis of starch, decrease wort viscosity and β-glucan [49]. However, maltsters may prefer to increase the steep water pH to leach out proanthocyanidins, with improvements for flavour and colloidal stability of the beer [141] and also reduce DON and *Fusarium* biomass [54].

2.2 Control of *Fusarium* growth during malting

From a malting perspective, a small percentage of infected grains within a batch can easily cause detrimental effects on the final malt quality. Different control measures have been employed in the control of *Fusarium* infection of wheat, barley, and sorghum in the field such as the development of plant varieties resistant to FHB, the application of fungicides and the use of plant cultivars that show passive resistance to FHB infection [142]. Further, the usage of biocontrol agents that have antagonistic activity against *Fusarium* spp. and crop management practices such as crop rotation and tillage have been employed [142]. Among these strategies, genome manipulation has had so far only limited success incorporating fungal resistance into wheat and barley varieties [109]. For post-harvest control, current research has been directed towards screening for biological agents to control the development of toxigenic fungal species, especially during steeping, and thus suppress the production of mycotoxins. In particular, for DON control, maltsters normally start the malting process with barley grains having DON levels < 0.5 mg/kg [143], as DON levels generally decrease during steeping due to the washing effect of steeping water and remain low to below the detection limit in the final malt (see discussion of DON under unfavourable effects of microbiota). However, this is not always the case. Using artificially inoculated and or infected barley grains with *Fusarium* spp., DON levels were recorded to decrease during steeping and then increase in the final malt to levels higher than those detected in the raw grains [144, 145]. This latter case portrays a case scenario of harvest years when FHB is rampant. Research into methods to suppress the growth of *Fusarium* during malting and also inhibit the biosynthesis of mycotoxins constitute a way for the utilisation of such crops during bad harvest years.

A. Use of essential oils as novel antifungal agents/biofungicides in malting

One such emerging method is the application of essential oils (EO) as oil-in-water nanoemulsions during the first steeping step to suppress the growth of *Fusarium* and inhibit the biosynthesis of mycotoxins during malting. So far two studies have successfully demonstrated this proof of concept. Application of a hop essential oil (HEO) nanoemulsion during the first steeping phase was effective in suppressing fungal growth and production of DON in naturally FHB infected barley grains at all stages of the malting process [146]. A similar study reported that clove oil Tween 80-stabilised nanoemulsion was also effective in reducing the growth of *Fusarium graminearum* and suppressed the production of DON at all stages of malting while having a negligible influence on the germinative energy of the barley grains [143].

The antifungal activity of these essential oils is mainly associated with their chemical constituents [146]. It is generally proposed that these essential oils and their constituent bioactive compounds inhibit microbial growth by; (i) alternation of the cell wall composition i.e., lipid synthesis, fatty acid composition; (ii) disturbance of cytoplasm membrane permeability and integrity, and reduction of proton-motive force (PMF) of bacteria [146]. In sum, these successful trials shed light on the future use of essential oil-in-water nanoemulsions in the control of mycotoxin production in FHB-infected barley grains during malting. Limitations in applicability of this technology are due to the allelopathic effects of essential oils on seed germination (essential oils normally decrease seed germination) and the flavour compounds from the essential oil transferred to malt that might have a negative impact on the sensory profile of the beer produced thereafter.

B. Use of biological control agents as starter cultures in malting

Over the past decades, research efforts have been dedicated to screening for potential microbial candidates to be used as biocontrol agents in the management of toxigenic fungal growth during malting. The use of such biopreservation methods in the food industry has been mainly driven by the growing consumer demand for clean-label food products that are perceived to be natural and eco-friendly [147]. Several fungal (i.e., *Geotrichum candidum*, *Aureobasidium pullulans*, *Candida saitoana*, *Candida sake*, and *Wickerhamomyces anomalus*) [148] and bacterial (i.e., *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus amylovorus*, *Lactobacillus amylolyticus*) strains have been studied in past years for their potential antifungal or antibacterial effects and their subsequent effect on enhancing particular malt characteristics [33, 149, 150]. Among these candidates, researchers have focused greatly on lactic acid bacteria (LAB). Given that LAB have a GRAS (Generally Recognized as Safe) status, they have been widely exploited in the food and feed industry because of their biopreservation capabilities [151, 152]. The biopreservative capacity of LAB is attributed mainly to the synergistic interaction between its several fermentation end-products such as lactic acid, acetic acid, proteinaceous metabolites (i.e., cyclic di-peptides), carbon dioxide, hydrogen peroxide, 3-hydroxyl fatty acids, propionate, phenyl-lactate,hydroxyphenyl-lactate (OH-Phenyllatic acid), Benzoic acid, ethanol, and bacteriocins (i.e., acidocin, sakacin, nisin, reuterin, enterocin, and pediocin) [140, 152–155].

