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Biocontrol Using *Pythium oligandrum* during Malting of *Fusarium*-Contaminated Barley

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Abstract: This study investigates the potential of *Pythium oligandrum* (strains M1 and 00X48) as a biocontrol agent in suppressing the growth of *Fusarium culmorum* and the production of mycotoxins during the malting of naturally contaminated barley (*Hordeum vulgare*). The effects of the biocontrol agent on *F. culmorum*-infected barley malt (BM) were evaluated through real-time PCR and its impact on mycotoxin production was determined by quantitative analysis of deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G). The effect of treatment on BM and beer quality were also determined through European Brewery Convention (EBC) standard methods. Optimal treatment with *P. oligandrum* strains M1 and 00X48 yielded a 59% and 48% reduction in *F. culmorum* contamination, by 37% and 17% lower DON, and 27% and 32% lower D3G, respectively. BM treated with both *P. oligandrum* strains exhibited quality enhancement; beer produced from the BM treated with *P. oligandrum* strain M1 resulted in no quality deterioration and with 26% and 18% less DON and D3G, respectively, transferred to the final product.

Keywords: *Fusarium culmorum*; *Pythium oligandrum*; malting; barley

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1. Introduction

Fusarium head blight (FHB) in barley is of economic concern due to crop yield losses [1] and reduced quality, leading to downgrading of grains to low-value feed use, instead of malting applications, or outright rejection for malting purposes [2]. The prevalence of FHB is important in malting and brewing with negative impacts on malt and beer quality caused by the fungal contamination, including decreased wort filterability [3] and gushing of beer [4].

Aside from quality effects, FHB is also related to food safety issues due to the production of mycotoxins in infected grains [5]. Of the mycotoxins produced by the fungi, deoxynivalenol (DON) and its modified forms, such as deoxynivalenol-3-glucoside (D3G), are the most abundant and are the greatest concern for food safety [6]. The temperature and humidity to which the grains are subjected during malting are ideal for fungal growth [7], leading to elevated mycotoxins in the final malt [8]. Variations in global temperature and humidity brought about by climate change are also expected to increase on-field *Fusarium* infection and to elevate mycotoxin levels in harvested cereals [9].

Barley is one of the most important cereals produced worldwide, with approximately 141 Mt produced in 2017, of which 30% was used for malting [10]. The demand for the crop is continually growing, with an estimated 54% increase in global production required to meet worldwide requirements by 2050 [11]. The supply problem is further complicated due to an increasing incidence of FHB, which causes devastating losses to malting barley. Between 1997–2014, significant economic losses resulted from FHB in barley with $18 million average annual losses in revenue reported in the United States [12]. The malting industry needs an effective biological tool to control FHB.

Due to limitations in traditional chemical and physical methods to reduce the effects of FHB in malting, biological control methods have gained interest. Chemical treatment methods are harsh treatment methods that affects malt quality and could lead to the formation of unwanted byproducts and residues, while physical treatment methods have been described to negatively affect barley germination [13]. A promising biocontrol agent against *Fusarium*-infection in barley malting is *Pythium oligandrum*, which has shown exceptional qualities against *Fusarium* in various crops [14–16] and has also shown its capability of suppressing growth of *Fusarium* during the malting of wheat for the brewing industry [17].

P. oligandrum is a soil-born oomycete that has exhibited antagonistic activity against a variety of pathogenic fungi. It has been observed to protect plants from fungal infections through direct and indirect mechanisms and has attracted attention as a promising biocontrol agent [13]. The microorganism has also received approval from the EU and US EPA for plant protection in agriculture applications [13].

This study explores the application of *P. oligandrum* during the malting of barley, the main cereal for the brewing industry. The suppression of *F. culmorum* growth and mycotoxin production during malting of naturally contaminated barley was determined together with the effect of the biological control treatment on malt and beer quality.

