EFFECT OF ANTIMICROBIAL METABOLITES OF LACTIC ACID BACTERIA FOR DETOXIFICATION OF FUSARIUM SPP MYCOTOXINS IN MALTING WHEAT

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INTRODUCTION

The health and safety of crops, and the eventual grain yield and quality, are highly dependent on the implementation of preventive measures, such as guaranteed pathogen-free seeds and appropriate clean handling by agriculture procedures. Microbes and ubiquitary fungi such as *Fusarium* spp. could have a big negative impact on the sprouting capacity when seeds are stored for a longer time. However one promising and economic strategy is treatment of seeds with an anti-fungal bio-product that can decontaminate a wide range of crops after storage. The objective of the study was to investigate the effect of bio-treatment using dairy by-product (cheese permeate) fermented with antimicrobial active LAB [1] for detoxification of *Fusarium* spp. mycotoxins in malting wheat grains. Additionally, the impact of bio-treatment on microbial contamination and germination capacity of grain was analysed.

MATERIALS

Contaminated grains of spring wheat cultivars Arktis LEU 60210 (protein content 11–11.5%) grown and harvested in 2012 (contaminated with *Fusarium* spp., the dominant species being *F. culmorum*) were collected from various farms in Lithuania (the Plunge region).

Dairy industry waste samples (cheese permeate) were obtained from Lithuanian dairy companies and the Institute for Food Technology in Novi Sad (Serbia).

The processing of bio-products for detoxification of seeds has been carried out with one stage fermentation using Bacteriocins Like Inhibitory Substances (BLIS) produced by lactic acid bacteria (LAB). (Lactobacillus sakei KTU05-6, Pediococcus acidilactici KTU05-7, Pediococcus pentosaceus KTU05-8, KTU05-9 and KTU05-10) have been isolated from spontaneous rye sourdoughs [1].

METHODS

Lactic acid bacteria: KTU05-6 was propagated at 30 °C; KTU05-7 and KTU-10 at 35 °C; KTU05-8 and KTU05-9 strains at 25 °C were cultivated in MRS media for 24 h and used for the malting grain detoxification and for further experiments.

Mycotoxins determination: The determination of trichothecene mycotoxins DON, T-2 and HT-2 in malting wheat grains was performed by HPLC-MS method.

Antifungal activity of LAB supernatants: For antifungal activity evaluation, LAB strains were propagated for 24 h in MRS media at optimal temperatures (as presented above). The cells were harvested by centrifugation (6000 g, 10 min, 4°C), whereas the supernatant was filtered through a 0.2 µm sterile Millipore filter. The pH value of the supernatant was adjusted to 6.5 with 5 mol/L NaOH to eliminate the effects of the organic acids. The antimicrobial activity of the LAB supernatants were evaluated by agar well diffusion assays according to the method [2].

Antifungal test of bio-products based on cheese permeate: Various substances for the bio-treatment of grain and evaluation of their antifungal activity and germination capacity were prepared using fermentation with tested LAB.

Bio-treatment of wheat grains: Bio-treatment of wheat grains was performed by mixing individual bacterial culture suspension with grain samples (100 ml suspension for 500 g seeds) and thoroughly shaken for 30 min at room (18°C) temperature until uniform distribution of bacteria or fermented permeate were achieved.

Germination analysis: The seeds were germinated between two layers of moist filter paper strips. One hundred seeds per roll were placed in rows at regular 5–6 cm intervals from the top edge, leaving 3–4 cm gaps on the sides, covered with a 7–8 cm wide moist strip of filter paper, then with a 15 cm wide moist parchment paper, and loosely rolled. Four rolls per treatment were prepared. The rolls were placed in glass beakers with distilled water (covering the bottom 3 cm of rolls) and incubated at a room temperature (18°C) for 3 days.

RESULTS AND DISCUSSION

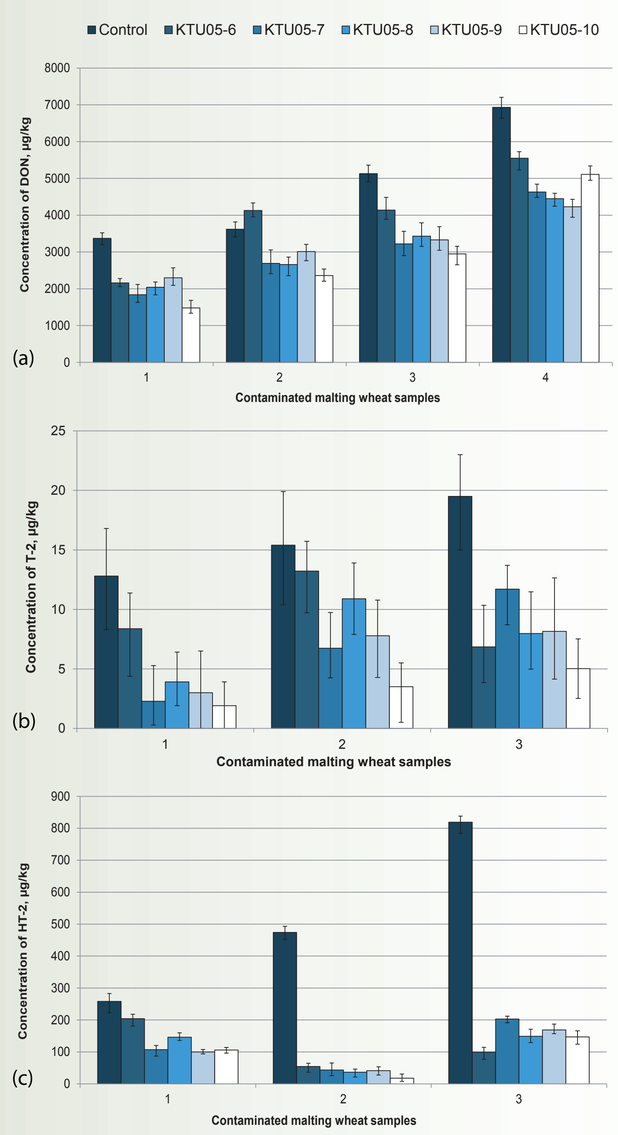

Fig.1.*Detoxification effect of treatment with LAB bio-products on DON (a), T-2 (b) and HT-2 (c) in malting wheat samples.*

