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Microflora during Malting of Barley: Overview and Impact on Malt Quality

Malt is an important industrial product with a huge market outlet. The diverse microbial communities naturally colonizing barley grains greatly influence malt quality and subsequently other products in the malt value chain, in particular beer. In this manuscript, an overview is given of current knowledge of barley and malt-derived microorganisms and their impact on malt properties. In addition, emphasis is put on strategies to enhance the malting process, including the use of starter cultures and the management of endogenous microflora. Molecular studies on microbial community composition and function throughout the process will contribute to efficient implementation of these strategies, ultimately leading to more efficient wort production and enhanced beer flavour quality and stability. More specifically, these studies may lead to the discovery of novel, industrially important microbial strains or enzymes.

Descriptors: microbial characterisation, microbial community, microflora management, process optimisation, starter culture

Introduction

Malted barley is the major raw material used in brewing of most beers. Of the total malt production, approximately 90 % is produced from barley. About 94 % of malt is used for making beer, 4 % for distillation and 2 % for other food processes. Malt production (or malting) is a complex biological process involving many biochemical and physiological reactions in which the microbial communities colonizing barley grains play a crucial role. The malting environment can therefore be considered as a complex ecosystem involving two metabolically active components: the germinating grains and the microbial communities colonizing the grains. The main goal of malt production is to produce enzymes that are capable of degrading the grain macromolecules into soluble compounds [6]. Malting technically involves three steps: steeping, germination and kilning. After cleaning and calibration of the kernels, the grains are submerged and aerated until a water content of 42–46 % is reached. This process is called steeping. In general, water temperatures of 10–15 °C and steeping times of 24–48 hours are used. The grains are then allowed to germinate under humid and aerobic conditions at 16–20 °C for 3–6 days resulting in enzymatic breakdown of endosperm cell walls and proteins. During germination, temperate aeration through the grain bed is used to control the temperature. In addition, the grain bed is turned regularly to avoid temperature gradients and entangling of barley rootlets. Germination is ended by controlled drying the grains (kilning) for approximately 21 hours at temperatures increasing gradually from about 50 to 85 °C or more, depending on the equipment and type of malt. Kilning stops the biochemical reactions and ensures stability and storability of the dried product (final moisture content 3–4 %). During this step several colour and flavour compounds are produced via Maillard reactions, thereby influencing the characteristics of the final beer [49]. In addition to the germinating grains, a diverse microbial community including bacteria, yeasts and fungi [26, 45, 47, 54] represents a second metabolically active compound in the malting ecosystem. Microbes greatly affect malting performance and malt quality and thus have an important impact on beer quality. Depending on the nature and extent of the microbes present, their effects may be either beneficial or disadvantageous to the process and/or the final product [12, 20, 26, 42, 48, 51, 79, 81, 82]. Consequently, well-characterised microbial communities consisting of barley and malt-derived microbes open up several possibilities to improve malt characteristics and to ensure the safety of the malting process and the resulting beer [49].

In this manuscript, an overview is given of current knowledge of the barley and malt-derived microorganisms and their impact on malt properties. Emphasis is put on strategies to enhance the malting process, including the use of starter cultures and the management of endogenous microflora. However, note that the microflora on each grain is unique, while the available data presents averages for standard industrial barley and malt [54].

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Figures and tables see Appendix

1 Origin and diversity of the microflora during malting

Microbial communities during malting may be diverse and change during the process. Additionally, the community may continue to change when the malt is stored and finally transported to the breweries [3]. Below the diversity of the microflora during malting is elaborated, starting with field and storage microflora, because of their influence on the microbial load of the starting material in the malting process. An extensive list of the microbial genera and species found on barley, barley malt intermediates and malt is displayed in *Noots et al.* [54].