As earlier discussed (see under unfavourable effects of microbiota), growth of *Fusarium* during malting leads to production of lower quality malt products associated with mycotoxin contamination, premature yeast flocculation, and high proteolytic levels. Suppression of *Fusarium* growth and reduction of mycotoxin production during malting is therefore of paramount necessity to ensure food safety and the production of high-quality food products. Species from *Lactobacillus* have been most exploited for their antifungal effect in the malting industry [153]. These LAB species are part of the natural microflora colonising the barley grains and some of the recent potential LAB species that have been screened for their potential application in the management of fungal contamination and mycotoxin production in industrial-scale malting include, *Lactobacillus brevis* R2Δ [140], and *Lactobacillus reuteri* R29 [156]. The addition of *Lactobacillus reuteri* R29 cell-free-supernatant (obtained from a 3 °P wort substrate) was effective in suppressing *Fusarium* growth and DON mycotoxin production by more than 33 and 58 %, respectively [156]. The authors attributed the antifungal effect of *L. reuteri* R29 to the synergistic effect of lactic acid, phenyllactic acid (PLA) and benzoic acids since these were the major metabolites detected in the cell-free-supernatant. Similarly, the application of acid-fermented worts of *Lactobacillus brevis* R2Δ as starter cultures during steeping and germination was demonstrated also to be effective in controlling the growth of *Fusarium* and mycotoxin production [140]. In this study, it was also highlighted that the delivery system of the inoculum onto the barley grain during malting is of importance. The authors demonstrated that the addition of an acidic substrate (cell-free-supernatant obtained after fermentation of wort with a LAB strain) combined with living cultures of *L. brevis* R2Δ, was more effective in suppressing *Fusarium* and reducing the mycotoxins load of artificially contained barley grains with *Fusarium culmorum* than when only acids or pasteurised wort or merely LAB cultures were applied. LAB strains can reduce mycotoxin in infected grains by inhibiting their biosynthesis in fungi (i.e., via the production of "anti-mycotoxigenic" metabolites during LAB growth) and also by binding mycotoxins to their cell wall structures [157, 158]. This suggests that whereas bacterial metabolites are vital for inhibiting fungal growth, the presence of LAB cells in the inoculum is essential to enhance mycotoxin reduction.

Although LAB starter cultures have been reported to enhance malt processability by improving the wort filterability [12], they can also reduce grain germination [140, 159]. The metabolites produced by LAB such as organic acids can interfere with grain metabolism and slow down germination [159]. A slow germination could also lead to suboptimal production of relevant brewing enzymes. Findings from a study conducted by [159] showed that the application of *Lactobacillus plantarum* starter cultures during malting led to lower alpha-amylase, limit dextrinase, and β-glucanase enzymatic activities than the control. It is noteworthy that the bioprotective potential of LAB start cultures and their impact on malt quality is highly influenced by the LAB strain used as different LAB strains produce different antifungal compounds and possess different endo-protease, xylanase, and β-glucanase activity potential [33, 140, 150, 153, 160].

Besides LAB, *Pythium oligandrum* has also been evaluated as a potential biocontrol agent in the malting of wheat and barley. The fungicidal potential of *P. oligandrum* has been demonstrated in various crops such as peas, cucumber, tomatoes, pepper, sugar beet, wheat, rice, strawberry, and tobacco where it has been applied to control the growth of fungal pathogens such as *Pythium ultimum*, *Fusarium oxysporum*, *Phytophtora parasitica*, *Verticillium dahliae*, etc. [reviewed in 153]. The biocontrol properties of *P. oligandrum* are attributed to its ability to directly interact with fungal pathogens through distinct or combined modes of actions such as mycoparasitism, antibiosis, nutrient and space competition, and/or indirectly by inducing resistance in the plants [161, 162]. The mode of attack elicited by *P. oligandrum* depends on the target fungal pathogen [161]. The most common mode of attack on fungal pathogens is via mycoparasitism. *P. oligandrum* achieves mycoparasitism by actively growing along the hyphae of the host species and secreting hydrolytic enzymes such as chitinases, cellulases, endo-glucanases, and proteases that degrade the cell wall of the host species [161, 162]. This allows *P. oligandrum* to penetrate inside the host and kill the cell [161]. *P. oligandrum* can also destroy pathogenic microbes via antibiosis. This is defined as the specific interaction in which the prey is destroyed by toxic secondary metabolites produced by antagonistic microorganisms [161]. However, this is not a common mode of action used by *P. oligandrum* on fungal or yeast pathogens as oomycetes display a limited ability to produce secondary metabolites [162]. In the malting industry, a few studies have so far shown the potential use of *P. oligandrum* as a biofungicide in the management of fungal growth and mycotoxin production. [163] demonstrated that the application of 1 g of *P. oligandrum*/100 ml of water in the third steeping step was more effective in suppressing the growth of three *Fusarium* species (*F.culmorum*, *F.oxysporum*, and *F.graminearum*) in naturally infected barley during the malting process than *Geotrichum candidum*. In a similar study, it was reported that application of 0.6 – 1 g of *P. oligandrum*/ kg of wheat before steeping was effective in restricting the growth of *Fusarium culmorum* and production of mycotoxins in artificially infected wheat with *Fusarium culmorum* [164]. The authors documented reduction levels above 86 % for both *Fusarium culmorum* contamination and the mycotoxins DON and its modified form, deoxynivalenol-3-glucoside (DON-3G). Furthermore, the final malts obtained using the *P. oligandrum* starter culture had a lower wort viscosity and lower free amino nitrogen (FAN) and soluble nitrogen than the untreated wheat malt. Production of malts with reduced levels of soluble nitrogen (free amino nitrogen and soluble protein) could be advantageous for the brewing industry as such malts could deliver fewer staling precursors into the brewing process which is pivotal for enhancing the flavour stability of beer and prolonging its shelf life [4]. However, in a different study conducted by [165], the authors reported nonsignificant differences in the wort viscosity and free amino nitrogen in barley malt using a starter culture of *P. oligandrum*. This discrepancy was attributed by the authors to the difference in the morphology of barley and wheat; wheat lacks the thick barley husk [166]. Further, the thick barley husk could have provided shelter to fungal hyphae thus shielding them from the mycoparasites [165]. This could also explain the lower efficiency levels (35 – 59 %) reported for suppression of fungal growth in the production of barley malt using *P. oligandrum* in comparison to those that were obtained with wheat malt [165]