2. Materials and Methods

2.1. Malting Process

Malting was carried out in a micromalting device (Ravoz, Olomouc, Czech Republic) equipped with software (Proteco Ltd., Pardubice, Czech Republic) to control the malting parameters, following malting parameters described by Postulkova et al. [16]. Malting was performed in boxes containing 1 kg of barley grains (*Hordeum vulgare*, Lodestar variety, harvest 2020) obtained from the locality of Hrubcice, Czech Republic. The germinative capacity of used barley was 96% (EBC 2004 3.5.2), while DON and D3G contents were 1320 and 1030 µg/kg, respectively. The malting program started with a 48 h steeping step at 15 °C with alternating wet/air rest intervals at 8/12/8/12/4/4 h, followed by a 48 h germination stage with a gradual temperature decrease from 21 °C to 18 °C, ending with a 24 h kilning step starting at 45 °C for 6 h with gradual increase to a final temperature of 80 °C.

2.2. Brewing Process

Brewing trials were executed in the pilot brewery (50 L) at the University of Chemistry and Technology Prague (Prague, Czech Republic), following standard procedure from the pilot brewery with adjustments [18]. Detailed brewing conditions are summarized in Table 1. Briefly, milled grains were mashed in a single decoction process, after which the spent grains and sweet-wort were separated through a traditional lautering process. This was followed by wort boiling with the addition of hops and cooling. Fermentation was carried out with a bottom fermenting yeast, *Saccharomyces pastorianus* (SafLager W-34/70, Fermentis), and the beer was then left to mature.

2.3. Application of Pythium Oligandrum

Pythium oligandrum samples (strains M1 and 00X48, 1×10^6 oospores/g) were obtained from Biopreparaty Ltd. (Prague, Czech Republic). *P. oligandrum* suspensions were prepared by adding 0.1 to 1 g samples in 100 mL of distilled water and mixed with the barley grains (1 kg dry weight) at the following malting stages: (i) with the first steeping water, (ii) with the second steeping water and (iii) before germination. All malting experiments were performed in three biological replicates. Collected replicates were thoroughly mixed and samples for analysis were taken from the mixtures. The mixing of biological replicates is based on the industrial practice of malthouses aiming at averaging the malt properties.

Table 1. Summary of the brewing process.

Brewing Stage	Experimental Conditions
Mashing	Malt grist (5.3 kg) was mixed with 25 L water and a single decoction process was carried out. First rest of the mash was at 52 °C and the second rest was at 73 °C
Lautering	Traditional lautering was carried out to separate the spent grains and wort. A total of 35 L sweet-wort was collected including one sparging with hot water (70 °C)
Wort boiling	Saaz hop pellets (70 g, T90, Bohemia Hop, Czech Republic) were added to the boiling wort. A total of 60 min boiling was performed and approximately 30 L were obtained after the process
Wort cooling	Trub was removed and the wort was cooled to between 14–15 °C prior to yeast pitching
Fermentation	Bottom fermentation was carried out at 12 °C for 5 days
Maturation	Maturation at 1 °C for 21 days

2.4. Isolation and Quantification of Fungal DNA

A HighPure PCR Template Preparation Kit (Roche Applied Science, Prague, Czech Republic) was used to isolate the DNA from the samples. Isolated DNA was analyzed by real-time PCR using the LightCycler 2.0 (Roche Applied Science) to determine relative *Fusarium* contamination in the samples. Sample preparation, DNA isolation and real-time PCR analysis were performed according to the detailed procedure described by Ng et al. [17], with the primer sequences and Universal ProbeLibrary (UPL) probes for *F. culmorum* and *F. graminearum* as described by Postulkova et al. [16].

2.5. Mycotoxin Quantification

Deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) in the barley malt (BM) samples were determined through a Donprep immunoaffinity column (R-Biopharm AG, Darmstadt, Germany) after purification of the samples following the method described by Havelka et at. (2019) [19]. Identification and quantification of the mycotoxins were carried out by HPLC (Finnigan Surveyor) coupled to an ion trap LCQ Advantage (Thermo-Fisher, USA) with atmospheric pressure ionization, and validated according to the method described by Belakova et al. [20].

