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Methods for suppressing *Fusarium* infection during malting and their effect on malt quality

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Abstract: The incidence of *Fusarium* head blight (FHB) in cereal grains such as barley and wheat is of growing concern due to climate change threatening food safety. Further processing of cereals by malting provides an ideal environment for the growth of *Fusarium*, leading to food safety concerns due to the production of mycotoxins, production challenges with the negative effects to malt and beer qualities, and economic loss owing to the field yield reduction. To improve food safety and product quality, different methods of fungal control have been investigated and reported in the literature. Traditional methods to control fungal growth and mycotoxin production have included chemical and physical methods, but these treatments led to worsened malt properties, limiting their applicability to the brewing industry. Biological control methods have, therefore, attracted wide interest as alternative treatments due to their ability to limit *Fusarium* growth and mycotoxin production in malting cereals without toxic by-products, thus exhibiting promise for improving food safety. Various biological agents have been investigated and applied in malting and have shown the potential to suppress *Fusarium* spp. growth and mycotoxin production. These agents include several lactic acid bacterial (LAB) species and *Geotrichum candidum*. Another promising biocontrol agent for malting control is *Pythium oligandrum*, which has successfully limited *Fusarium* infection in other agricultural crops. The review outlines the *Fusarium*-control methods reported referenced for the brewing industry and the present prospects in biological control applications on the promise of *P. oligandrum* as a novel agent for malting.

Keywords: mycotoxins; malting; biological control; *Pythium oligandrum*

Malted barley is one of the traditional raw materials in beer production. It is prepared by controlled steeping, germination and kilning of barley under regulated conditions of moisture, temperature, time and air flow. Barley malting starts with steeping, where the grains go through alternating stages of wet steeping and, air rests to increase the grain moisture from around 12% to 45% w/w with periods to release excess carbon dioxide and supply fresh oxygen. The steeped grain is then transferred to germinate at 15–20 °C with periodic aeration of humidified air to develop malt enzymes from kernel modification. The germination process is then terminated by drying through temperature, humidity and air flow-controlled kilning to a final moisture content of approximately 4–5% w/w. The final stage of kilning, where malt is cured at higher temperatures, leads to the formation of colour and flavour compounds (Wolf-Hall 2007; Contreras-Jiménez et al. 2017).

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The temperature, humidity and airflow conditions subjected to barley in malting provides an ideal environment for microbial growth (Van Nierop et al. 2006). Grains, including barley, are rich in microflora and are affected by a range of toxigenic filamentous fungi, of which the most common are *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* species (Foszczyńska et al. 2004; Oliveira et al. 2014). The main concern with a fungal infestation of barley in malting and brewing is associated mainly with the negative effects they impart on the quality of the final product (Rouse and Sinderen 2008). Among the fungi present, *Fusarium* species are the most important in malting and brewing because of the prevalence of *Fusarium* head blight (FHB) disease in the crop (Wolf-Hall 2007). Frequently detected *Fusarium* species in head blight diseases of small grain cereals, including barley in Europe, are *F. graminearum*, *F. cumlorum*, *F. avenaceum* and *F. poae*. *Fusarium* contamination of barley is a problem in the production of beer due to the presence of mycotoxins in the infected barley, which can be further elevated through fungal growth in final malt, causing concerns for food safety (Foszczyńska et al. 2004; Sarlin et al. 2005; Wolf-Hall 2007; Rouse and Sinderen 2008; Dalié et al. 2010; Oliveira et al. 2014; Habler et al. 2017; Ksieniewicz-Woźniak et al. 2019).

Aside from the formation of toxic metabolites, *Fusarium* infection also negatively impacts malt quality for brewing use. The presence of cell-wall-degrading enzymes from *Fusarium* leads to decreased wort filterability, which hinders the use of the malt in brewing (Sarlin et al. 2005; Lowe et al. 2006; Mastanjević et al. 2018). The presence of the fungal contaminant may also lead to gushing of beer utilising the infected malt (Schwarz et al. 1996; Lowe and Arendt 2004; Lowe et al. 2005; Sarlin et al. 2007; Rouse and Sinderen 2008; Shokribousjein et al. 2011; Khalesi et al. 2015; Peyer et al. 2016). *Fusarium* moulds also produce small, surface-active proteins classified as hydrophobins, which lead to the gushing, or spontaneous over-foaming, of carbonated beverages such as beer (Shokribousjein et al. 2011). The presence of *F. culmorum* was observed to cause altered wort properties, with yeast prematurely flocculating, resulting in increased concentrations of off-flavour compounds (Oliveira et al. 2012).

Fusarium-infection in cereals also causes yield loss in the field, which is an important economic concern (Linkmeyer et al. 2016; Polišenská et al. 2019; Timmusk et al. 2020). *F. graminearum* infection of barley has led to the reduction of barley quality below standards. This lead to significant price reductions due to the high mycotoxin levels and gushing, making them unsuitable for brewing. That results to downgrading the product as feeds or outright rejection of the grains for use (McKee et al. 2019). Reduced grain plumpness and germination capacity due to FHB limit malting and brewing applications, leading to negative economic consequences (Nielsen et al. 2014). Additionally, FHB in barley brings safety concerns because of high levels of mycotoxin contamination.

A previous study demonstrated a positive correlation between *F. culmorum*/*F. graminearum* and deoxynivalenol (DON) content in FHB-barley originated in the United Kingdom (UK). This exhibits the possibility of decreasing DON in malting by suppressing the growth of *Fusarium* in the process (Nielsen et al. 2014). *F. culmorum* and *F. graminearum* infection was also observed to have a more pronounced effect on the malt quality when compared to other fungi (Sarlin et al. 2005), which highlights the need to minimise the effects of these two *Fusarium* species to improve food safety and malt quality of infected malts, which highlights the need to minimise the effects of the two *Fusarium* species to improve food safety and malt quality of infected malts.

Various methods have been proposed to suppress the effects FHB pathogens in barley malting. Pre-malting solutions were suggested, including chemical and physical treatments, and malting control with biological agents have also been explored (Wolf-Hall 2007). Due to the harsh effects of chemical and physical treatment, biological control methods have gained interest for malting applications. This review details current trends on the control of FHB in cereal grains used for malting and presents future trends with the promising biological agent *Pythium oligandrum* as a novel solution for controlling *Fusarium* infection in malt.

CLIMATE CHANGES AND OCCURRENCE OF *FUSARIUM* INFECTION

Fusarium infection is a growing issue due to the expected weather changes caused by global warming, with extreme weather differences in relation to the average. Variations in temperature, humidity and precipitation associated with climate change are expected to increase *Fusarium* infections and the levels of mycotoxins in cereals, posing a significant threat to food safety (Tima et al. 2016; Vaughan et al. 2016; Habschied et al. 2019). The Northern European climate is expected to become more humid, which is favourable for the spread of FHB in cereal grains (Parikka et al. 2012). Forecasts for the Northern European climate

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is on a milder and more humid future moving towards 2050, which benefits the spread of *F. graminearum* and the production of DON, leading to safety issues in future harvests (Moretti et al. 2019).

Hofer et al. (2019) observed the incidence of *Fusarium* in barley from Bavaria over a few decades. The authors observed that climate changes, such as rising temperatures, tend to increase the severity of *Fusarium* infection in barley, especially in spring varieties. Accordingly, Beccari et al. (2017) reported greater *Fusarium* incidences in barley samples exposed to higher temperatures. Outbreaks in the fungal disease have been associated with warm and humid climate conditions, and future projections point to further increases in warmer weather that will lead to devastating yield loss, worsening seed quality and increased food safety concerns (Timmusk et al. 2020). UK climate change projections presented by Madgwick et al. (2011) also expect to see a gradual increase in FHB to 2050 that will lead to significant economic loss and food safety problems due to projected increase in temperatures and with more rainfall. Weather effects are not limited to the Northern hemisphere; Nogueira et al. (2018) studied the weather effects in fungi associated with FHB in Argentinian barley. The predominant species were *F. graminearum* and *F. poae*, with a higher incidence of *F. graminearum* in barley exposed to warmer and moist growing conditions, leading to the formation of higher levels of type B trichothecenes.