Another emerging biocontrol agent for fungal growth and reduction of mycotoxin production during malting is *Erwinia gerundensis*. *Erwinia gerundensis* is a motile, non-spore forming and facultatively anaerobic bacterium first isolated from pear tree leaves in Spain [167] although also naturally occurring on barley kernels [168]. The potential application of using *Erwinia gerundensis* strain as a biocontrol agent for controlling fungal development and mycotoxin production during the malting process of barley was demonstrated by [168]. The authors observed that the application of the bacterial strain at the beginning of the steeping step reduced the fungal load of the final malt by 67 – 72 % and its ENN and DON contents by 32 – 50 % and 15 – 33 % respectively depending on the dosage rate of the inoculum. The anti-mycotoxin effect was attributed to several mechanisms such as competition for resources and space, inhibition of ENN biosynthesis pathways, and transformation of ENN into non-toxic metabolites. However, a few limitations were highlighted in their study; (i) *E. gerundensis* showed varying effectiveness in suppressing the growth rate of different strains of *Fusarium tricinctum*. For instance, the growth of *F. tricinctum* 2399 was reduced by 82 % while that of *F. tricinctum* 2502 was unaffected by *E. gerundensis*. (ii) the deoxynivalenol-3-glucoside (DON-3G) content was higher for the *E. gerundensis* treated samples in comparison to the untreated samples. Also increasing the concentration of *E. gerundensis* in the inoculum from 10^4 to 10^{10} led to an increase in the T-2 + HT-2 toxin concentration. This was suggested to possibly be an effect of the high concentrations of the biological agents eliciting a stress response in *Fusarium* species resulting in an enhancement of secondary metabolite production (mycotoxin). Nevertheless, in a validation experiment using a two-row spring barley cultivar that was naturally contaminated by ENN and T-2 & HT-2 toxins, the authors showed that at the optimal concentration dosage of 4×10^4 CFU/kg applied at the beginning of the steeping step, *E. gerundensis* was effective in reducing the concentration of ENN, T-2 & HT-2 toxins by approximately 67 % and 69 % respectively. It is also worth noting that in this study the steeping temperature range used (16 – 18 °C) was not within the favourable growth temperature range (20 – 45 °C) of *E. gerundensis* since it is a mesophilic strain. Higher steeping temperatures (~ 20 °C) could increase the effectiveness of *E. gerundensis* in suppressing fungal growth and also its mycotoxigenic effect.

3 Detection and quantification of microbial communities using meta-omics approaches

So far, most studies relied on culture-based methods to profile the microbial communities associated with barley and malt or quantitate a specific microorganism of interest. The main limitation of these culture-based methods is that more than 99 % of microorganisms in any given environmental sample observed under the microscope are not cultivable by the use of standard plating methods [169]; Some microbes are strictly anaerobic and will not be cultured in the presence of oxygen; Some microbes display a slow growth rate such that visible colonies do not appear within the normal incubation period of a few days; Some require cross-feeding or metabolic cooperation between species for the provision of nutrients and some require community communication through a network of signals which can only be found in natural ecosystems [170–172]. This means that the vast majority of microbial communities found in environmental samples (characterized by diverse and complex microbial interactions) have not been obtained in pure culture to allow their characterization [171]. With the use of cultural plating techniques, only a limited diversity of the microbial communities associated with ungerminated and germinated barley can be achieved. This in effect, hampers our understanding of the evolution of these microbial species during the industrial malting process which is vital for process control and optimization. In recent years, there has been a tremendous advancement in the omics and sequencing technologies that have birthed a new era of microbial ecology. Most of the molecular techniques used in characterizing the microbial community are based on direct isolation and analysis of nucleic acids, and proteins and include genetic fingerprinting, metagenomics, metaproteomics, and metatranscriptomics [173]. These techniques circumvent the need for isolation and cultivation of microbial species and are vital for discovering and cataloguing microbial diversity and understanding their interaction with the brewing grains and their impact on the quality of malt. In the following, current meta-omics approaches for the comprehensive characterization of microbial communities (microbiome) will be briefly presented. First, metagenomics using next generation sequencing (NGS) is discussed, followed by metatranscriptomics and -proteomics approaches, including their individual advantages and limitations.

3.1.1 Metagenomics

Metagenomics using high-throughput NGS is a recent approach for the identification of members of microbial communities. For this, genetic material is harvested directly from the samples, eliminating the need for microbial cultivation. This is of major advantage for the identification of microbes which are viable but cannot be cultivated using classical plating methods. Two approaches are commonly employed: 1) PCR based reporter gene amplification and sequencing (also called DNA-metabarcoding) and 2) direct sequencing of the entire microbial DNA (shotgun metagenomics). For the PCR based methods, there are several drawbacks: The chosen primer pairs for reporter gene amplification can introduce biases regarding the identified phyla, and different (universal) primer pairs are recommended for *Archaea*, *Bacteria* and *Eukarya* 16S/18S rDNA sequencing [174]. Further, care has to be taken when amplifying a mixture of reporter gene templates, as primers can exhibit preferential binding and might distort the relative reporter gene quantities in the final product leading to distorted microbial quantification [175], also caused by the self-annealing of abundant templates towards the end of the PCR [175]. A further complication is that different organisms contain different copy numbers of the 16S gene, which biases microbial abundance estimation and should thus only be employed for closely related taxa [176]. But not only the abundance of the individual microbes can be overestimated, the number of the members of the microbial communities can be artifactually inflated by errors which are introduced in reporter amplicon sequences by the employed DNA polymerase [177–179]. A major factor contributing to this is the generation of chimeric amplicons, stemming from unfinished PCR products acting as primers for a heterologous sequence. These resulting amplicons consist of the target gene sequence of more than one species, which can lead to the spurious identification of novel organisms [180]. Another source of overestimation of the sample microbial complexity is introduced by the formation of heteroduplex molecules which can lead to mosaic sequences in the amplicons [181]. These error sources therefore necessitate that identified 16S sequences are grouped into operational taxonomic units (OTUs), and the resulting classification that > 95 % sequence identity represents microbes from the same genus, while > 97 % identity can be regarded as the same species [182].