2.6. Malt and Beer Quality Parameters

Malt quality was determined based on the following technological parameters: total nitrogen of malt (EBC 2004 4.3.1), extract of malt (EBC 2004 4.5.1), diastatic power (EBC 2010 4.12), final attenuation (EBC 1999 4.11.1), free amino nitrogen (EBC 1997 4.10), soluble nitrogen (EBC 1999 4.9.3), viscosity of wort (EBC 2004 4.8), beta-glucan (EBC 2007 3.10.2) and friability (EBC 2015 4.15). Overall brewing malt quality (BMQ) of the BM was calculated based on the parameters to describe the cytolytic, proteolytic and amylolytic activities of the samples [21], 1 being the worst and 9 being the best. The following beer quality parameters were analyzed: color (EBC 2000 9.6.), original, apparent and real extract (EBC 2004 9.4), alcohol (EBC 2008 9.2.6), and apparent and real attenuation (EBC 2000 9.7) [22].

2.7. Statistical Analysis

Statistical analyses were performed with Microsoft Excel. Data were presented as means ± standard deviations. Experimental data was statistically evaluated through t-test, and all statements of significance were based on a probability of $p < 0.05$.

3. Results

3.1. Effect of Pythium Oligandrum on Fusarium Culmorum Growth and Mycotoxin Production

Real-time PCR was carried out with both *F. culmorum* and *F. graminearum* primer and probe pairs to determine which species was the dominant fungal contaminant on the grains. *F. culmorum* was identified as the dominant *Fusarium* contaminant on the barley samples, yielding exponential growth curves from the real-time PCR analysis, and the absence of a growth curve for *F. graminearum*. Relative levels of *F. culmorum* contamination were determined by real-time PCR to demonstrate the effect of *P. oligandrum* on inhibiting the spread of the fungi in the malting process. The addition of *P. oligandrum* resulted in *F. culmorum* growth suppression by 35–59% relative to untreated BM (Table 2). These values indicate a statistically significant reduction in fungal growth as a result of biocontrol treatment ($p < 0.05$). The suppression of *F. culmorum* growth was also observed to be the most significant when *P. oligandrum* was added with the second steeping water and in the germination stage (Table 2).

Table 2. Relative *F. culmorum* contamination, deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) content in barley malt (BM) samples treated with *P. oligandrum* strain M1 (M1) oospores added at different malting stages (1st STW—first steeping water, 2nd STW—second steeping water, GE—germination). Each addition contained 1×10^6 oospores/kg barley.

Sample	Relative *F. culmorum* Contamination	DON (µg/kg)	D3G (µg/kg)	Decrease in Total DON Concentration
BM	100% ^{a}	701 ± 131 ^{a}	2260 ± 445 ^{a}	
BM + M1 (1st STW)	65% ± 6% ^{b}	501 ± 94 ^{b}	1470 ± 290 ^{b}	33%
BM + M1 (2nd STW)	41% ± 1% ^{c}	444 ± 83 ^{b}	1660 ± 327 ^{ab}	29%
BM + M1 (GE)	45% ± 2% ^{d}	467 ± 87 ^{b}	1780 ± 351 ^{ab}	24%

a–d values: Values with different letters in columns are significantly different ($p < 0.05$).

Together with the suppression of fungal growth during malting of barley, mycotoxin levels were also seen to drop with biocontrol treatment compared to untreated BM. Total DON levels decreased by 29% to 37% in the treated BM samples and all these decreases were statistically significant. The decrease in D3G in BM resulting from *P. oligandrum* treatment was 21% to 35%, being statistically significant when the biological treatment was applied to the first steeping water (Table 2).

Different concentrations of two *P. oligandrum* strains (M1 and 00X48) were applied in the second steeping water and similar significant reductions in *F. culmorum* growth were observed (Table 3). The application of strain M1 resulted in greater suppression of fungal growth than that with strain 00X48. Differences between efficiencies of the two strains were statistically significant. Reductions in fungal contamination levels with different *P. oligandrum* treatment concentrations were not significantly different for both the M1 and 00X48 strains (Table 3).

Total DON levels were also lower for BM samples with *P. oligandrum* treatment. Total DON decreased by 22% to 33% compared to untreated BM (Tables 2 and 3). However, the decrease in DON level was statistically significant ($p < 0.05$) only for BM treated with 1×10^6 oospores M1/kg barley, while D3G reduction was only statistically significant for BM treated with 1×10^6 oospores 00X48/kg barley (Table 3).