FHB and mycotoxins in grains are also influenced by precipitation. Rain intensity, duration and frequency influenced the dispersal of *Fusarium* spores infecting more grains on the field (Wenda-Piesik et al. 2017). Song et al. (2019) surveyed FHB-occurrence in winter wheat from samples collected over five decades and was able to observe elevated incidences of *F. culmorum* in years with excess precipitation. This is significant since the region is predicted to have increased precipitation in the future, which is a concern. Increases in strong precipitation events are also expected in Southern Germany due to climate change that supports FHB-occurrence (Hofer et al. 2019). Mycotoxins in barley were also shown to be influenced by climatic conditions. DON concentrations in some varieties were the greatest in years with high precipitation, while some had greater DON levels in warmer years. Aside from heavy precipitation, the other extreme of unusually dry conditions also influenced mycotoxin formations in cereals. Malachova et al. (2010) were able to observe that although DON levels decreased with less precipitation, abnormally high nivalenol (NIV) with elevated T-2 toxin and HT-2 toxin were collected in barley grown in the Czech Republic between 2005 to 2008, which was attributed to the stressed *Fusarium* species in the condition that lead to competition between species in the unusually dry environment. NIV was also seen to be predominant in barley grown in Argentina at dry field conditions due to elevated *F. poae* in the dry conditions (Nogueira et al. 2018).

Barley infected with FHB complex surveyed in Italy showed the formation of the mycotoxins DON and T-2 toxin (Beccari et al. 2017). In the American continents, barley harvested in a 17-year span (2001–2017) examined from Ontario also resulted in 83% of the samples infected with *Fusarium,* where *F. graminearum* was determined to be primarily responsible in the FHB outbreak years (Xue et al. 2019). In Southern Brazil, a survey on barley and wheat, grown over an 8-year span (2008–2015), showed a significant amount of samples with detected DON and zearalenone (ZEN), where 19% and 18% of the samples were found to have DON and ZEN levels respectively at concentrations above the limits (Mallmann et al. 2017). While recently, Piacentini, et al. (2019b), testing 64 brewing barley from 2016 harvested grains from the largest barley producing states in Brazil, had 90.6% of the samples with DON detected, and 87.5% detected with ZEN, where 86% of the samples had ZEN levels above safety limits. Type B trichothecenes such as the acylated forms of DON in 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) have also been detected in barley from Uruguay due to infection with *F. gramunearum* species-complex (FGSC) and NIV from *F. poae* (Garmendia et al. 2018). Iwase et al. (2020), meanwhile, detected the prevalence of *Fusarium* species belonging under the *F. sambucinum* species complex (FSAMSC) in Brazilian barley samples, with FGSC as the major contaminant from the complex. All the *Fusarium*-infected samples were contaminated by mycotoxins, including beauvericin (BEA), DON, enniatin (ENN), fumonisin B (FB), NIV and ZEN. Furthermore, samples in one of the regions surveyed had 21% and 33% of the samples containing DON and ZEN above the established safety limits, respectively.

Due to the higher prevalence, trichothecenes have been pointed to be the most economically important of the *Fusarium* mycotoxins. In cereals, the type A trichothecenes are the most acutely toxic mycotoxin, but the less potent type B forms are more widespread (Munkvold 2017). In the brewing applications, concentrations of HT-2 (type A trichothecene) was observed to decrease during the barley to malt transformation,

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while DON (type B trichothecene) has been seen to increase after malting, making them a greater concern (Janssen et al. 2018). Of the mycotoxins, DON is of greatest concern due to *F. culmorum* and *F. graminearum* key role in FHB outbreaks. Both these species are the predominant producer of DON (Wolf-Hall 2007). DON have demonstrated acute and chronic toxicity on humans and animals, leading to food safety issues in barley and malt (Janssen et al. 2018).

EFFECT OF *FUSARIUM* INFECTION ON MALTING AND BREWING

Fusarium infection in cereal grains leads to negative impacts on the malting and brewing process. *Fusarium* infection's economic damage can be traced to a decrease in grain quality, making it unsuitable for brewing. Effects on expression of malting genes have been observed where fungal infection affected quality parameters such as enzymatic activity, availability of nitrogen and foam stability, decreased germination, as well as the transfer of mycotoxins to the final beer (Oliveira et al. 2014; Hofer et al. 2016, Habler et al. 2017, 2018; Hückelhoven et al. 2018). Alterations in the expression of malting-quality genes have been observed as early as the onset of infection, which demonstrates the need to control *Fusarium* as early as possible to prevent negative qualities to the malt, such as the enhancement of proteolysis that leads to technological problems in processing, and on food safety with the production and transfer of mycotoxins to the product. The infection also significantly altered enzymes involved in starch and sugar processes, such as amylase and limit dextrinase, reduced diastatic power, but did not affect other processes, such as lipid metabolism (Lowe and Arendt 2004; Hofer et al. 2016; Geissinger et al. 2017). The presence of mycotoxins has been observed to inhibit the growth of rootlets, slow protein synthesis and affect the synthesis of α-amylase in malt, responsible for a starch breakdown during mashing (Schapira et al. 1989).

Sarlin et al. (2005) demonstrated the effects of *Fusarium* infection in barley on the quality of the malt. Induced gushing was observed for the *Fusarium*-infected samples, with increased wort colour and decreased filtration rate compared to non-infected samples. Increased proteinase, β-glucanase and endoxylanase activities leading to decreased malt quality were attributed to *F. graminearum* and *F. poae* infection in malt barley (Janssen et al. 2018). Similarly, *F. culmorum* resulted in the malt with altered qualities due to the infection affecting proteolytic activity and gibberellins that negatively impacted germination (Spanic et al. 2017). FHB in UK barley has also resulted in decreased germination capacity and increased barley water sensitivity, increased wort free amino nitrogen (FAN), and decreased extract was also observed from infected malt that adversely affects the applicability for malting and brewing (Nielsen et al. 2014). A similar reduction in germination capacity, with increased protein and nitrogen in malt, was mainly attributed to *F. graminearum* and *F. poae* infection in Argentinian barley (Nogueira et al. 2018). The reduced malt quality causes colour and flavour changes in the final beer which are undesirable (Piacentini et al. 2019b).

The infection caused premature yeast flocculation, leading to the lack of further processing of ketones and acetaldehyde by the flocculated yeast and resulting in an increase in esters, fusel alcohols, fatty acids and dimethyl sulfide. These negatively affected the flavour profile of the final beer, although the final beer extract and attenuation were not affected (Oliveira et al. 2012).

The mycotoxin T-2 was observed to strongly inhibit the growth of *Saccharomyces cerevisiae*, which leads to delayed fermentation and the inhibition of ethanol synthesis (Foszczyńska et al. 2004). Boeira et al. (2002) also observed the adverse effects of the mycotoxins DON and ZEN produced from *Fusarium* infection on *S. cerevisiae* where the yeast growth was observed to be sensitive to the mycotoxins, and high DON and ZEN concentrations resulted in decreased cell number and viability.