16S sequencing is most commonly performed using short read techniques (e.g. Sanger sequencing, Illumina), which have an upper read limit of around 600 bases, resulting in the analysis of only small subregions of the 16S gene. While the bacterial 16S gene is around 1500 base pairs in size and is composed of 9 hypervariable regions [183]. For taxonomic identification using short read sequencing techniques usually primers spanning either single or several regions (e.g. V4, V6, V1-V3 and V3-V5). However, usage of these subregions will cause bias in the identified taxa and is only sufficient for identification at genus level or above and further, cannot resolve intragenomic 16S variants [184]. These drawbacks are overcome by whole 16S gene [184] or even 16S-ITS-23S gene sequencing [185] using long-read techniques like PacBio or Oxford Nanopore. Together with consensus [186, 187] and de-noising algorithms [188], single nucleotide resolution is achievable [189].

As mentioned above, intragenomic 16S variants and variable gene copy numbers between microbes make an accurate estimation of the abundance of the identified taxa difficult. This can be overcome by shotgun metagenomic sequencing, where the PCR amplification is omitted, and short-read [190] as well as long-read techniques can be employed [191]. An important parameter which needs to be decided on using this technique is the sequencing depth: Shallow sequencing consists of around 0.5 million sequences per sample, deep sequencing of around 10 million [192, 193] and ultra-deep sequencing can reach 2.5 billion sequences [194]. Interestingly, shallow sequencing has lower technical variance and a higher taxonomic resolution than 16S sequencing and at lower cost than deep sequencing [195], while there was a 97 % correlation with species composition and KEGG Orthology group profiles in comparison with ultra-deep sequencing [194]. However, an important parameter associated with sequencing depth is coverage, i.e. to what extend the present microbiome is identified [196]. Nonetheless, there are several challenges associated with this technique: Contamination with foreign DNA (e.g. human), highly abundant host DNA (e.g. barley), problems with taxa quantification [197] and the inability to distinguish between alive and dead microbes as well as no information about actual gene expression, which is the field of metatranscriptomics and metaproteomics.

3.1.2 Metatranscriptomics

Metatranscriptomics studies the set of expressed genes via identifying and quantifying their respective mRNA or cDNA products [198]. This gives insight into activated metabolic pathways of individual taxa at the point of sampling [198–200]. However, this technique has several innate challenges: The extraction of high-quality mRNA is of utmost importance as mRNA is degraded easily [201], which is further complicated by the fact that in contrast to the genome, the transcriptome is highly dynamic and sampling strategies and sample preparation protocols need to account for this [202]. For metatranscriptomic studies it is also important to consider that different taxa exhibit different susceptibility to cell lysis and thus RNA extraction efficiencies. This can lead to wrong species abundance estimations.

Another factor is that highly abundant ribosomal RNA which can make up over 80 % of total cellular RNA [203] requires depletion prior analysis to improve sequencing depth [204]. Further, when studying prokaryotes, eukaryotic host or contaminating mRNA can be depleted by poly(A)-tail capture/removal [205]. For the actual transcriptomic analysis, two workflows currently exist, one employing reverse transcription of mRNA into cDNA and PCR amplified before (short read) sequencing, the other omitting these steps and using a direct, long read sequencing shotgun approach. One major drawback of the first method is that cDNA amplification by PCR introduces biases, e.g. sequences rich in Adenine/Thymine or exhibiting very high Guanine/Cytosine content are often poorly amplified [206, 207] or the amplification of sequence errors towards the later cycles of the PCR (see also the Metagenomics paragraph above). These drawbacks are overcome by direct, long-read RNA sequencing, while additionally offering identification of RNA base modifications and information about splice variants [208, 209]. However, usage of the Nanopore technology has some inherent drawbacks, e.g. uncertainty about the terminal 10 – 15 bases of the 5' end of the mRNA [209], and difficulties with erroneous detection of indels and substitutions in homopolymeric regions [210] , however newer versions of the Nanopore flow cell design have improved on this [211]. While metatranscriptomics is capable to identify and quantify mRNA transcripts, it cannot answer the question if these transcripts are translated into protein, which is one of several reasons [212] behind the surprisingly low correlation of transcriptomics and proteomics quantification data (Spearman correlation rank $\rho \sim 0.4$) [213]. For the elucidation of these questions metaproteomics is a useful technique.

3.1.3 Metaproteomics

The proteome is defined as the set of expressed proteinogenic gene-products of a cell or the set of proteins inherent to a system under specified conditions (e.g. time, temperature, osmolarity, ...). Thus, metaproteomics aims to identify and quantify the dynamic set of all proteins belonging to a microbiome. This poses some inherent challenges: 1) The sample preparation strategy needs to prevent artifactual changes to the proteome to avoid misleading results, 2) protein extraction strategies need to account for the fact that some microbes (e.g. yeasts) are very resistant to cell lysis, 3) there might be a massive cross-contamination with host (e.g. barley) proteins which leads to 4) massive undersampling of microbial peptides, necessitating the usage of pre-fractionation methods [214] and 5) not all peptides are unique to their host species making the identification of taxa and their protein expression difficult. This problem is furthered by the computational methods for the identification of peptides derived from LC-MS/MS approaches. For peptide identification, the theoretically expected masses of peptides derived from amino acid sequence databases (FASTAs) are matched to the experimentally derived mass spectra. The quality of the match is then expressed as a numerical value [215, 216] which then can be further transformed into a probability of the match being a random event [217, 218] , or the probability of a correct match [219, 220]. However, these probabilities are estimated by the usage of a decoy database which (normally) contains the reversal of the amino acid sequences found in the target FASTA [221]. For this, spectra matching to entries in the decoy FASTA are used to calculate false-positive discovery rates of peptide identification.