A Field microflora

The field microflora consists of those microorganisms found on barley prior to harvest and is related to the microflora of soil, vegetation and air [29]. Gram-negative bacteria numerically dominate the microbial community of barley [24, 28]. During plant growth, *Erwinia herbicola* is, for example, reported as the most common and abundant bacterial species on barley. Yeasts are generally the second most abundant microorganisms in preharvest barley [54]. Infestation and degree of infestation are dependent on multiple factors, including plant variety, agronomic practices, climate and growing conditions [22]. Climate is believed to be the dominant factor in determining the extent and composition of the microflora [38]. *Fusarium* species, for example, are mainly present in humid areas, like Northern-America and the North of Europe [25, 38], while they are less present under warmer and drier conditions. Extensive mould growth, especially of fusaria, has a negative impact on the quality of barley and malt as *Fusarium* is associated with the production of mycotoxins and gushing factors [67, 68, 69, 75]. An overview of the most important microbial genera found on barley immediately after harvest using traditional culture-based isolation and identification techniques is displayed in table 1.

B Storage microflora

After harvest and prior to malting, grains are stored to overcome seed dormancy [62]. If necessary, before storage, barley is dried to a moisture content below 13 %. A difference as little as 0.5 % in grain moisture content can make a significant difference in microbial growth and the composition of the microbial community [70]. Temperature is another important factor as the water activity (a_w), which is inherently linked to microbial growth, increases with temperature at constant moisture content of the grain. In general, bacteria do not grow well during good storage of grains since they are less xerophilic than yeasts and fungi [15]. Spore forming bacterial species survive better during storage [59]. In addition, encapsulated yeasts were reported to survive during long-term storage of barley [16]. Storage fungi are generally saprophytes with low specificity, whose ability to grow and persist is mainly determined by a_w and temperature. The storage fungi comprise xerophilic members of, for example, the genera *Aspergillus*, *Eurotium*, *Micropolyspora, Penicillium, Rhizomucor, Thermoactinomyces* and *Thermomyces* [38, 60]. Also *Absidia*, *Rhizopus* and *Mucor* fungi may develop at elevated moisture contents [3]. When *Aspergillus* and *Penicillium* species develop, their metabolic activity is responsible for the release of water by respiration. They can cause elevated temperatures in the grain mass. When the grain mass is aerated insufficiently, these factors initiate a chain reaction of outgrowth of other microorganisms that are less xerophilic and more thermophilic. This microbial proliferation results in further heating of the grain which is known as 'hot and smelly barley' [25]. It is clear from the above that the microbial communities on the kernels may change drastically during storage compared to those found in the field and that a microflora specific for the storage conditions may develop.

C Malt microflora

Malting conditions are favourable for microbial growth. Species dynamics in the malting process are determined by the initial microbial load of the (stored) barley, inter- and intraspecies interactions, the varying substrate characteristics, specific process conditions, and additives. As such, this may lead to a microflora which is specific to a given malting plant (in-house microflora) [22, 38, 46, 54, 57]. In figure 1 the dynamics of some major microbial groups in the malting ecosystem, as determined by classical plate counts, are presented [86].

a Microflora during steeping

Steeping is probably the most critical stage at which microbial proliferation begins and is often regarded as the most critical step in malting with respect to both microbial activity and microbial safety [45, 54]. Bacteria and yeasts rapidly grow (Fig. 1), mould mycelium develops while dormant spores are activated resulting in new growth that can extend from kernel to kernel. Although some of the microbes are washed away along with the steeping water, the viable numbers increase substantially during steeping (Fig. 1). Especially lactic acid bacteria (LAB) heavily develop because of the limited oxygen availability during steeping, while they constitute only a minority of the bacterial community in freshly harvested barley. At the end of the steeping phase, LAB concentrations of up to 10^8 cfu/g are reported, which are generally dominated by heterofermentative *Leuconostoc* species [14, 57]. The fungal microflora is generally dominated by basidiomycetous yeasts that prefer growth temperatures beneath 20 °C [17, 45, 49]. As for the LAB, a strong increase of yeasts was observed during steeping. Aeration during steeping enhances proliferation, resulting in a coat of bacteria, yeasts and fungal spores on steeped grains, but particularly on damaged kernels [51]. With regards to filamentous fungi related to barley and malting, the genus *Fusarium* is considered as the most important group. Intensive *Fusarium* growth has been observed during steeping, even when the original barley had only a low level of *Fusarium* contamination [43].