Studies on malted rye also demonstrated consistently negative effects of *F. graminearum* on wort quality. The spread of the fungi was tracked through the concentration of DON in rye seeds. The study found that the DON concentrations in malted rye were significantly negatively correlated with the concentrations of β-glucans and arabinoxylans, showing a consistent decrease in malt quality with increased viscosity related to the fungal infection (Jin et al. 2018).

Aside from the production of mycotoxins, another fungal product that adversely affects malt quality are hydrophobins which lead to decreased beer quality. Hydrophobins are small surface-active proteins produced by filamentous fungi, which can form hydrophilic--hydrophobic interfaces and stabilise large amounts of gas microbubbles resulting in the spontaneous over foaming phenomenon, so-called gushing (Lowe and Arendt 2004; Peyer et al. 2016). Gushing is a phenomenon characterised by a spontaneous and wild over foaming at the onset of opening carbonated beverages,

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such as beer. Gushing can be due to several factors, but hydrophobin-induced gushing is classified as a primary cause of gushing (Shokribousjein et al. 2011; Postulkova et al. 2018). The hydrophobins interact with the CO_2 in carbonated beverages, such as beer, forming nanobubbles. Then, when the pressure drops to atmospheric due to bottle opening, enough energy is released, causing massive growth of CO_2 nanobubbles (Khalesi et al. 2015). Because gushing is of concern to carbonated beverage stability, numerous studies have investigated the prevention of gushing. Various solutions have been introduced, such as the use of antifoam agents, and reduction of *Fusarium* is one of the options (Shokribousjein et al. 2014; Postulkova et al. 2016).

Like mycotoxins, most hydrophobins are also formed during steeping and germination, with an observed ten-fold increase from barley to malt, and despite only 10% of these fungal proteins being transferred to the finished beer, vigorous gushing can still be observed (Shokribousjein et al. 2011). The most problematic source of primary gushing in beer are *Fusarium* species, of which *F. culmorum* is the most challenging. Aside from *F. culmorum*, other *Fusarium* species, such as *F. graminearum* and *F. poae* have been observed to induce gushing (Lowe et al. 2005; Rouse and Sinderen 2008). Sarlin et al. (2007) investigated barley infected with *F. culmorum*, *F. graminearum* and *F. poae* in the field were observed from malting to brewing. Using an enzyme-linked immunosorbent assay (ELISA) method the researchers developed, the study showed that hydrophobins start to form as the grain grows, but the production accelerates in malting with the fungal metabolites transferring to wort in mashing. Substantial losses in hydrophobin concentrations are observed with the spent grains, cold break, yeast and in filtration, but levels enough for gushing were still measured in the final beer.

Fusarium infection adversely affects malting also through decreased barley quality. Reduced crop yield, smaller barley kernels and reduced germination capacity were noted due to *Fusarium* infection leading to significant economic losses; reductions of as much as 20% of yield were observed (Sarlin et al. 2005). Different species of *Fusarium* were observed to have varying degrees of effect on the barley. *F. culmorum* and *F. avenaceum* were observed to cause significant yield reductions, with *F. avenaceum* leading to smaller ear sizes, while *F. graminearum* was associated with decreased grain quality and toxin contamination rather than with yield reduction, with it producing most of the DON in barley (Linkmeyer et al. 2016; Polišenská et al. 2019; Timmusk et al. 2020). Apart from the reduction of crop yield, fungal contamination in the field results in barley that falls below quality standards, significantly reducing prices due to downgrading use for feeds or outright rejection of the grains for use (McKee et al. 2019).

FUSARIUM MYCOTOXINS

Various mycotoxins produced by *Fusarium* in cereals lead to health and economic concerns associated with the infection. *F. graminearum, F. culmorum, F. avenaceum* and *F. poae* have been pointed as the *Fusarium* species most associated with small grain cereals in Europe. These unwanted microorganisms on cereals lead to the production of different mycotoxins. Common mycotoxins detected in cereals includes type A trichothecenes (T-2 and HT-2), type B trichothecenes (DON, NIV), ZEN, BEA, ENN and moniliformin (MON) (Oliveira et al. 2014; Beccari et al. 2017). Aside from the above, *F. sporotrichioides* and *F. langsethiae* have also been observed in cereals to produce mycotoxins (Linkmeyer et al. 2016). Mycotoxins produced by the different *Fusarium* species have been studied from various regions around the world, with some selected studies summarised in Table 1.

These mycotoxins are important in the context of food safety due to their effects on human and animal health. Trichothecenes are classified as type A-D based on structural differences, where only the type A and B forms are produced by *Fusarium*. Type A trichothecenes, such as T-2 and HT-2 toxins, in FHB-symptomatic barley, has been shown to possess potent immunotoxic, genotoxic and neurotoxic properties, while type B trichothecenes, including DON and NIV, have exhibited acute and chronic effects on the gastrointestinal, nervous and immune systems (Janssen et al. 2018). ZEN is another common mycotoxin and has low toxicity but shows estrogenic effects that lead to reproductive abnormalities (Shephard 2011). MON is considered an emerging mycotoxin and has been linked to rapid death in poultry and swine due to the ability to inhibit protein synthesis, cause chromosomal damage and its cytotoxicity. While BEA and ENN are other emerging mycotoxins currently with limited information available on them (Munkvold 2017).

Among the *Fusarium* species tested, *F. graminearum* produced the most mycotoxin, followed by *F. culmorum* and *F. poae*. Habler et al. (2018) observed the presence of a variety of *Fusarium* mycotoxins in malt barley. Toxic compounds such as DON and its derivatives such as deoxynivalenol-3-glucoside (DON-3G),

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Table 1. *Fusarium* species common in cereals and their produced mycotoxins

Fusarium species	Mycotoxins produced	Reference
F. avenaceum	AURO BEA CHLAM ENN FUC MON	(Beccari et al. 2017) (Jestoi et al. 2008) (Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2019) (Munkvold at al. 2021) (Nielsen et al. 2014) (Piacentini et al. 2019b)
F. culmorum	AURO BUT CHLAM CUL Cyclonerodiol DON ENN FUC MON NIV ZEN	(Beccari et al. 2017) (Jestoi et al. 2008) (Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2019) (Munkvold at al. 2021) (Nielsen et al. 2014) (Shephard 2011) (Spanic et al. 2017)
F. graminearum	AURO BUT CHLAM CUL Cyclonerodiol DON FUC NIV* ZEN	(Beccari et al. 2017) (Jestoi et al. 2008) (Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2019) (Munkvold at al. 2021) (Nielsen et al. 2014) (Piacentini et al. 2019b) (Shephard 2011)
F. langsethiae	BEA DAS ENN HT-2 T-2	(Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2021) (Shephard 2011)
F. poae	AURO BEA BUT CUL Cyclonerodiol DAS ENN FUC NIV	(Beccari et al. 2017) (Garmendia et al. 2018) (Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2019) (Munkvold at al. 2021) (Nielsen et al. 2014) (Piacentini et al. 2019b) (Sarlin et al. 2005) (Shephard 2011)
F. sporotrichioides	AURO BEA BUT CUL DAS ENN FUC HT-2 MON T-2	(Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold 2017) (Munkvold at al. 2019) (Munkvold at al. 2021) (Shephard 2011)
F. tricinctum	AURO BUT CHLAM ENN FUC MON	(Beccari et al. 2017) (Jestoi et al. 2008) (Langseth et al. 1999) (Linkmeyer et al. 2016) (Munkvold, 2017) (Munkvold at al. 2021) (Nielsen et al. 2014)

**F. graminearum* has been described as a species-complex that is predominantly DON producer with limited species producing ZEN (Piacentini et al. 2019b); AURO – aurofusarin; BEA – beauvericin; CHLAM – chlamydosporol; ENN – enniatin; FUC – fusarin; MON – moniliformin; BUT – butanolide; CUL – culmorin; DON –deoxynivalenol; NIV – nivalenol; ZEN – zearalenone; DAS – diacetoxyscirpenol

3-ADON and 15-ADON; and ZEN, along with ENN, BEA and HT-2 were found in samples contaminated with *F. culmorum*, *F. graminearum* and *F. avenaceum*. *F. culmorum* was determined to be a major DON and ZEN producer, while *F. graminearum* was found to form the most mycotoxins in contaminated barley.