This poses a problem for metaproteomics, as the construction of the target FASTA containing all relevant protein sequences requires the knowledge of the set of taxa contained within the sample. In case the target FASTA contains only a small subset of the sample taxa, a mass spectrum can be assigned a wrong peptide sequence with an excellent matching probability. As the real sequence, with an likely better matching probability, is not contained in the target database, a too sparse FASTA leads to a false positive peptide identification. However, the opposite problem arises if the target FASTA contains large numbers of protein sequences of taxa which are not part of the microbial sample. As the target FASTA is too vast, spurious identification will increase the portion of false-positive peptide identifications (loss of specificity) [222], but the decoy FASTA will also be inflated. This means the probability of experimentally derived spectra matching peptide sequences in the decoy FASTA is inflated as well, leading to distorted peptide false discovery rates with the consequence of the introduction of potentially large numbers of false-negative identifications (loss of sensitivity) [223]. In conclusion, for metaproteomics a well-curated target FASTA needs to be available and for which prior metagenomic identification of the expected taxa in the sample can be employed.

An additional problem for metaproteomic approaches is that, as mentioned above, the identified peptides might not be unique to taxa. To overcome this problem an approach to derive the so-called lowest common ancestor (LCA) was introduced [224]. The LCA is defined as the lowest taxonomic lineage to which the identified peptide sequence is unique. By this, peptides, and by extension, proteins can be assigned to their LCA, and functional contribution of the related taxa can be made. Further, by using tandem mass tag labelling, peptides from several samples can be multiplexed and quantified to increase the throughput of metaproteomics approaches [225, 226]

4 Conclusions, Future Directions and Perspectives

In conclusion, a great amount of research has been conducted to unravel the microorganisms associated with barley during germination. This has led to important contributions regarding their role in malt quality (e.g. wort filterability) and their control, especially in view of suppression of mycotoxin production. However, it is clear that simple identification of the members of the microbiome does not give robust insight into the function of these microbes during malting. In recent years, the advent of the so-called meta-omics techniques offers the promise that the microbiome can not only be comprehensively identified, overcoming the problem of the viable-but-nonculturable microorganisms, but also the expressed genes and produced proteins can be quantified. This gives insight not only into the functionality of the microbes but also the potential to steer malting processes towards the production of beneficial microbial products, e.g. glucanases and xylanases, while controlling the suppression of substances detrimental to the final malt quality (mycotoxins, gushing- and PYF factors), not only by enumeration of the associated microorganisms, but by quantification of the involved gene products. This will lead to in-depth insights into the individual contributions of the involved taxa onto the quality of the produced malt and their control not only in terms of numeric abundance, but also in-situ active (beneficial and detrimental) biological pathways.

Finally, using non-culturing dependent techniques will also allow to study microbial community interactions during germination, not only in their negative forms (amensalism and antagonism) to e.g., supress unwanted microorganisms, but also in their positive form (commensalism and synergism) to study their influence onto the quality of the produced malt and downstream applications, but also to give more accurate microbe enumeration due to the absence of plating specific biases. This will open the door to study the species-level resolved barley and malt production/storage microbiome as a function of barley cultivar, geographical location, weather conditions, the microbes introduced by the malting plants, as well as by the storage and processing facilities of the breweries themselves.