3.2. Effect of Pythium Oligandrum on Barley Malt Quality Parameters

Aside from the decreased *F. culmorum* and mycotoxin concentrations, the application of the biocontrol agent resulted in improved diastatic power, apparent final attenuation and friability compared to untreated BM when added at the different malting stages (Table 4). However, the malt quality parameter differences were not significant ($p > 0.05$).

Table 3. Relative *F. culmorum* contamination, deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) in barley malt (BM) samples treated with different concentrations of *P. oligandrum* M1 (M1) and *P. oligandrum* 00X48 (00X48) oospores added in the second steeping water (number of oospores/kg barley).

Sample	Relative *F. culmorum* Contamination	DON (µg/kg)	D3G (µg/kg)	Decrease in Total DON Concentration
BM	100% ^{a}	701 ± 131 ^{a}	2260 ± 445 ^{a}	
BM + 1 × 10^6 M1/kg	41% ± 1% ^{b}	444 ± 83 ^{b}	1660 ± 327 ^{ab}	29%
BM + 5 × 10^5 M1/kg	39% ± 3% ^{b}	603 ± 113 ^{ab}	1730 ± 341 ^{ab}	22%
BM + 1 × 10^5 M1/kg	41% ± 6% ^{b}	629 ± 118 ^{ab}	1660 ± 327 ^{ab}	23%
BM + 1 × 10^6 00X48/kg	52% ± 1% ^{c}	587 ± 110 ^{ab}	1540 ± 303 ^{b}	28%
BM + 5 × 10^5 00X48/kg	52% ± 1% ^{c}	624 ± 117 ^{ab}	1680 ± 331 ^{ab}	22%
BM + 1 × 10^5 00X48/kg	52% ± 1% ^{c}	670 ± 125 ^{ab}	1610 ± 317 ^{ab}	23%

a–c values: Values with different letters in columns are significantly different ($p < 0.05$).

Table 4. Malt quality parameters of barley malt (BM) and the effect of the treatment with *P. oligandrum* M1 (M1) oospores added at different malting stages (1st STW—first steeping water, 2nd STW—second steeping water, GE—germination). Each addition contained 1 × 10^6 oospores/kg barley.

Sample	Viscosity (mPa·s)	AFA (%)	DP (u·WK)	FAN (mg/L)	BG (mg/L)	Extract (%)	RE (%)	KI (%)	Friability (%)
BM	1.442 ± 0.010	79.3 ± 1.0	220 ± 10	183 ± 20	195 ± 49	82.9 ± 0.3	40.5 ± 1.3	45.4 ± 6.4	79.7 ± 2.4
BM + M1 (1st STW)	1.445 ± 0.010	79.6 ± 1.0	225 ± 10	189 ± 21	180 ± 45	82.4 ± 0.3	41.0 ± 1.4	45.5 ± 6.4	80.7 ± 2.4
BM + M1 (2nd STW)	1.442 ± 0.010	80.0 ± 1.0	234 ± 10	191 ± 21	216 ± 54	82.6 ± 0.3	41.9 ± 1.4	46.5 ± 6.5	80.7 ± 2.4
BM + M1 (GE)	1.440 ± 0.010	79.4 ± 1.0	225 ± 10	180 ± 20	168 ± 42	82.6 ± 0.3	40.3 ± 1.3	43.2 ± 6.0	80.1 ± 2.4

AFA—Apparent final attenuation, DP—Diastatic power, FAN—Free amino nitrogen, BG—Beta-glucans in wort, RE—Relative extract at 45°C, KI—Kolbach index. Note: Values in columns are not statistically different ($p > 0.05$).

Different treatment concentrations of both M1 and 00X48 strains added with the second steeping water also resulted in improved diastatic power and apparent final attenuation (Table 5). The friability of BM was also greater for the treated samples, except for BM, with 1 × 10^6 oospores M1 per kg barley. The greatest friability was observed for the BM with 1 × 10^6 oospores 00X48 treatment. The beta glucan concentrations were also generally lower for the treated samples, except for BM with 1 × 10^6 oospores M1 per kg barley, which recorded concentrations greater than the untreated BM. Regardless of the differences, most quality parameters were not statistically different ($p > 0.05$).