The co-occurrence of modified or masked forms of mycotoxins has also been established and also poses food safety risks. Lancova et al. (2008) monitored the transfer of mycotoxins from barley to malt, noting significant levels of the DON-3G in beer. The favourable malting conditions leads to *Fusarium* growth and, subsequently, the production and transformation of mycotoxin products. The enzymes released during the mashing stage could also result in the release of DON-3G as the cell wall, membrane-bound proteins and starch depots are degraded. These masked forms are important due to the physiochemical differences compared to the 'free' form allowing them to elude conventional analysis (Berthiller et al. 2013). These modified forms can be transformed back to the bioavailable 'free' forms during digestion that could lead to potential adverse health effects (Freire and Sant'Ana 2018).

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The gylcosidic bond between DON and the glucoside group can be cleaved in the gastrointestinal tract that increases bioavalability of the masked form of DON (Zachariasova et al. 2012). Aside from DON, other *Fusarium* mycotoxins such as ZEN have been observed to convert into a masked form as zearalenone-16-O--glucoside. This masked form of ZEN also evades routine analysis and can be hydrolysed by gastrointestinal microorganisms and free up the mycotoxin for absorption (Paris et al. 2014).

Of the toxins, DON is the most abundant and most commonly detected mycotoxin in malted barley and has been the main mycotoxin focused on in studies (Foszczyńska et al. 2004; Wolf-Hall 2007; Rouse and Sinderen 2008; Dalié et al. 2010; Oliveira et al. 2014). Nielsen et al. (2014) presented DON as the predominant mycotoxin in UK barley samples, followed by NIV and ZEN. *F. graminearum* complex responsible for FHB in Southern Brazil has shown DON and NIV as the mainly produced mycotoxins (Mallmann et al. 2017). While DON was the most produced toxin in Finnish grains infected with *F. culmorum* and *F. graminearum* (Jestoi et al. 2008). DON was also found to be the most widespread toxin which was detected in 83% of the examined barley cultivars in the Czech Republic harvested over three years (Malachova et al. 2010). Ksieniewicz-Woźniak et al. (2019) surveyed *Fusarium* mycotoxins in malt and beer and highlighted the abundance of DON and its modified form DON-3G. Both were found in more than 90% of the malt and beer samples at significant levels, with other tested mycotoxins at concentrations several times lower. (Habler et al. 2017). *Fusarium* strains mostly investigated for the production of DON, and its derivatives are *F. culmorum* and *F. graminearum,* which have been found to grow faster in malting, with the latter observed to produce the most mycotoxins and malt quality was more negatively affected than with the other species (Sarlin et al. 2005).

During malting, steeping is a critical stage for microbial proliferation on barley because the moisture and temperature conditions are favourable for the rapid growth of mycelium, and activation of dormant spores has been observed. Steep aeration also adds to the growth of microbial contaminants, coating the steeped grains with microbial biofilms (Lowe and Arendt 2004). While the steeping process removes DON initially present in the grains due to its water solubility, the rest of the malting process leads to the production of mycotoxins to the final malt, in particular DON and ZEN, which are considered as the most hazardous mycotoxins by the industry (Piacentini et al. 2019a). Sarlin et al. (2005) observed the production of the mycotoxins DON, 3-ADON, NIV and ZEN, with the highest toxin count occurring in the green malt after germination. Mycotoxins are usually produced with the growth of *Fusarium* spp. in the germination stage. The temperature and moisture conditions in the malting process create an environment in which *Fusarium* growth is activated, leading to the formation of trichothecene mycotoxins as described above (Schwarz et al. 1995; Foszczyńska et al. 2004; Wolf-Hall 2007; Rouse and Sinderen 2008; Dalié et al. 2010; Oliveira et al. 2014).

The production of these fungal metabolites poses health risks due to substantial concentrations of the mycotoxins in the malt that are carried over into the final beer and spent grains from brewing, where levels of type B trichothecenes also pose a health risk to livestock (Habler et al. 2017).

Schwarz et al. (1995) showed that brewing with *Fusarium*-infected malt, a maximum of 93% of the DON produced after germination were transferred to the final beer due to the temperature-stable property of the mycotoxin. Although ZEN and 15-ADON were not detected in the beer, significant quantities were observed in the spent grains left after brewing, limiting their suitability for livestock use. The abundance of DON makes it the greatest source of food safety concern in the consumption of beer, thus making it the focus of most research (Lowe and Arendt 2004; Wolf-Hall 2007; Marin et al. 2013; Jin et al. 2018; Pascari et al. 2018). DON and their modified forms are described by the European Food Safety Authority (EFSA) as human and animal health risks with cereal grains and feeds as main sources in the diet. The mycotoxin has been described to have chronic and acute toxicity to humans and animals, with the EU setting maximum safety levels of 1 250 μg kg^{-1} for unprocessed cereals for human consumption and 8 mg kg^{-1} for cereal animal feeds (Sobrova et al. 2009; European Commission 2017), while the U.S. Food and Drug Administration (FDA) has a higher DON advisory at 30 mg kg^{-1} for brewer's grains (U.S. FDA 2010). DON control is important for food safety due to its acute and chronic effects on humans and animals (Janssen et al. 2018), and Brazil sets DON limits of 1 000 μg kg^{-1} for barley and 750 μg kg^{-1} in malt (Mallmann et al. 2017).

METHODS OF CONTROL OF *FUSARIUM* INFECTION IN BARLEY AND MALT

Correlation between *Fusarium*-infection and mycotoxins in grain and malt was found (Spanic et al.

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2019). This shows the need to suppress *Fusarium* species in barley before or during malting to suppress the formation of mycotoxins and improve the quality of the final malt. Methods to control fungal growth before malting have been proposed, such as the cleaning and removal of thin and discoloured kernels, but were ineffective since *Fusarium* were detected even with healthy-looking grains and was not able to decrease mycotoxin levels after malting (Schwarz et al. 2006). In addition to literature research, a survey was conducted on methods of controlling fungal contamination among Czech malt houses. All respondents wished to remain anonymous, so these parts of the review are referred to as industrial practice information. It is important to understand that the industrial practice mentioned in this work may differ from that in other countries due to local legislation, tradition and climatic conditions.

Chemical control methods

To minimise the negative consequences of *Fusarium* infestation on malt, various chemical control methods have been proposed. Common chemical agents that have been investigated include sodium bisulfite, chlorine gas, ozone and ammonia, among a number of chemicals, and have successfully suppressed *Fusarium* infection and reduced DON in malted barley (Wolf-Hall 2007). The application of various agricultural fungicides has also been observed to limit the production of T-2 toxins and DON by different *Fusarium* species (Hrubošová et al. 2015).

Gaseous ozone and hydrogen peroxide to control *Fusarium* in barley for malting have been investigated as possible chemical control agents. Kottapalli et al. (2005) measured *Fusarium* survival on infected seed, counting after five days cultivation on lactic acid – acidified potato dextrose agar (PDA), and found gaseous ozone significantly decreased *Fusarium* survival by 61% and 53% at treatment doses of 11 mg and 26 mg of ozone respectively per gram of barley, with 15 min exposure times. Hydrogen peroxide also decreased *Fusarium* survival between 50–98% at different concentrations; both substances, therefore, showed promise without having any significant effect on germinative energy and left no chemical residues after treatment.