The germination capacity of Fusarium contaminated grains (1800 µg/kg DON) decreased on average by 9%, compared to healthy grains (<1.8 µg/kg DON) (Fig. 3). The bio-products had no significant effect on the germination capacity of healthy grains, while the germination capacity of contaminated grains was increased on average by 7.5% compared to untreated grains.

Table 1. *Unmolded grain content after different bio-treatments*

Grain sample	Unmoulded grain, %
Control*	21.7±1.2
Permeate	45.0±1.6
Permeate + *L. sakei* KTU05-6	93.3±1.7
Permeate + *P. acidilactici* KTU05-7	91.5±2.1
Permeate + *P. pentosaceus* KTU05-8	87.5±2.3
Permeate + *P. pentosaceus* KTU05-9	88.0±2.0
Permeate + *P. pentosaceus* KTU05-10	88.3±1.5

*Control – grain treated with sterilized distilled water.

The treatment of malting wheat grains with LAB bio-products had the significant impact on DON, T-2, and HT-2 reduction ($p<0.05$). Mycotoxins contents were found to be 1.9, 1.8 and 5.1-fold lower compared to the untreated grains, respectively (Fig 1 a,b,c). The mycotoxins reduction level depended on the type of LAB used for bio-product fermentation and the grain contamination level. The highest mycotoxins reducing effect showed KTU05-7 and KTU05-10 strains.

LAB metabolites showed a broad spectrum of antimicrobial activity against fungi, especially *Fusarium culmorum* and *Fusarium poae* (Fig. 2).

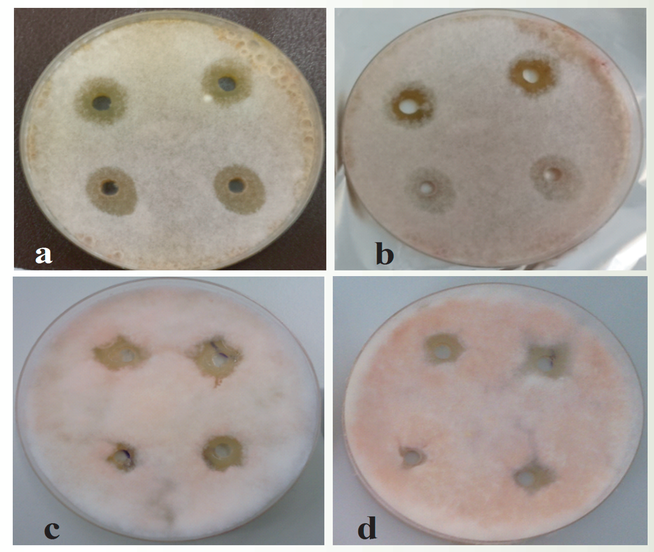

Fig. 2. *Antimicrobial activity of P. pentosaceus KTU05-9 supernatants (top) and neutralised supernatants (bottom) against F. poae (a and b) and F. culmorum (c and d) after 3 days (a and c) and 10 days (b and d) of cultivation at 27°C.*

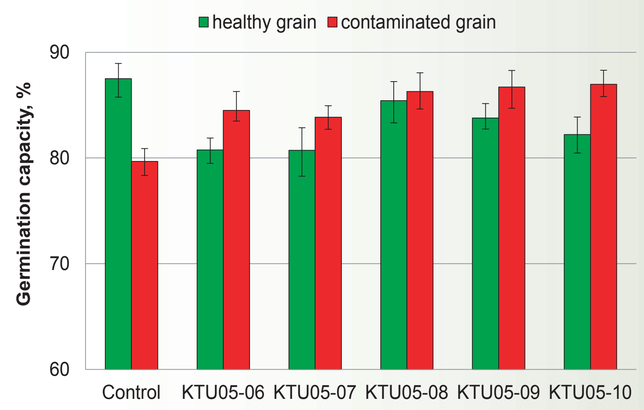

Fig. 3. *The effect of bio-products based on fermented by different LAB permeate on wheat grain germination capacity after 3 days of germination. DON contaminated in contaminated grain 1800 µg/kg, healthy grain <1.8 µg/kg DON).*

The antifungal test has shown that the grain treatment with pasteurized cheese whey permeate fermented by LAB significantly ($p < 0.05$) improved the grain resistance to mould infection, with more than 88% (KTU05-8,9,10), and on average 92% (KTU05-6,8) of unmoulded grains compared to the control (Table 1).

CONCLUSIONS

An environmental beneficial plant-protection strategy was developed through bio-treatment of malting grain seeds using BLIS producing LAB. This study demonstrates that contamination in malting grains could be reduced by bio-treatment with lacto fermented new cheap cheese permeate media without affecting the malting wheat germination capacity.

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