b Microflora during germination

Major metabolic changes in the barley kernel take place during germination, including conversion of residual carbohydrates to fermentable sugars. In parallel, during germination, an increase in microbial counts is observed [Fig. 1; 59]. Bacteria constitute the dominant microflora showing values of 10^8–10^9 cfu/g for Enterobacteria and *Pseudomonas* spp. [21, 28, 54, 57, 59]. Whereas *Leuconostoc* species were the predominant LAB during steeping, in this phase particularly lactobacilli have been found as the major

LAB. Nevertheless, great variation in species diversity has been observed between different malting houses [14, 47, 57]. Unlike during steeping, ascomycetous yeasts are dominant at the end of germination [49]. For the filamentous fungi, in addition to the general presence of *Fusarium*, great variation in fungal communities has been observed, which can particularly be explained by differences in malting practices. The levels of field fungi such as *Alternaria* and *Cladosporium* usually decline during germination [2, 21, 26, 28].

c *Microflora during kilning*

The final process during malting is crucial to microbial stability and quality of the malt. The kilning temperature and procedure strongly influence the microbial load of the malt produced. As presented in Fig. 1, total counts in the final malt are typically reduced by a factor 10–100 compared to the start of the kilning. Compared to the amount of microbes in barley, microbial load of the malt is significantly higher [54]. An overview of the most important microbial genera found in malt is given in table 2. Certain heat-resistant fungi, such as *Rhizopus* and *Mucor*, and some Ascomycetes continue to grow during the early hours of kilning [21, 49, 74]. Their organisation in biofilms might explain their high resistance to heat [64].

In general, the microbial load and composition of barley and malt have been determined using traditional microbiological methods based on plating, counting and identifying colonies. However, as these techniques rely on the cultivability of the organisms, our view on the total microflora in the malting ecosystem is probably heavily biased and might be very different from reality [34, 63, 73]. In contrast, a much more accurate picture of the total microbial community can be obtained using culture-independent molecular methods. To our knowledge, only a few research groups have been using such methods to characterise the microbial communities on barley and during malting. *Laitila et al*. [46] used denaturing gradient gel electrophoresis (DGGE), complemented by sequencing of key DGGE bands, to investigate the dynamics of the bacterial community in the malting process and to study the effects of the microbial community on malting performance. Several antibiotics were added to the steeping water, resulting in the development of different microbial communities during malting and different malt quality. Within the same research group, PCR denaturing high pressure liquid chromatography (PCR-DHPLC) was evaluated as a promising tool for culture-independent profiling of microbial communities in the malting and brewing process [36]. Furthermore, Kaur [38] described the use of terminal restriction fragment length polymorphism (T-RFLP), complemented with sequencing of clone libraries to characterise the bacterial and fungal community of Australian barley and malt in search for new tools to assure microbial safety and malt quality. Besides being successful in stipulating a fungal fingerprint as a tool for detecting premature yeast flocculation (PYF), new insights on the barley/malt associated microflora were gained in this PhD thesis [38]. For example, it was found that both filamentous fungi and yeasts show greater spatial distribution than bacteria in barley. Additionally, factors such as ‘climate’ and the ‘malting protocols’ in different malting houses were confirmed to affect the microbial community. Furthermore, malting was shown to reduce the microbial diversity, indicating selection and dominance by process-dependant microorganisms. Even novel microbial species that were previously not associated with the barley malting ecosystem were found. These included, for example, strains of the fungal genera *Davidella*, *Glonium*, *Tiarosporella* and *Udeniomyce,* and the bacterial genera *Acinetobacter, Brachybacterium, Frigoribacterium, Kineococcus, Lactococcus, Leucobacter, Massilia* and *Sphingomonas* [38]. Clearly molecular biology has offered new opportunities to widen our knowledge on ‘what is out there?’ and, even more interesting, on ‘which process-borne microflora could be useful for quality control or process optimisation?’