Another study utilising gaseous ozone against fungal contamination resulted in 96% fungal spore inactivation on PDA plates at a treatment dose of 0.16 mg of ozone per gram of barley for 5 min, without affecting the germination of barley (Allen et al. 2003). Ozone was also applied in the steeping process at 26 mg cm^{-3} for 120 min resulting to only slight reductions in the mould and yeast counts, and aerobic plate counts of the malt incubated on Petrifilm plates; while no significant decreases in DON concentrations were observed attributed to the low levels of DON in the starting barley (Dodd et al. 2011). However, the detoxification of mycotoxins by ozone has also been described with the inactivation of fungal contaminants by degradation or chemical modification of the mycotoxins (Tiwari et al. 2010). Savi et al. (2014) noted the elimination of DON in the pericarp and endosperm of *F. graminearum*-infected wheat after 120 min of ozone treatment. Aside from degrading DON, the modified form DON-3G was observed to significantly decrease in durum wheat after ozonation (Agriopoulou et al. 2020). The formation of free radicals from ozonation led to direct and indirect oxidation of the mycotoxin leading to degradation (Feizollahi and Roopesh 2021).

Another chemical agent studied for barley fungal control is peracetic acid. The application of peracetic acid was successful in reducing bacteria, yeasts and filamentous fungi at doses of 500 mg L^{-1} water added to barley before the malting steps (Rood et al. 2018). Sodium bisulfite, added in the malting stage, has also been proposed and was shown to reduce DON production from *Fusarium*-infected barley without compromising β-glucanase and α-amylase activities. However, this did hinder the germination of the grain at higher concentrations. DON levels after malting were reported to decrease 86% for the untreated sampled due to leaching out of the toxin with the steeping water, while treatment with sodium bisulfite treatment resulted in further reductions to 93% (Lake et al. 2007).

Formaldehyde negatively affected proteolysis, as observed by Foszczyńska et al. (2004). In addition, friability and glassiness were worse for treated malt and decreased diastatic power was also observed. Wort quality was also negatively affected, with worsened extract yields, higher β-glucan concentrations and increased viscosity compared to untreated samples (Foszczyńska et al. 2004).

Despite the effective control of *Fusarium*, chemical control is a harsh treatment that can be too severe for industrial acceptance of treated grains. These procedures may also result in the formation of unstable reaction by-products and residues, which would be unacceptable to brewers. Some methods that are effective in controlling *Fusarium* infection may be expensive and are only effective when applied within a narrow period of time (Wolf-Hall 2007). In addition, some fungal strains are also capable of building resistance against chemical treatments (Dalié et al. 2010). These drawbacks will hinder

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the acceptability of utilising chemical methods for controlling fungal contamination of barley during malting.

The industrial practice in Czech malt houses, in the case of *Fusarium* contamination, permits the use of chlorine-based products. Typical is the use of chlorinated lime, sodium hypochlorite and potassium permanganate into steeping water. Chlorine dioxide is also used for the disinfection of steeping water. The chlorine-based products were characterised by industrial practice as chemical agents only slowing the development of fungi. One maltster reported a small effect of chlorine-based products on the genus *Fusarium*, while the risk of residual chlorine odour in malt was assessed as high. One malt house excluded the use of chemical control methods, and one admitted the use of chlorine dioxide only under extraordinary circumstances.

Physical control methods

Heat and irradiation have also been explored as alternatives to chemical fungicides. Kottapalli et al. (2003) studied the effects of hot water treatment and gamma radiation on *F. graminearum* infection in barley. Gamma radiation, measured in absorbed radiation dose [kilogray (kGy)], was effective in significantly reducing infection at doses of at least 4.3 kGy, but germination energy decreased significantly with doses from 6.9 kGy and above. Hot water treatments also limited *Fusarium* infestation. The absence of *Fusarium* was observed at 55 °C after 10 min and after 5 min at 60 °C. Through aerobic plate counts on PDA plates, 15 min water treatment at 45 °C yielded a significantly decreased *Fusarium* seed contamination count, from 32% to 1% (Kottapalli and Wolf-Hall 2008). Other irradiation sources were also tested. A ^{60}Co source for gamma radiation was utilised, and at irradiation doses of 4–10 kGy, most *Fusarium* spp. were eliminated without affecting the germination of barley (Ramakrishna et al. 1991). An electro-beam as an irradiation source was also proposed by Kottapalli et al. (2006). Infected barley irradiated by an electron-beam showed significantly decreased *Fusarium* infection at a minimum dosage of 6 kGy. Irradiation of the barley also resulted in reduced DON levels in finished malt, decreasing from 54–100% compared to untreated barley, corresponding to 4–10 kGy radiation dosages, resulting from the decreased fungal contamination. However, radiation treatments did not have a significant effect in degrading mycotoxins directly at low dosage. At a high radiation dosage of 50 kGy, only 17.6% DON reduction was noted on dry wheat and was ineffective on dry distiller's grain (Calado et al. 2014).

Hot water treatments were also successful in reducing DON with the suppression of *Fusarium* growth, with DON reductions were observed from 54–71% on PDA plate incubation when treated with 45 °C and 55 °C water for 1 min. A longer treatment time of 20 min resulted in significant decreases in *Fusarium* infection on barley of 65–92% at 45 °C and 71–98% at 50 °C reductions, resulting in malted barley with greater DON reductions, from 79–93% at 45 °C and 84–88% at 50 °C compared to untreated barley. Detectable DON in barley is important for malt safety and marketability because it is the predominant FHB associated mycotoxin indicating the presence of *Fusarium* infection(Kottapalli and Wolf-Hall 2008).

Despite the effectiveness of the physical control methods, some negative impacts on germination and malt quality were observed. Hot water treatments were successful in significantly removing *Fusarium*; however, significant decreases in germination energies were also observed at elevated temperatures (Kottapalli et al. 2003; Kottapalli and Wolf-Hall 2008). Utilising electron-beam radiation was also an effective method, but it substantially decreased the growth rate of the irradiated seeds and decreased the germination energy (Ramakrishna et al. 1991; Kottapalli et al. 2006). Malt quality was also compromised by irradiation, with observed decreases in FAN (14% decrease) and soluble proteins (20% decrease), decreased α-amylase activity (11% decrease) and diastatic power with doses as low as 2 kGy and increased viscosity (6% increase) at higher radiation doses. This was due to the reduction in hydrolytic enzyme activity after irradiation, leading to the reduced breakdown of the endosperm and decreased hydrolysis of the protein matrix (Kottapalli et al. 2006). Quality parameters evaluated by the industry are based on the amylolytic, proteolytic and cytolytic modification of the malt. Based on publications describing malt quality, amylolytic activity is evaluated through diastatic power; proteolytic quality is correlated with the soluble nitrogen in malt and cytolytic modification with β-glucans linked to the viscosity of the wort (Psota and Kosař 2002). Brewers seek to maximise malt's fermentability which is measured with diastatic power and α-amylase, where decreased values lead to decreased fermentability. Lautering performance is also important for brewhouse efficiency, and increased viscosity hinders lautering, which limits applicability in brewing (Evans et al. 2014). While dissolved nitrogen from a proteolytic activity is important to product quality, a decrease in FAN and soluble nitrogen could have unfavourable effects on yeast growth

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and fermentation, while a decrease in soluble protein also adversely affects foam stability hindering industrial applicability of malt with decreased proteolytic activity (Psota and Musilová 2020).