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Supplementary figures

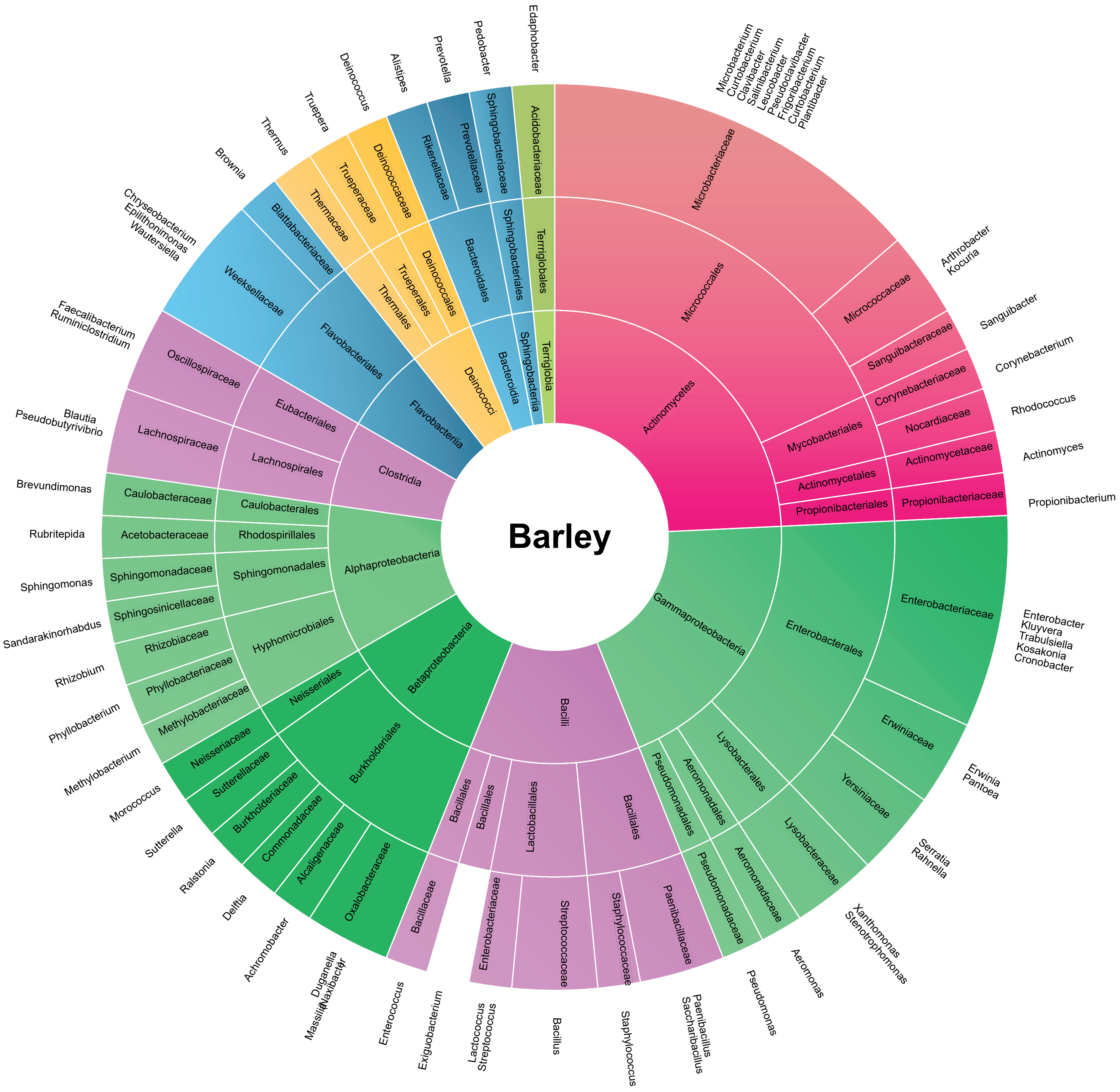

Fig. 1 **Taxonomic classification of the bacterial genera identified on/in raw barley using molecular techniques [16, 24–27, 33, 34, 36–38, 55, 56, 213]. From the centre towards the outside of the chart, the taxa are represented as the class, order, family and then the genus. The colours are coded according to the Phyla; Classes; Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria belong to Phylum Pseudomonadota; Classes; Bacteroidia, Flavobacteriia, and Sphingobacteriia belong to Phylum Bacteroidota; Classes Clostridia and Bacilli belong to phylum Bacillota; Class Actinomycetes to Phylum Actinomycetota; Class Deinococci to phylum Deinococcota, and Class Terriglobia to Phylum Acidobacteriota. The white colour mean no rank at the family level for that genus. N.B; The following phyla and genera were known formerly as: Phyllum Bacillota as Firmicutes; Phyllum Pseudomonadota as Proteobacteria; Genus *Candidatus Spencerbrownia* as *Brownia*; Genus *Massilia* as *Naxibacter*. The taxonomy classification of all the represented genera was obtained from the National Center for Biotechnology Information (NCBI) taxonomy browser (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi)**