2 Impact of microflora on malt properties: positive and negative effects

The diverse microbial community actively interacts with the grain, affecting microbial safety and technological, nutritional and organoleptic properties of the final product. Depending on the isolate or group and the amount of microbes, this may result in either deleterious or beneficial effects [47]. As a consequence, profound knowledge of the total microbial community as well as its specific characteristics is needed to guarantee malt quality and safety.

A Favourable effects

Grain-associated microbes may enhance the malting and brewing process in several ways, e.g. by the production of hormones and enzymes to stimulate germination [79], the production of antimicrobial factors in order to compete with other members of the microbial community [82] and the formation of several enzymes with positive effects on the malt characteristics, such as amylolytic, proteolytic and cell-wall degrading enzymes [13, 35, 80]. Hydrolytic enzymes play a key role in beer production by catalyzing the breakdown of biopolymers in malting and mashing. In terms of cell-wall degrading enzymes, more research has been done on the breakdown of β-glucans compared to arabinoxylans in regard to wort filtration problems. Nowadays, however, researchers recognise that the impact of arabinoxylans on viscosity and filterability is at least as important as that of β-glucans [5, 23, 66]. During malting, enzymes that hydrolyse arabinoxylans are generally reported to be produced late in the germination process, with the maximum of endoxylanase activity occurring only after 72 hours of germination [7, 50]. On the other hand, Kuntz and Bamforth showed the development of xylanases during the steeping of the barley and early in germination [5, 40]. The contribution of microbes to the “barley” β-glucanase pool has been estimated to be as high as 50–80 % [3, 83]. Likewise, a substantial part of the malt xylanolytic activity originates from the indigenous microbial community. Van Campenhout [80] reported that approximately 75 % of malt xylanase activity was derived from microbes and only 25 % from the grain. Consequently, the microflora during malting greatly affects the cell wall-degrading capacity and therefore has a significant effect on the modification of the malt. Finally, microorganisms can also contribute to the nutritional value of malted cereals. More specifically, microbes can remove antinutritive compounds, enhance the bioavailability of components such as minerals [33] or contribute to vitamin production [77].

B Unfavourable effects

Besides having positive effects on the malting process, microflora can also cause severe damage. In recent years, small grains such as barley have been greatly affected by Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, leading to significant yield losses and quality reductions. Flannigan [26] estimated the total losses for barley and wheat due to FHB between 1991–1996 as being in the order of $3 billion dollars in the United States alone. Another *Fusarium* species that causes FHB is *Fusarium culmorum*. Both *F. graminearum* and *F. culmorum* also may cause root rot, crown rot, foot rot and stem rot in wheat and barley. Two other *Fusarium* species, *F. poae* and *F. avenaceum*, also may cause kernel blight on barley. *Fusarium* species have been reported as the most toxigenic fungi in northern temperate regions [51]. Among storage fungi such as *Aspergillus* and *Penicillium, Fusarium* species produce mycotoxins such as trichothecenes, fusarins, moniliformin, zearalenone, and fumonisins with varying toxicological properties [78]. Several of these mycotoxins have been associated with human and animal diseases and are known to survive the malting and brewing process [51]. Contamination of barley by mycotoxigenic and active gushing inducing fusaria are of particular concern to both maltsters and brewers. Gushing of beer is regarded as one of the most negative consequences of moulds with respect to the quality of malt and beer. For a complete and well-documented overview of this gushing phenomenon we refer to *Shokribousjein et al.* [75]. Furthermore, trichothecene mycotoxines such as deoxynivalenol (DON) are inhibitory for both top (ale) and bottom (lager) fermenting yeast in the brewing process [10]. In addition, *Schapira et al.* [71] reported that DON as well as other trichothecenes affects the development of rootlets and coleoptiles, and enzyme synthesis during malting. Nevertheless, *Schwarz et al.* [74] found that steeping, particularly changing of steeping water, greatly reduced the level of DON in the subsequent malt. Some LAB have the ability to restrict the growth of *Fusarium* moulds, with *Lactobacillus plantarum* as a classical example [51]. Quality control protocols require that malting barley is free of mycotoxins such as DON or ochratoxin A (OTA). Mycotoxin analysis procedures, which are rapid, accurate and toxin-specific are relatively easy to perform, but are also expensive. *Kaur et al.* [37] improved the cost efficiency of those assays by developing DON and OTA immunoaffinity columns which could be safely reused for five times.