Table 5. Malt quality parameters of barley malt (BM) and the effect of the treatment with different concentrations of *P. oligandrum* M1 (M1) and *P. oligandrum* 00X48 (00X48) oospores added in the second steeping water (number of oospores/kg barley).

Sample	Viscosity (mPa·s)	AFA (%)	DP (u·WK)	FAN (mg/L)	BG (mg/L)	Extract (%)	RE (%)	KI (%)	Friability (%)
BM	1.442 ± 0.010 ^{a}	79.3 ± 1.0 ^{a}	220 ± 10 ^{a}	183 ± 20 ^{a}	195 ± 49 ^{a}	82.9 ± 0.3 ^{a}	40.5 ± 1.3 ^{a}	45.4 ± 6.4 ^{a}	79.7 ± 2.4 ^{a}
BM + 1 × 10^6 M1/kg	1.450 ± 0.010 ^{a}	80.1 ± 1.0 ^{a}	238 ± 10 ^{a}	187 ± 21 ^{a}	231 ± 58 ^{a}	82.9 ± 0.3 ^{a}	41.7 ± 1.4 ^{a}	46.6 ± 6.5 ^{a}	79.6 ± 2.4 ^{a}
BM + 1 × 10^5 M1/kg	1.446 ± 0.010 ^{a}	79.6 ± 1.0 ^{a}	222 ± 10 ^{a}	199 ± 22 ^{a}	133 ± 33 ^{ab}	82.3 ± 0.3 ^{b}	45.0 ± 1.5 ^{b}	47.8 ± 6.7 ^{a}	81.6 ± 2.4 ^{a}
BM + 1 × 10^6 00X48/kg	1.431 ± 0.010 ^{a}	80.3 ± 1.0 ^{a}	231 ± 10 ^{a}	204 ± 22 ^{a}	78 ± 20 ^{b}	82.2 ± 0.3 ^{b}	48.9 ± 1.6 ^{c}	50.2 ± 7.0 ^{a}	83.7 ± 2.5 ^{a}
BM + 1 × $10^5$00X48/kg	1.444 ± 0.010 ^{a}	78.5 ± 1.0 ^{a}	220 ± 10 ^{a}	186 ± 20 ^{a}	183 ± 456 ^{a}	82.6 ± 0.3 ^{ab}	42.3 ± 1.4 ^{ab}	47.5 ± 6.7 ^{a}	80.9 ± 2.4 ^{a}

AFA—Apparent final attenuation, DP—Diastatic power, FAN—Free amino nitrogen, BG—Beta-glucans in wort, RE—Relative extract at 45 °C, KI—Kolbach index. a–c values: Values with different letters in columns are significantly different ($p < 0.05$).

Overall barley quality was evaluated with the system proposed by Psota and Kosar (2002) [21]. A scale from 1 to 9 was used to quantify BM quality, considering the cytolytic, proteolytic and amylolytic properties calculated from weights given to each quality parameter based on industry requirements. The overall BMQ was generally seen to improve with the addition of *P. oligandrum*. The best BMQ (4.1) was found for BM with both 1 × 10^6 oospores M1 and 00X48 per kg barley treatment compared to that of untreated BM (3.1) (Tables A1 and A2).

3.3. Effect of Pythium Oligandrum on Beer Quality Parameters

Beer was brewed from BM samples both with and without *P. oligandrum* strain M1 treatment. The treated BM had a treatment dose of 1×10^6 oospores M1/ kg barley and resulted in significantly higher values ($p < 0.05$) in the color, apparent extract, and alcohol, while the fermentation rates and pH were statistically unchanged (Table 6).

Table 6. Quality parameters of beers brewed from barley malt (BM) with and without *P. oligandrum* M1 (M1) oospores treatment added in the second steeping water (1×10^6 oospores M1/kg barley). The original extract of both beers was 13.1 ± 0.06%.