These methods are regarded by the majority of Czech maltsters as academic topics. Only one malting plant reported that it had some information on the use of hot water to control fungal barley contamination without affecting germination capacity. Maltsters generally agreed that instead of physical methods, the following treatments were recommended in order to decrease the development of fungal contamination: careful cleaning and sorting of barley (removal of small grain), proper storage conditions (max. 14% grain humidity, ideally 15 °C and max. 20 °C), thorough cleaning of silos and steeping vessels (especially after processing contaminated barley), careful washing of barely (increasing the washing effect by shorter first seeping with intensive aeration), removal of light non-germinating grains, thorough separation of the remnants of the husks, dust and dirt. For more significantly contaminated barley, these interventions are used in combination with chemical or biological methods.

Biological control of *Fusarium* infection

With the limited applicability of chemical and physical control methods for barley, biological control methods have been proposed as alternative treatment procedures. Biological control of fungal infections, especially *Fusarium* spp., have gained a lot of interest due to their potential to improve the safety and quality of cereals and beverages. *Fusarium* multiplies throughout healthy barley grains during malting and produces mycotoxins that negatively affect the quality of malt. The prospect of a natural antifungal biological agent for malting is therefore an attractive one in hindering *Fusarium* growth in the malting of grains to lessen its impact on malt quality and safety (Oliveira et al. 2015a).

Yeasts. Yeasts are abundant in the malting ecosystem, second to bacteria in terms of culturable microbes. In the industrial malting ecosystem, 25 species belonging to 10 genera of ascomycetous yeasts and 18 species from 7 genera of basidiomycetous yeasts have been identified (Laitila et al. 2011).

Yeasts have been tagged as possible biocontrol agents for malting, based on the observed antifungal activities of certain strains of ascomycetous yeasts. Strains including *Aureobasidium pullulans, Candida sake, Candida saitoana, Geotrichum candidum, Wickerhamomyces anomalus* and *Meyerozyma guilliermondii* have shown antifungal activity. Several yeast strains are also commercially available as biocontrol agents, having been successfully applied against fungal diseases in fruits and vegetables. These strains include *Candida oleophila, Cryptococcus albidus* and *Metschnikowia fructicola* (Laitila et al. 2011).

Seven ascomycetous and five basidiomycetous yeast strains isolated from industrial malting were studied, and five strains of ascomycetous yeasts, *C. saitoana* C524, *Geotrichum* sp. D559, *W. anomalus* C564 and C565, *M. guilliermondii* C568, possessed prominent antagonistic activity against *Fusarium* fungi. *W. anomalus* C565, when inoculated on barley through the addition to steeping water, prevented gushing of beer, exhibiting successful suppression of fungal growth. The antifungal action was attributed to the yeast strain's ability to compete for space with *Fusarium* and faster seed colonisation (Laitila et al. 2011). The yeast also did not have significant effects on grain germination but did affect mash filterability, yielding 10% less filtrate. This was attributed to the suppression of β-glucanase and xylanase activities, thus increasing wort viscosity; this limits the applicability of *W. anomalus* C565 in malting (Laitila et al. 2007). The application of various yeast species to malting demonstrates the promise of effective 'biocontrol' of fungal infection in malting. Limitations in applicability are due to a decrease in the malt quality required for brewing. However, the pool of potential candidates is sufficient, and further microorganisms can be investigated for their fungal suppressive capabilities without having a negative effect on the quality of the malt.

Geotrichum candidum. *G. candidum* is another yeast strain that is naturally present in the barley microflora and is effective in inhibiting the growth of toxicogenic species during malting. In addition, when applied, it has been observed to improve the biochemical and physicochemical quality of malt (Boivin and Malanda 1999). It is a fast colonising yeast that has been observed to effectively limit unwanted microflora growth and has the potential to fully inhibit the growth of *Fusarium* when inoculated as a starter culture into steeping water (Foszczyńska et al. 2004).

Boivin and Malanda (1999) have successfully applied *G. candidum* to malt through inoculation of the steeping water. Inhibitory effects on undesirable flora, including *Fusarium* (from 40% to 5% seed infection after treatment), *Penicillium* (from 10% to 5% seed infection after treatment), *Alternaria* (from 5% to 0% seed infection after treatment) and *Aspergillus* (from 15% to 0% seed infection after treatment) were observed via aerobic plate counts. After malting with *G. candidum,* inhibi-

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tion led to a decrease in the production of secondary metabolites, including a significant decrease in the mycotoxin ZEN (Boivin and Malanda 1999). The fungi also reduced fatty acids in the wort and improved mash filterability as additional improvements in the quality of the malt, with the absence of toxic or mutagenic effects on the grain. The antagonistic effect of *G. candidum* on fungal contaminants was attributed to the presence of lytic enzymes, specifically β-1,3-glucanase and chitinase enzymes (Piegza et al. 2014).

Aside from limiting *Fusarium* growth on malted cereal, *G. candidum* also imparted positive qualitative properties to the malt and wort. A decrease in proteases, improved amylase activity and stimulation of cytolytic activity were brought about by *G. candidum*, resulting in malt with enhanced properties (Piegza et al. 2005, 2014). Foszczyńska et al. (2004) likewise observed *G. candidum* improving the enzymatic activity of malts, stimulating β-glucanase activity, α-amylase activity, better diastatic power, protein degradation similar to untreated malt, comparable Kolbach index values, friability and glassiness.

However, the strain of *G. candidum* should be selected carefully. Various strains have been observed to produce a variety of lipases (Boutroua and Guéguen 2005) and lipase activities (Piegza et al. 2005). High lipase activity is not suitable for brewing due to the formation of unwanted oxidation products (Boivin and Malanda 1999; Postulkova et al. 2018). Aside from lipase activity, some strains have also been shown to produce toxins, such as clavinet alkaloids, that can be harmful to human health (Wolf-Hall 2007; Anderson et al. 2019).

The only biological method used on an industrial scale in the Czech Republic is the application of the yeast *G. candidum*. According to the Czech malt industry, this yeast is able to effectively inhibit mould growth but not completely prevent it. Some malt houses use it preventively in small doses; others use it in higher doses for heavily biologically contaminated barley; in both cases, first into steeping water. Some customers regulate the use of *G. candidum* by malt houses.

Lactic acid bacteria. Lactic acid bacteria (LAB) have been applied as biopreservation agents in food products due to their antagonistic activities against a wide range of moulds, including *Fusarium* species. In a review by Perczak et al. (2018), the main *Fusarium* sources of mycotoxins were isolated from various food products: *F. poae, F. sporotrichioides, F. acuminatum, F. equiseti, F. graminearum, F. culmorum, F. cerealis, F. verticillioides,* and *F. proliferatum*. Cereal grains are one of main sources of mycotoxins in the food chain, and various methods to control mycotoxin formation and improve the quality of food products using LABs have been explored. LABs can be divided into four main genus: *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*, that can be used as biological antagonists for mould control (Dalié et al. 2010; Oliveira et al. 2014), with the *Lactobacillus* species being the most explored (Oliveira et al. 2015b). However, some strains from the *Streptococcus* and *Wissella* genre have also been effective in reducing *Fusarium* spore growth in malt (Oliveira et al. 2014).

LABs have been proposed as biocontrol agents in malting since they are naturally present on the surface of barley. They have been seen to impart a positive effect on the quality of barley malt and have been applied as biological control agents to minimise the occurrence of mycotoxigenic and gushing-inducing *Fusarium* fungi in malting and as a promising alternative to chemical agents that most brewers oppose (Lowe and Arendt 2004; Rouse and Sinderen 2008; Mauch et al. 2010).