The production of extracellular substances, like exopolysaccharides, causing filtration problems in the brewing process, represents another microflora-related problem [30, 39]. In a study of *Laitila et al.* [42], mash filtration difficulties observed in the presence of split barley kernels were found to be caused by an intensive growth of exopolysaccharide-producing bacteria such as *Pseudomomas* species. It has been shown that even small amounts of bacterial polysaccharides had a negative impact on wort separation [39]. Importantly, the release of bacteria and their exopolysaccharides from the grain matrix during mashing depends on agitation. Consequently, different technical treatments result in different filtration behaviour. Wort separation is often the rate-limiting step in the brewery and poor run-offs cause production losses [47]. Furthermore, non-viable malt-derived bacteria have been shown to perturb both wort and beer separation and to cause visible hazes in the beer produced [84]. Among the bacteria identified, strains of the genera *Pantoea*, *Erwinia*, *Micrococcus* and *Bacillus* were predominant.

Brewers sometimes face the problem of premature yeast flocculation (PYF), meaning that the brewer's yeast prematurely settles at the bottom of the fermentation tank leading to an incomplete fermentation and undesirable beer flavour [9, 38, 82]. Natural variation occurs between brewer's yeasts in sensitivity to PYF factors, some lager yeasts being more sensitive than others. The PYF phenomenon has been associated with the presence of fungi in barley [9, 81]. Breakdown of the husk arabinoxylans by fungal enzymes may result in the formation of factors inducing PYF [81]. PYF factors can be produced in the field and/or proliferated/generated during malting [4, 9, 81]. *Blechova et al.* [9] reported that PYF tendency was also closely correlated with gushing tendency and was increased when barley was artificially inoculated with *F. graminearum* and *F. culmorum*, whereas fungicide treatment of barley during the growth period reduced PYF tendency. Nevertheless, no clear cause and/or solution could be stated. Recently, Kaur [38] used molecular fingerprinting methods like T-RFLP complemented by cloning and sequencing to identify the microbial taxa that cause PYF. A significant breakthrough was made with this approach and a concept was developed identifying substantial differences between PYF positive and PYF negative malts. The results revealed that more than one fungal taxon is associated with PYF, which perhaps explains why researchers previously were facing problems identifying the causal microbial agent(s). Further research is ongoing to identify the causal fungal taxa. Ultimately, this should result in the development of specific PCR primer sets that will facilitate rapid and efficient identification of PYF positive malts and barleys.

Microbes are occasionally responsible for inhibited grain germination during malting. Especially aerobic bacteria compete with barley for dissolved oxygen during the steeping phase. Furthermore, uncontrolled bacterial multiplication may lead to biofilm formation, which may inhibit germination [20, 46, 80].

Finally, the diverse microflora during malting may result in changing concentrations of organic acids. As a consequence, the pH of the wort may vary with inconsistent brewing qualities as a result [30, 76].