Sample	Color (EBC)	AE (%)	RE (%)	Alcohol (%)	AA (%)	RA (%)	pH
BM	10.6 ± 0.5 [a]	2.60 ± 0.02 [a]	4.62 ± 0.06 [a]	4.40 ± 0.04 [a]	80.1 ± 0.6 [a]	64.7 ± 1.2 [a]	4.47 ± 0.05 [a]
BM + M1	11.2 ± 0.5 [b]	2.51 ± 0.02 [b]	4.54 ± 0.06 [a]	4.43 ± 0.04 [a]	80.7 ± 0.6 [a]	65.2 ± 1.2 [a]	4.49 ± 0.05 [a]

AE—Apparent extract, RE—Real extract, AA—Apparent attenuation, RA—real attenuation. a–b values: Values with different letters in columns are significantly different ($p < 0.05$).

DON and D3G transfer to the final beer samples were also measured (Table 7). The sample prepared from the *P. oligandrum* (M1) treated BM led to a final product with both lower DON and D3G levels by 26% and 18%, respectively, compared to the beer prepared with untreated BM. However, these differences were not statistically significant ($p > 0.05$).

Table 7. Deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) in beer prepared from barley malt (BM) with and without *P. oligandrum* M1 (M1) treatment added in the second steeping water (1×10^6 oospores M1/kg barley).

Sample	DON (µg/kg)	D3G (µg/kg)
BM	104 ± 19	205 ± 40
BM + M1	77 ± 15	168 ± 35

Note: Values in columns are not statistically different ($p > 0.05$).

4. Discussion

Due to the link between FHB infection in grains and growing mycotoxin levels [23], a variety of methods have been tested to limit the spread of *Fusarium* and control the production of mycotoxins [6]. Chemical control agents and physical control methods have been proposed but have limited industrial applications due to the formation of unwanted byproducts and the negative affect on grain quality after treatment [13]. Because of this, biological control agents have gained increasing interest for improving the quality and safety of grains and beverages [24]. The application of biological fungicides is also in line with global food security and sustainability goals of minimizing the impact of pesticides by shifting to non-chemical alternatives [25]. Several biological agents have been proposed for malting applications to limit the spread of *Fusarium* and decrease mycotoxin production. Lactic acid bacteria (LAB), which are naturally present on the surface of barley, have been proposed and shown to successfully minimize the occurrence of *Fusarium* fungi during malting [26,27]. However, some LAB strains are undesired microbial contaminants in beer, resulting in reluctance to apply them industrially [13]. *Geotrichum candidum* is another microorganism used as a biocontrol agent on an industrial scale and has also shown promise in limiting *Fusarium* growth and in decreasing mycotoxin production in malt [28]. However, careful strain selection is required due to some strains possessing high lipase activities resulting in the formation of unwanted oxidation products [27], while some strains could form byproducts such as clavinet alkaloids, which are toxic to human health [6,29].

Pythium oligandrum is a non-pathogenic soil-born oomycete that has attracted attention as a biocontrol agent against pathogenic fungi in plants, including *Fusarium* species [15,30]. The biocontrol ability of *P. oligandrum* applied during malting is here demonstrated by decreasing the relative levels of *F. culmorum* on naturally contaminated barley. This is

consistent with the potential of *P. oligandrum* to decrease artificial *Fusarium* infection in BM, as first described by Postulkova et al. [16]. The anti-fungal properties of *P. oligandrum* can be explained by its mycoparasitic properties, allowing abundant growth on *Fusarium* hyphae and resulting in growth inhibition of fungi [31]. Ng et al. applied *P. oligandrum* during the malting of wheat, resulting in greater suppression of *F. culmorum* contamination than observed in the case of barley [17]. This higher efficiency can be attributed to the absence of a thick husk on wheat compared to barley, which could provide shelter to fungal hyphae [32]. *Fusarium* spores start forming on barley husk surfaces and fungal hyphae grow over the surface of the grain and extend to the interior of the grain through crevices, which protects them against mycoparasites and allows them to thrive throughout malting [33,34].