The promise of LABs has been demonstrated in research where the spread of undesirable moulds was inhibited, leading to a reduction in mycotoxin production, decreased gushing, and improvements in the quality of the malt and wort when the LAB were inoculated into the steeping water (Lowe and Arendt 2004; Rouse and Sinderen 2008).

LABs can be isolated from various sources, with some strains possessing inhibitory properties on different *Fusarium* species. LAB strains and their efficacy against specific *Fusarium* species in different food sources are summarised in Table 2.

Despite the efficacy of certain LAB species, their effect on different *Fusarium* strains has been found to be strain-dependent. This can be illustrated by the study of Laitila et al. (2002) testing *Lactobacillus plantarum* strains against 20 different cereal grain-derived *Fusarium* strains. Two *L. plantarum* strains were effective in suppressing *Fusarium* mould, in general, but had varying levels of effectiveness (with fungal growth measured by turbidometer in potato dextrose growth medium); *F. avenaceum* (29–38% reduction in fungal growth), *F. culmorum* (26–41% reduction in fungal growth) and *F. oxysporum* (~50% reduction in fungal growth). *F. graminearum*, meanwhile showed mixed results, with the LABs resulting in 26–54% inhibition of affected strains, while some strains (D13, D82, D136, D169) were observed to be resistant to the antimicrobial action (Laitila et al. 2002).

Oliveira et al. (2015a) investigated the antifungal capabilities of *L. amylovorus* DSM19280 and *L. reuteri* R29

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Table 2. *Fusarium* inhibiting LABs strains from the literature

Fusarium species	LAB strain	Reference
F. culmorum	*Lactobacillus plantarum* CCDM 181, CCDM 583, MP2, UFG 121	(Russo et al. 2017) (Horackova et al. 2018)
	Lactobacillus acidophilus CCDM 151	(Horackova et al. 2018)
	Lactobacillus reuteri R29 *Lactobacillus brevis* R2Δ	(Schmidt et al. 2018)
	Streptococcus alactolyticus TMW 1.1001 b, TMW 1.101 *Lactobacillus pontis* TMW 1.1101 *Lactobacillus sanfranciscensis* TMW 1.854, TMW 1.855 *Lactobacillus salivarius* TMW 1.962 *Lactobacillus reuteri* TMW 1.975 *Weissella paramesenteroides* TMW 1.983 *Pichia membranaefaciens* TMW 3.076	(Liske et al. 2000)
F. oxysporum	*Lactobacillus plantarum* KCC-24	(Vijayakumar et al. 2015)
F. graminearum	*Lactobacillus paracasei* LUHS244 *Lactobacillus plantarum* LUHS135 *Lactobacillus brevis* CRL 772, CRL 796	(Bartkiene et al. 2018)
	Pediococcus acidilactici DSM20284 *Lactobacillus brevis* ATCC367 *Pediococcus pentosaceus* ATCC25745 *Lactobacillus paracasei* NBRC15889 *Lactobacillus uvarum* (strain 8)	(Jukonyte et al. 2018)
F. avenaceum	*Pediococcus acidilactici* DSM20284 *Lactobacillus brevis* ATCC367 *Pediococcus pentosaceus* ATCC25745 *Lactobacillus paracasei* NBRC15889 *Lactobacillus uvarum* (strain 8)	(Jukonyte et al. 2018)
F. verticillioides	*Lactobacillus plantarum* M9Y2, M5MA1, M9MM1, M9MG6, M9MM4 *Lactobacillus fermentum* T3M3 *Lactococcus mesenteroides* T1M3	(Yépez et al. 2017)
	Lactobacillus plantarum E2 *Lactobacillus pentosus* E4 *Lactobacillus paralimentaris* Q2 *Lactobacillus fermentum* Q4 *Lactobacillus buchneri* Q6	(Kharazian et al. 2017)

LAB – lactic acid bacteria

in malting by plate assays against *F. culmorum*. Both LAB strains were found to have strong antifungal activities, with *L. reuteri* R29 having exceptional antifungal activity with 68% inhibition of fungal mycelial growth measured by microtiter plate well assay. Laitila et al. (2006) investigated the effect of *L. plantarum* and *Pediococcus pentosaceus* starter cultures in barley malting. Both were seen to be selective, effectively restricting the growth of *Fusarium*, although failing to inhibit *Alternaria*, *Cephalosporium, Cladosporium* and *Drechslera*. Through agar plate counting, *Fusarium* contamination, measured by kernel plating on Czapek-Dox agar containing iprodion and dichloral, was decreased by 20% by the application of the LABs. Malt quality was also improved by both LABs, with a 30% decrease in β-glucans in the wort, lower viscosity, improved mash filterability and enhanced proteolytic activity, with *L. plantarum* inducing additional xylanase activ-

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ity. The application of *L. plantarum* TMW 1.460, *L. amylolyticus* TMW 1.268, and *L. amylovorus* FST 1.1 also enhanced β-glucanase activity of the malt, and mash filterability, without affecting extract levels and fermentability. However, germination of the grain was inhibited, negatively affecting the modification of the malt (Lowe et al. 2005). *Lactobacillus brevis* PS1 is another strain that has exhibited bioprotective properties against *Fusarium* in barley (Mauch et al. 2010).

Various mechanisms have been attributed to the antifungal activities of LAB. These include the production of organic acids, bacteriocins and other low molecular weight compounds and competition for nutrients (Lowe and Arendt 2004; Dalié et al. 2010; Perczak et al. 2018). For *Lactobacillus* strains, *L. paracasei* LUHS244 and *L. plantarum* LUHS135 that inhibited *F. graminearum*, their antifungal activities were attributed to the production of organic acids such as lactic acid (Bartkiene et al. 2018), acting through a lowering of pH that leads to reduced intracellular pH and disruption of the cytoplasmic membrane (Rouse and Sinderen 2008; Oliveira et al. 2014). Laitila et al. (2006) tested both *L. plantarum* and *P. pentosaceus* on *Fusarium* spp.-contaminated malted barley, compared with artificial acidification of MRS-agar substrate with lactic acid. They showed that lactic acid inhibited *F. avenaceum* growth but did not restrict *F. culmorum* or *F. graminearum*, while the LABs successfully suppressed all *Fusarium* species. This result suggests that while lactic acid is a major contributor to the antifungal action of LABs, other compounds work synergistically in inhibiting pathogenic fungi (Oliveira et al. 2014).

Aside from lactic acid, other organic acids, such as acetic, caproic, formic, propionic, butyric and *n*-valeric acids, synergistically contribute to the antifungal activity of LABs (Corsetti et al. 1998). Phenolic acids, such as phenyllactic acid and benzoic acid, have also been identified as antifungal compounds produced by LABs (Rouse and Sinderen 2008; Oliveira et al. 2015a,b). Others identified benzeneacetic acid and 2-propenyl ester as antifungal organic compounds produced by LAB strains (Wang et al. 2012). This effect is attributed to the carboxylic acids usually having a higher pKa, allowing them to remain protonated in an acidic medium, which improves their diffusion across fungal membranes before dissociation, leading to their added antifungal properties (Peyer et al. 2016).

A synergistic mechanism was observed with artificial spiking of phenolic acids into wort with and without LABs. Schmidt et al. (2018) demonstrated that these compounds worked synergistically with the bacteria. The addition of phenyllactic acid to *L. reuteri* R29 enhanced the antifungal activity of the LAB, showing a synergistic effect of phenyllactic acid with other LAB metabolites, rather than just the action of one compound. Pure acetic acid or lactic acid without *L. brevis* PS1 did not inhibit the growth of the fungi (Mauch et al. 2010).