3 Inoculation of microflora for process optimisation

The idea of exploiting natural microflora to control undesired microbial growth is widespread in many food applications. Also in malting, many scientists have tried to identify and isolate key microorganisms to optimise the malting and/or the subsequent brewing process, ending up with a selection of yeasts such as *Geotrichum candidum* and *Wickerhamomyces anomalus* (synonym *Pichia anomala*), filamentous fungi such as *Rhizopus,* and LAB [12, 49, 51, 55, 65]. Being adapted to the substrate, well-characterised microorganisms can be used or inoculated into the system because of biocontrol properties or specific enzymatic activities. Several groups of microorganisms appear as interesting inoculation candidates with each of them having specific advan-

tages. LAB are commonly exploited for their bio-preservation capabilities of various foods, feed and beverages. This capacity is attributed to the production of fermentation end-products such as lactic acid, diacetyl, acetaldehyde and hydrogen peroxide, which possess the ability to eliminate or retard the growth of many spoilage organisms. In addition, some LAB exhibit antimicrobial activities in the form of small, heat-stable, ribosomally synthesized antimicrobial peptides called bacteriocins [18, 56]. In the malting and brewing industry, indigenous LAB have been extensively screened for their industrial use. Dixon claimed already in 1959 a procedure for the biological acidification of malt by the addition of LAB [19]. Many researchers further described LAB as possible biocontrol agents during malting [31, 32, 41, 51]. For example, LAB have been shown to restrict the growth of toxigenic fungi like fusaria, thereby reducing the formation of harmful toxins. Furthermore, certain LAB produce antimicrobial substances which restrict the growth of harmful Gram-negative and Gram-positive bacteria. These Gram-negative and -positive bacteria compete with grain tissue for dissolved oxygen and may also retard mash filtration [51, 80]. Also Laitila and co-workers reported an enhanced malt processability after the addition of *Lactobacillus plantarum* and *Pediococcus pentosaceus* [44, 64]. A reduction in wort viscosity and β-glucan content as well as enhanced xylanase and microbial β-glucanase activities were observed upon inoculation of these bacteria. Furthermore, lautering performance was improved. Besides LAB, it has also been reported that the addition of *Pseudomonas herbicola* during the steeping stage may result in a reduction of the germination period by one day [58]. Also eukaryotes have been used to enhance the malting process. Boivin and Malanda [11], for example, proposed inoculation of selected strains of *G. candidum* before or during steeping. This yeast is often naturally present on barley and has also been cultured for use in certain branches of the food industry, e.g. the manufacture of soft cheeses. The addition of *Geotrichum* to the first steep inhibited growth of mould fungi of the genus *Fusarium* throughout the malting process, producing a malt free from *Fusarium* mycotoxins. Treatment with *Geotrichum* also resulted in a higher and more homogeneous degree of malt modification, with consequent improvements in brewhouse performance [12]. Also in Laitila et al. [49], the importance of a yeast starter culture to improve the malting process has been reported. In their study, *W. anomalus* showed strong antagonistic activity against field and storage moulds. This yeast restricted hydrophobin production during malting and prevented beer gushing. In order to broaden the antimicrobial activity and to improve malt brewhouse performance, the yeast could be combined with other inocula such as *L. plantarum*, resulting in improved germination and cell wall breakdown during malting, followed by a faster wort filtration. Beside standard inoculation of specific cultures in the process, Noots et al. [55] report microbial inoculation using *Rhizopus* sporangiospores following sterilization of the barley. The barley was sterilized by gamma radiation, resulting in sterile and non-germinating barley. Upon inoculation, increased β-glucanase and xylanase activities were measured with higher and more consistent resulting cell wall modifications during malting. The level of microbial enzymes produced was related to the amount of barley kernels infected with the starter culture. However, activation of the sporangiospores was found to be the critical part in this approach.