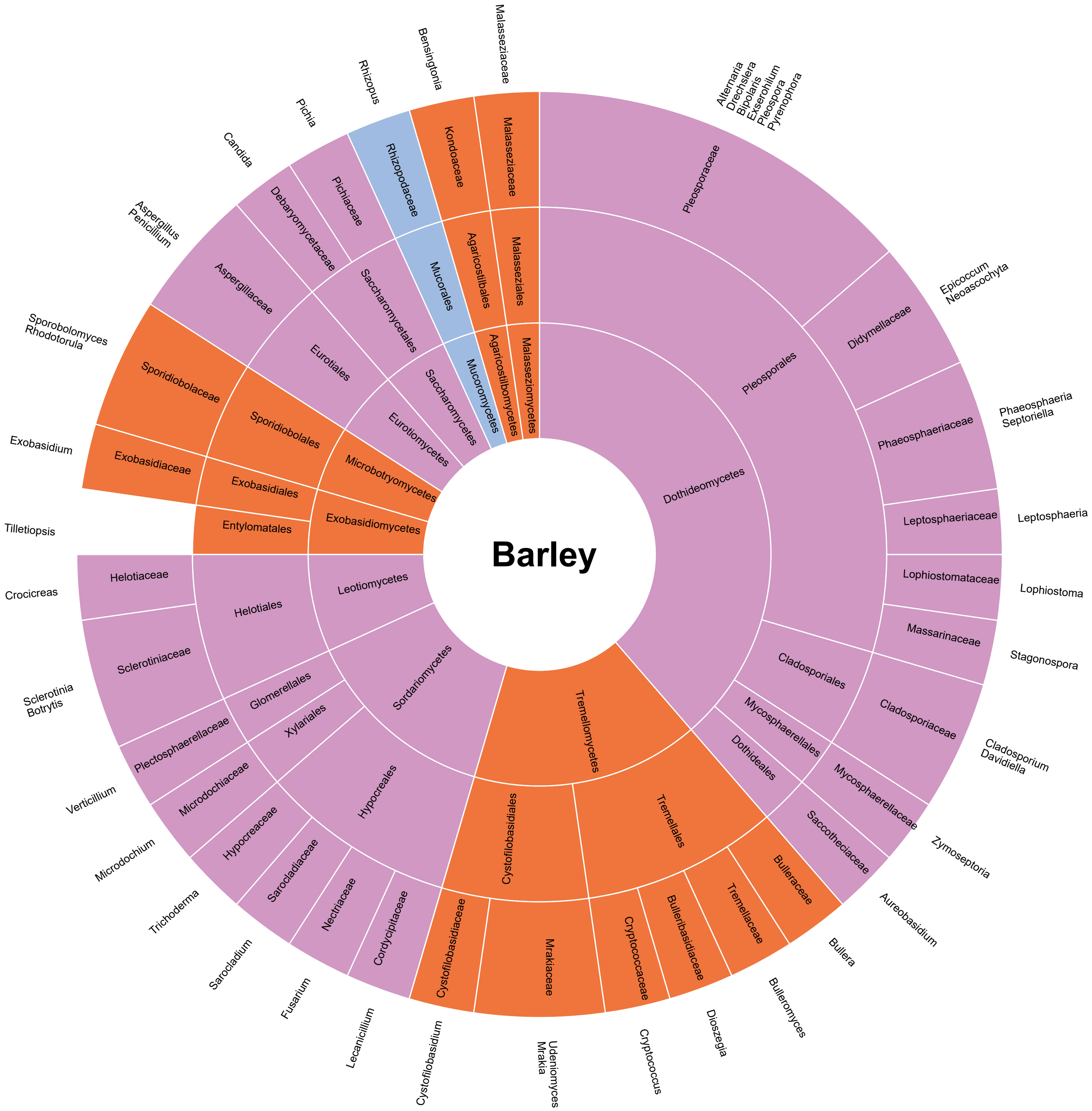

Fig. 2 **Taxonomic classification of the fungal genera identified on/in raw barley using molecular techniques [16, 24, 27, 36, 41, 214]. From the centre towards the outside of the chart, the taxa are represented as the class, order, family and then the genus. The colours are coded according to the Phyla; Classes Saccharomycetes, Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Leotiomycetes belong to Phylum Ascomycota While classes Microbotryomycetes, Malasseziomycetes, Tremellomycetes, Agaricostilbomycetes, Exobasidiomycetes, belong to Phylum Basidiomycota; while Class Mucoromycetes belongs to Phylum Mucoromycota. The white colour means no rank at the family level for that genus. N.B. The following fungal genera were formerly known as: *Bipolaris* as *Cochlibolus*; *Exserohilum* as *Setosphaeria*; *Fusarium* as *Gibberella*; *Septoriella* as *Vagicola*; *Mrakia* as *Mrakiella*.**