However, organic acids are not the sole metabolites produced by LABs that have antifungal activities. Juodeikiene et al. (2018) investigated the reduction of *Fusarium* and mycotoxins in the malting of wheat with various LABs. Treatment with *L. sakei*, *Pediococcus acidilactici* and *P. pentosaceus* strains decreased mycotoxin concentrations compared with untreated samples. *P. acidilactici* was active against *F. solani*, due to the production of non-acidic metabolites by the LABs. Decreased levels of mycotoxins were attributed to the binding and detoxification of mycotoxins by the LABs in the grains, along with a reduction in fungal contamination in malting.

The production of reuterin (also known as 3-hydroxypropionaldehyde) and fatty acids by the LABs were also suggested as additional metabolites imparting antifungal effects (Liske et al. 2000; Rouse and Sinderen 2008).

The addition of proteolytic enzymes (Proteinase K and Proteinase E) reduced antifungal activities, suggesting that apart from organic acids, proteinaceous metabolites were also responsible for the antifungal activity of *L. brevis* PS1 (Mauch et al. 2010). The effect of *L. amylovorus* DSM19280 on *F. culmorum* was also observed to occur by a different antagonistic mechanism. Aside from producing 12 antifungal metabolites, cell lysis of the bacteria was also observed to contribute to the antifungal potential of LABs. Cellular material from cell lysis of the bacteria could inhibit fungal growth, which might be due to the release of protein-based compounds (Oliveira et al. 2015a).

Comparing data from the literature, the antifungal activities of LABs can be seen to be a complex phenomenon that is strain-dependent, with numerous synergistic mechanisms acting to inhibit fungal growth (Mauch et al. 2010) and imparting excellent mycotoxin detoxification properties (Oliveira et al. 2015a,b).

Some maltsters shared some information on the protective role of LABs when added into steeping water (suppressed growth of fungi and mycotoxin formation, improved enzymatic activity of malt). Otherwise, LABs are not used in the Czech malt industry. This may be due to the resistance of the brewing industry to malt treated with LAB, as some LAB strains are undesirable microbial contaminants of beer.

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Pythium oligandrum. This is a soil-born oomycete and is a promising biological control agent exhibiting antagonistic and parasitic activity against many pathogenic fungi. It has attracted attention due to being a non-pathogenic microorganism capable of acting against pathogens and directly and indirectly protect plants from fungal infections (Takenaka 2015).

P. oligandrum has also been observed to show efficacy against *Fusarium* species in various crops. Benhamou et al. (2001) observed the production of oligandrins by *P. oligandrum* to prevent *F. oxysporum* colonisation on tomato roots. In addition, rapid lysis of *Fusarium* cytoplasm and cell wall was observed as *P. oligandrum* mycelium continued to develop abundantly (Benhamou et al. 1999). Potato wilt caused by *F. oxysporum* was also seen to be reduced significantly with the application of commercially available Polyversum, applied to the roots of the plant (Ayed et al. 2007).

The antagonistic action of *P. oligandrum* is due to various mechanisms, depending on the pathogenic specie. Recorded behaviour includes growth inhibition of host mycelium, mycoparasitism and antibiosis (Benhamou et al. 1999), the production of antibiotic metabolites (Yacoub et al. 2016), and through coiling around the host hyphae and subsequent penetration (Ikeda et al. 2012). *P. oligandrum* has also been observed to indirectly act on fungal pathogens by stimulating the plant's defense system and through growth promotion (Al-Rawahi and Hancock 1998; Takenaka 2015).

P. oligandrum was applied as a starter culture to malting barley by Postulkova et al. (2018) in order to investigate its potential as a biological control agent against barley *Fusarium*. *P. oligandrum* was found to significantly decrease the levels of *F. culmorum* and *F. graminearum*, expressed through quantitative reverse transcription polymerase chain reaction (RT-PCR) deoxyribonucleic acid (DNA) measurements, in artificially contaminated barley when inoculated into the steeping water for malting. *P. oligandrum*, was also found to be more efficient in suppressing *Fusarium* growth compared to *G. candidum,* due to the mycoparasitic action of the fungi, leading to the destruction of the hyphae. There is no information on the effect of *P. oligandrum* on the technological process of malting and the final quality parameters of treated malts. In the malting of *F. culmorum*-infected wheat, the application of *P. oligandrum* in the steeping stage yielded suppression of fungal growth. Treated malted wheat resulted in *F. culmorum* contamination levels 20% relative to untreated wheat malt, with DON and D3G at 17% and 21%, respectively, after *P. oligandrum* treatment. The application of the biocontrol agent also resulted in no deterioration in the quality of the finished malt (Ng et al. 2021).

Biocontrol agents are promising alternatives to chemical intervention methods for suppressing *Fusarium* infection in malting. Various microorganisms have been investigated and have shown effectiveness in controlling fungal growth, leading to a reduction in mycotoxin production, but with limited applicability for brewing due to their effect on the technological properties of the malt. *P. oligandrum* is a promising microorganism for malting applications, showing efficacy against Fusarium growth in crops, and has been commercially approved by the European Parliament and Council Regulation (EC) and the U.S. Environmental Protection Agency (EPA) for plant protection, although limited studies have been done on it as a biocontrol agent for malting.

CONCLUSIONS

Fusarium infection in barley is a serious problem for the malting and brewing industries. The suppression of the formation of *Fusarium*-related mycotoxins and hydrophobins is seen to be a crucial health and quality aspect of malt and beer. The projected weather changes brought by climate change further aggravates the problem, increasing the incidence of FHB in barley that would be damaging economically. The increasing occurrences of *Fusarium* contamination would result to decrease field yield and decreased grain quality unsuitable for brewing and downgrading of the grains to less economical use as feeds.

Food safety is also a growing concern with the rising mycotoxin contamination in grains also due to climatic change. Processing of contaminated grains in malting creates a favourable environment yielding more problematic mycotoxin levels in the final malt. Various methods have been proposed as possible solutions to the fungal contamination in grains for malting. Pre-malting control methods through the use of chemical control agents and physical methods have been studied and have shown good results in minimising the growth of *Fusarium* and reducing the production of mycotoxins during malting. However, these methods also possess drawbacks that prevent acceptance for industrial applications. Chemical control agents has limited industrial acceptability due to possible unwanted by-products and residues, while physical control methods, such as hot water and radiation treatments, are considered only academic topics by maltsters due

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to cost prohibitions in the application of these methods. Consequently, from previous studies, biological control agents seem to be promising solutions due to the lack of chemical residues and the lower cost of application to minimise fungal growth during the malting of barley. Biological control agents have shown promise in the malting control of grains.

Of the proposed control microorganisms, LABs have been the most extensively studied with promising results. LABs have been observed to suppress the growth of *Fusarium* and minimise the production of mycotoxins when applied as control agents during malting. However, LABs, particularly *Lactobacillus* and *Pediococcus* species, are considered spoilage microorganisms and thus would have difficulty being accepted by brewers. Other microorganisms such as *G. candidum* and *P. oligandrum*, have shown promise for possible applications in malting. *G. candidum* have been observed to successfully inhibit *Fusarium* growth in malting, along with improving the quality of the malt. However, careful selection of the *G. candidum* strain is necessary due to the high lipase activities and toxin production by some strains. *P. oligandrum* is a promising biocontrol agent for malting, showing efficacy against *Fusarium* growth in crops. Preliminary applications to malting have also shown promising ability to suppress *Fusarium* growth in the malting of barley, and further studies can be done to investigate the effect of *P. oligandrum* on reducing mycotoxin production and on the technological quality of malt and beer.

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