Many efforts have been undertaken to screen the original microflora of barley during malting in the search for efficient inoculation cultures or mixtures, for example by *Rouse et al.* [65] who tested 77.793 LAB colonies from 11 commercial malting companies and brewing facilities. However, until now, mainly culture-based techniques have been used in this process. As a result, as these techniques are known to underestimate the microbial species diversity, an overwhelming part of the process-borne microflora during malting may still be unexplored or unknown [34, 63]. Therefore, culture-independent molecular methods such as T-RFLP, clone library sequencing or novel sequencing technologies to deeply characterise microbial communities (e.g. 454 pyrosequencing [53]) are increasingly used in search for new inoculation mixtures. These molecular methods may precisely map the microflora during malting and open up the black box of process-borne microflora, enabling the discovery of interesting strains for inoculation and process optimisation.

4 Future perspectives

It is generally accepted that a profound characterisation of the microbial communities in the malting ecosystem will seriously enhance our understanding of the malting process [36, 38, 47]. Knowledge of the most important community members constitutes the basis for well-thought selection of starter cultures or microflora management during malting. Therefore, because of the limitations associated with traditional, culture-based detection and identification techniques, we are convinced that highly advanced molecular techniques will further find their way in malting and brewing research, as exemplified by both Laitila [47] and Kaur [38].

Starter cultures in the malting industry have been selected either for their biocontrol capabilities or for the production of extracellular enzymes. Production of, for example, xylanases is of particular interest since the remaining arabinoxylans in the malt have a negative impact on the lautering performance. The addition of heat-stable exogenous xylanases at mashing and during germination was found to be an effective strategy to degrade arabinoxylans, resulting in a reduced mash viscosity and an increased wort filtration rate [1, 52]. Because of their importance, several industries including the malting and brewing industry are interested in new (microbial) xylanases or the producers thereof. These enzymes can be used in a variety of processes to improve processing and/or product quality, including food and feed applications such as breadmaking, gluten-starch separation, animal feeds, clarification of juices, and liquefaction of fruits and vegetables. They can also be applied in non-food related processes such as prebleaching of kraft pulps, preparation of textile fibers and bioconversion of lignocellulosic materials to biofuels [8, 61]. Apart from characterising the members present in a microbial community, their functionality can also be assessed, enabling the discovery of new or interesting enzymes. Xylanase diversity can be explored by the same molecular tools as those used for microbial community analysis [85] and may complement microbial community studies in the search for potential starter cultures with interesting xylanase activity. Microbial xylanases belonging to the glycosyl hydrolase (GH) family 10 (GH 10) are, for example, not inhibited by the barley endoxylanase inhibitor HVXI (in contrast with GH11 xylanases) which makes

them highly interesting for malting process optimisation [27, 52]. Obviously, final selection of xylanases or xylanase producers will be based on knowledge of the biochemical characteristics of the enzyme and process requirements.

Nowadays, instead of adding exogenous starter cultures, there is the tendency that breweries increasingly desire to work according to 'clean label technology', meaning that no external cultures or products are added to the process [72]. As a consequence, these companies will need to manage the original barley or malting microflora in such a way that the crucial microbial populations are favoured. Desired microorganisms might be enriched for example in a semi solid-state fermentation system in which for instance the irrigation water used for germination could be circulated, allowing the beneficial microflora to become predominant.

In conclusion, because of the advances in molecular biology, detailed molecular investigations on microbial ecology will greatly contribute to our understanding and management of the malting process and lead to more efficient wort production and enhanced beer flavour quality, including flavour stability.