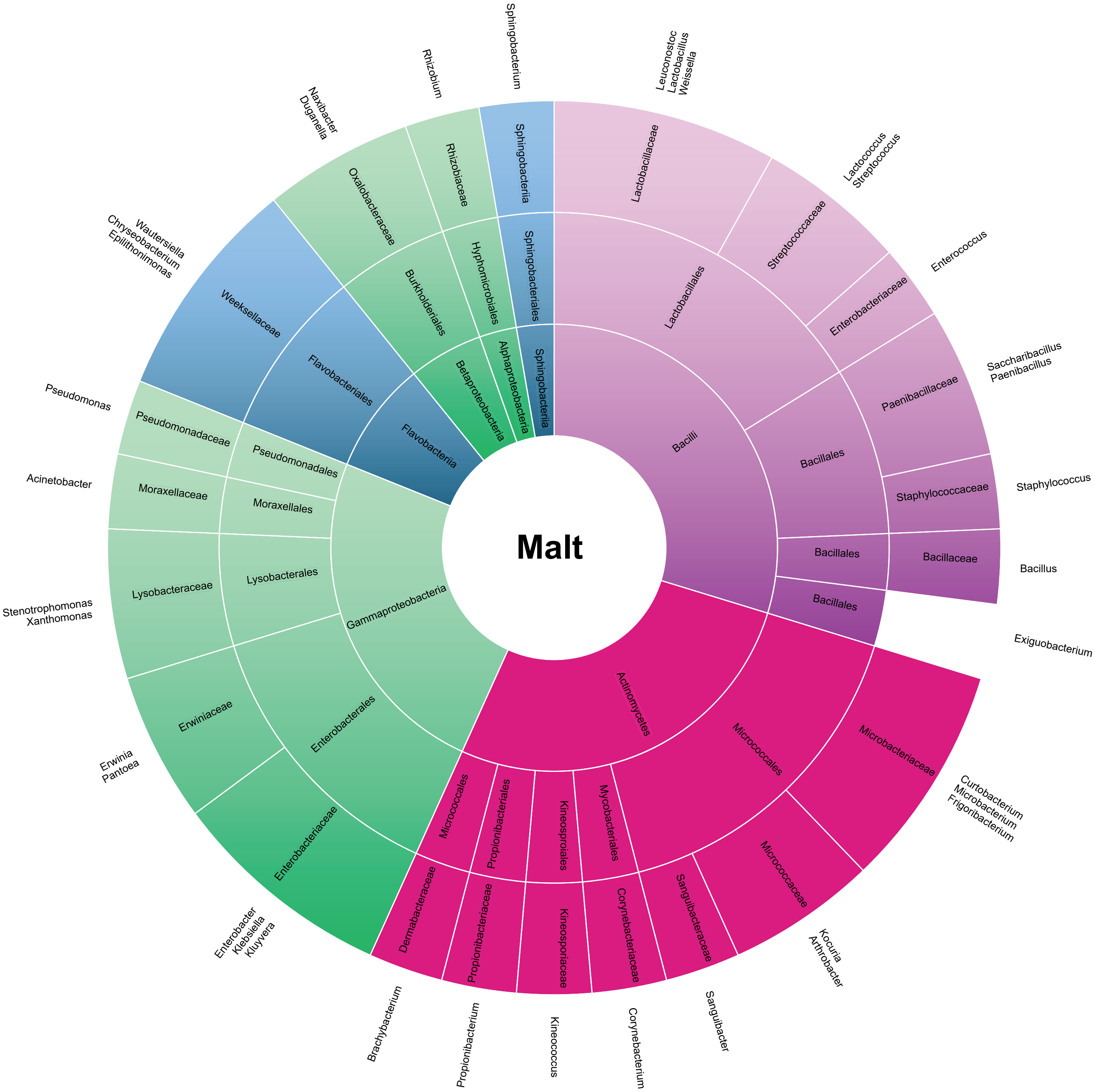

Fig. 3 Taxonomic classification of the bacterial genera identified on/in barley malt using molecular techniques [16, 24, 25, 41, 55]. From the centre towards the outside of the chart, the taxa are represented as the class, order, family and then the genus. The colours are coded according to the Phyla; Classes; Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria belong to Phylum Pseudomonadota; Classes; Flavobacteriia, and Sphingobacteriia belong to Phylum Bacteroidota; Class Bacilli belong to phylum Bacillota; Class Actinomycetes to Phylum Actinomycetota. The white colour means no rank at the family level for that genus.

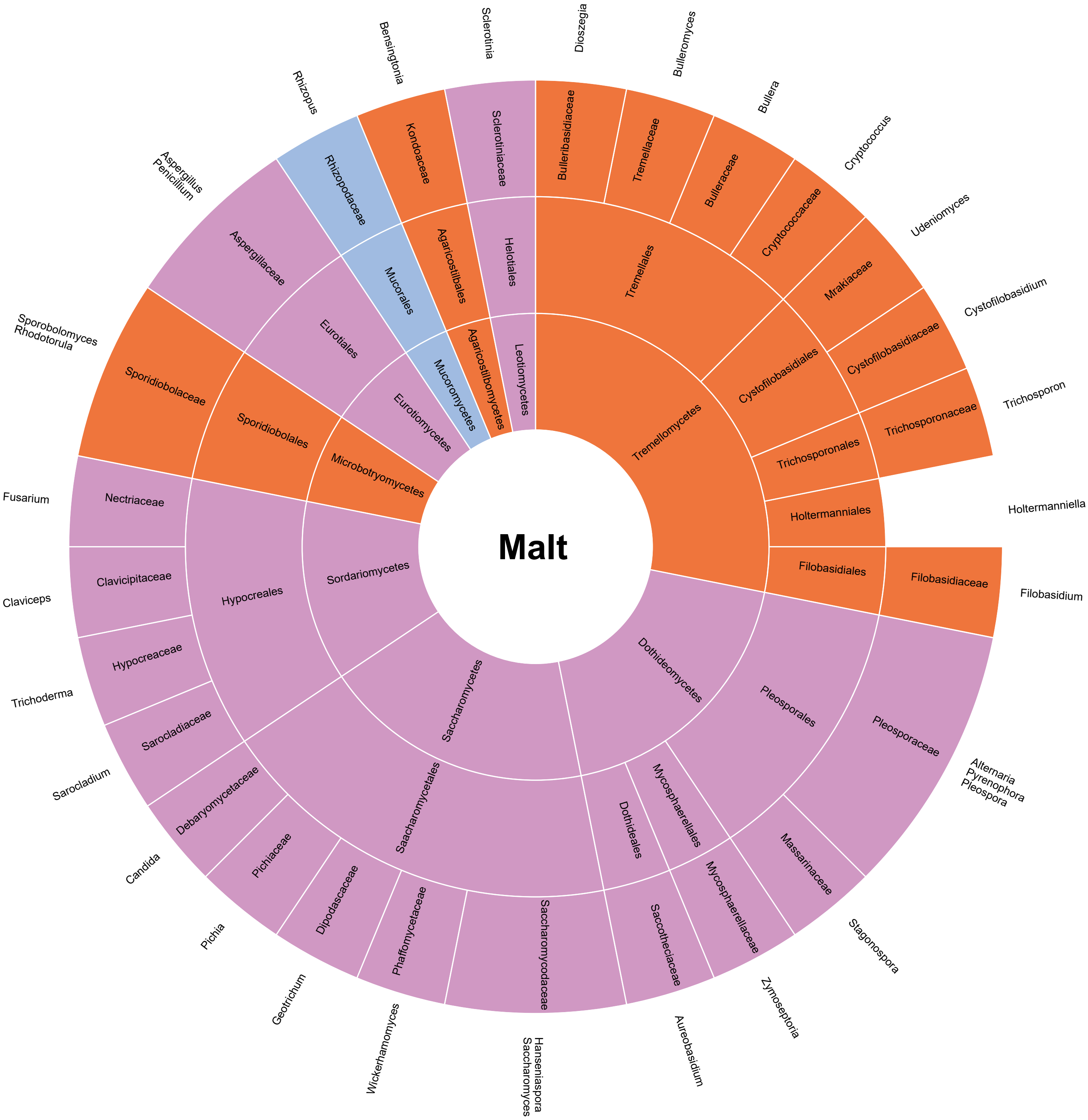

Fig. 4 Taxonomic classification of the fungal genera identified on/in barley malt using molecular techniques [16, 27, 41, 55, 214]. From the centre towards the outside of the chart, the taxa are represented as the class, order, family and then the genus. The colours are coded according to the Phyla; Classes Saccharomycetes, Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Leotiomycetes belong to Phylum Ascomycota While classes Microbotryomycetes,, Tremellomycetes, Agaricostilbomycetes, Exobasidiomycetes, belong to Phylum Basidiomycota; Class Mucoromycetes belongs to Phylum Mucoromycota. The white coded colour means no rank at the family level for that genus. N.B. Genera Pichia was formerly known as *Issatchenkia* or *Hansenula*.