The observed decrease in DON and D3G mycotoxin levels in *P. oligandrum*-treated BM were also in agreement with literature claiming a correlation between the production of mycotoxins and levels of *F. culmorum* contamination [35]. The decrease in *F. culmorum* content by *P. oligandrum* treatment led to decreased DON and D3G mycotoxins in the finished BM. These two *Fusarium* mycotoxins are the most abundant *Fusarium* mycotoxins and are the most frequently detected in malt and beer [8]. DON levels have also been observed to rise during the malting process, thus making them of greater concern [36].

From the BMQ data, diastatic power, apparent final attenuation and friability in *P. oligandrum*-treated BM samples tended to be higher than in untreated BM, although the differences were not statistically significant. Diastatic power and friability both describe the levels of amylolytic and cytolytic modification in the grains [22]. These higher values with biocontrol treatment point to increased malt modification, which can be traced to the ability of *P. oligandrum* to either stimulate enzymatic activity and plant growth, or/and act with its own enzymatic complement [30,37]. This is also consistent with the observed higher diastatic power obtained for the *P. oligandrum*-treated BM samples [38]. The enhanced proteolytic activity also resulted in the release of higher levels of free amino acids, which react with sugars, leading to the slightly darker color of the beer made from BM treated with *P. oligandrum* [38,39].

5. Conclusions

There is a global trend shifting away from using chemical fungicides to combat the adverse effects of *Fusarium* contamination on brewing barley quality. Accordingly, there is a growing interest in alternative biological control agents. A promising microorganism to control the spread of *Fusarium* in the malting of brewing barley is *P. oligandrum*, which has already received various EU and US EPA approvals for use in agriculture and human medicine. The suitably timed and dosed application of *P. oligandrum* during the malting of barley was able to significantly suppress the spread of *Fusarium* contamination and the formation of mycotoxins. Furthermore, the treatment resulted in enhanced barley malt quality and lower mycotoxin content in beer brewed from it. Although the use of biological control agents in the malting process requires the consent of the regulatory authorities, *P. oligandrum* is such a promising tool that major maltsters are currently seeking its registration in the Czech Republic.

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Appendix A. Supplemental Tables

Table A1. Brewing malt quality (BMQ) parameters of barley malt (BM) and the effect of the addition of *P. oligandrum* (M1, 1×10^6 oospores/kg barley) oospores in different malting stages (1st STW—first steeping water, 2nd STW—second steeping water, GE—germination).

Sample	BMQ	Protein	Extract	RE	KI	DP	AFA	Friability	BG
BM	3.1	9.0	8.5	9.0	9.0	1.0	1.8	1.8	3.9
BM + M1 (1st STW)	3.5	9.0	5.8	9.0	9.0	1.5	2.6	2.9	4.7
BM + M1 (2nd STW)	4.1	9.0	8.5	9.0	9.0	2.8	3.9	1.7	2.0
BM + M1 (GE)	3.5	9.0	6.9	9.0	9.0	1.5	2.1	2.3	5.4

RE—Relative extract at 45 °C, KI—Kolbach index, DP—Diastatic power, AFA—Apparent final attenuation, BG—Beta-glucans in wort.

Table A2. Brewing malt quality (BMQ) parameters of barley malt (BM) and the effect of treatment with different concentrations of *P. oligandrum* M1 (M1) and *P. oligandrum* 00X48 (00X48) oospores, added in the second steeping water (number of oospores/kg barley).

Sample	BMQ	Protein	Extract	RE	KI	DP	AFA	Friability	BG
BM	3.1	9.0	8.5	9.0	9.0	1.0	1.8	1.8	3.9
BM + 1×10^6 M1/kg	4.1	9.0	8.5	9.0	9.0	2.8	3.9	1.7	2.0
BM + 1×10^5 M1/kg	3.4	9.0	5.3	9.0	9.0	1.2	2.6	4.0	7.2
BM + 1×10^6 00X48/kg	4.1	8.9	4.7	7.6	5.5	2.1	4.5	6.4	9.0
BM + 1×10^5 00X48/kg	3.1	9.0	6.9	9.0	9.0	1.0	1.0	3.2	4.6

RE—Relative extract at 45 °C, KI—Kolbach index, DP—Diastatic power, AFA—Apparent final attenuation, BG—Beta-glucans in wort.

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