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Appendix

Table 1 Microorganisms (genera) detected on barley immediately after harvest [54]

Bacteria		Fungi/yeasts			
Gram⁻	***Gram⁺***	***Ascomycota***	***Basidiomycota***	***Zygomycota***	***Mitosporic fungi***
Clavibacter	*Actinomyces*	*Alternaria*	*Cryptococcus*	*Absidia*	*Acremoniella*
Enterobacter	*Arthrobacter*	*Arthrinium*	*Rhizoctonia*	*Mucor*	*Acremonium*
Erwinia	*Bacillus*	*Aspergillus*		*Rhizopus*	*Arthrobotrys*
Escherichia	*Corynebacterium*	*Botrytis*		*Syncephalastrum*	*Aureobasidium*
Flavobacterium	*Lactobacillus*	*Candida*		*Thamnidium*	*Cephalosporium*
Pseudomonas	*Leuconostoc*	*Chaetomium*			*Doratomyces*
Xanthomonas	*Micrococcus*	*Cladosporium*			*Epicoccum*
	Pediococcus	*Cochliobolus*			*Harzia*
	Streptomyces	*Curvularia*			*Helminthosporium*
	Thermoactinomyces	*Didymella*			*Hormodendrum*
		Drechslera			*Monilia*
		Eupenicillium			*Papulaspora*
		Fusarium			*Rhodotorula*
		Geotrichum			*Sclerotium*
		Gonatobotrys			*Septonema*
		Hansenula			*Spicaria*
		Hyphopichia			*Sporobolomyces*
		Hypocrea			*Thermomyces*
		Microdochium			*Thielaviopsis*
		Neocosmospora			*Torula*
		Nigrospora			*Trichothecium*
		Paecilomyces			*Ulocladium*
		Papularia			*Verticillium*
		Penicillium			*Wallemia*
		Phoma			
		Pithomyces			
		Pyrenophora			
		Saccharomyces			
		Scopulariopsis			
		Septoria			
		Sordaria			
		Stemphylium			
		Talaromyces			
		Thermoascus			
		Thielavia			
		Torulopsis			
		Trichoderma			
		Williopsis			

Table 2 Microorganisms (genera) detected on malt using traditional plating methods [54]

Bacteria		Fungi/yeasts		
Gram⁻	***Gram⁺***	***Ascomycota***	***Zygomycota***	***Mitosporic fungi***
Clavibacter	*Actinomyces*	*Alternaria*	*Absidia*	*Cephalosporium*
Enterobacter	*Arthrobacter*	*Aspergillus*	*Mucor*	*Epicoccum*
Erwinia	*Bacillus*	*Botrytis*	*Rhizopus*	*Helminthosporium*
Escherichia	*Lactobacillus*	*Candida*		*Rhodotorula*
Flavobacterium	*Leuconostoc*	*Cladosporium*		*Trichothecium*
Pseudomonas	*Micrococcus*	*Fusarium*		*Ulocladium*
	Pediococcus	*Geotrichum*		*Verticillium*
		Penicillium		
		Phoma		
		Pyrenophora		

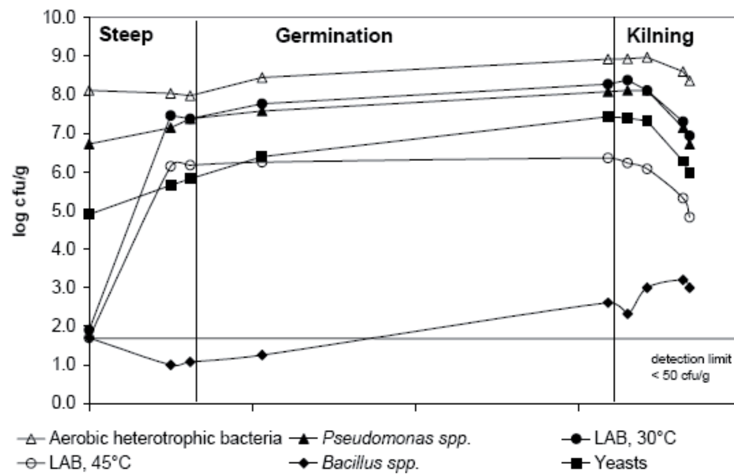

Fig.1 Growth of aerobic heterotrophic bacteria, *Pseudomonas* spp., mesophilic and thermophilic lactic acid bacteria (LAB), aerobic sporeforming bacteria, and yeasts during industrial scale malting [86]