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Cereal fungal infection, mycotoxins, and lactic acid bacteria mediated bioprotection: From crop farming to cereal products

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ABSTRACT

Lactic acid bacteria (LAB) metabolites are a reliable alternative for reducing fungal infections pre-/post-harvest with additional advantages for cereal-base products which convene the food market's trend. Grain industrial use is in expansion owing to its applicability in generating functional food. The food market is directed towards functional natural food with clear health benefits for the consumer in detriment to chemical additives. The food market chain is becoming broader and more complex, which presents an ever-growing fungal threat. Toxigenic and spoilage fungi are responsible for numerous diseases and economic losses. Cereal infections may occur in the field or post-processing, along the food chain. Consequently, the investigation of LAB metabolites with antifungal activity has gained prominence in the scientific research community. LAB bioprotection retards the development of fungal diseases in the field and inhibit pathogens and spoilage fungi in food products. In addition to the health safety improvement, LAB metabolites also enhance shelf-life, organoleptic and texture qualities of cereal-base foods. This review presents an overview of the fungal impact through the cereal food chain leading to investigation on LAB antifungal compounds. Applicability of LAB in plant protection and cereal industry is discussed. Specific case studies include *Fusarium* head blight, malting and baking.

1. Introduction

Cereals are one of the most important sources of food (FAO, 2002), which have contributed to human nutrition for millennia. However, cereals are exposed to numerous biotic and abiotic stress factors, from cultivation and throughout their life cycle to processing.

Toxigenic fungi are a major problem in cereal crops as they produce a multitude of toxic metabolites contaminating plants and food products. Fungi cause numerous crop diseases (Clark et al., 2012), which are responsible for economic losses amounting to billions of euros. Many phytopathogenic and spoilage fungi also cause several potential carcinogenic and mutagenic diseases in humans and animals due to mycotoxin production. Mycotoxins are secondary metabolites produced by moulds as a natural protection. Mycotoxins are generally thermostable (above 100 °C), and thus, can be transferred to food, even after microbial stabilization steps, such as heating and extrusion. Consequently, humans and animals are exposed to their toxic effects. Mycotoxins represent a substantive health hazard to the brewing, breakfast cereal, and baking industries (Araguás et al., 2005). Moulds have the ability to grow in a broad range of environmental conditions. It has been estimated that 5–10% of the world's food production is lost as a result of fungal spoilage (Pitt and Hocking, 2009). Mycological safety threats prevail in spite of continue efforts to the contrary and thus, methods to detect and quantify harmful fungi are ongoing, in particular, towards cereal plant pathogenic species (Ahmad et al., 2012; He et al., 2012; Mavungu et al., 2012; Prieto-Simón et al., 2012; Scauflaire et al., 2012).

Although it is not possible to prevent the introduction of pathogens into food processing facilities, it is crucial to minimize their presence (Akins-Lewenthal, 2012). The most common food preservation strategies applied in the food industry involve chemical or physical techniques. However, these methods only decrease fungal infections and fall short of contaminant elimination. In addition, current consumer trends focus on high-quality, minimally processed green-label foods, thus, driving the food industry towards a focus on natural preservation and stabilization approaches (Reis et al., 2012). Biopreservation technologies are being favoured to improve the safety, nutrition value, and the organoleptic properties of cereals, in response to consumer demands. Lactic fermentation represents one of the most important (Bourdichon et al., 2011). The fermentation microorganisms used in food production can antagonize spoilage contaminants, and are increasing in popularity due

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to their ability to enhance either the product quality, and/or its nutritional profile.

Lactic acid bacteria (LAB) as biopreservative organisms have been the focus of numerous studies. Generally, LAB are accepted as safe for use in food by the Food and Agricultural Organization of the United States (FAO) and by the European Food Safety Authority (EFSA) who have granted many species with Generally Regarded as Safe (GRAS) and Qualified Presumption of Safety (QPS) status, respectively (Franz et al., 2010). LAB are known to deliver desired technological properties and bioprotection in several different food matrices, concurrently enhancing organoleptic and textural qualities of the final product.

The objective of this review is to provide an updated perspective of the current and prevailing fungal infections of cereal crops and the resulting foods, with an overview of the antagonistic role that LAB can play. Specific examples to illustrate biocontrol of phytopathogenic fungi in the field will be discussed. Additionally, the effects of these phenomena in the cereal food processing industry will be illustrated.

2. Cereals

Cereals and cereal-base products are important human food resources and livestock feeds worldwide. The major cereal crops produced worldwide are wheat (*Triticum* spp.), rice (*Oryza* spp.), maize/corn (*Zea mays* L.), and barley (*Hordeum vulgare* L.) (USDA, 2013). Other cereals include millet, sorghum, rye, oat and triticale. Maize ranks first in quantity produced and cultivation area of cereals worldwide, followed by wheat, rice and barley (Table 1). Interestingly, barley presented the highest growth in industrial use in recent years, with a 39% increase in trade exports in 2011/12, while rye is the largest component of global coarse-grain trade market.

In developed countries, up to 70% of the cereal harvest is used as animal feed, while in developing countries cereal is mainly used for human nutrition (Awika, 2011). In fact, 50% of the world's calories are provided by rice, wheat and maize. Cereals are important in human nutrition as a source of protein, dietary fibre, and carbohydrates, as well as providing micronutrients such as, magnesium, zinc, and E and B complex-vitamins (McKevith, 2004).

Regular consumption of cereals is associated with health-promoting effects, in particular whole grains (Angelov et al., 2006). These are associated with the prevention of chronic diseases such as coronary heart disease, diabetes and colorectal cancer (McKevith, 2004). Conversely, whole grain cereals may also contribute with anti-nutrients such as phytate and tannins, while processed cereals contribute to sodium intake (McKevith, 2004).

Cereals are also used to produce oils, starch, flour, sugar, syrup, malt, alcoholic beverages, gluten and renewable energy. The primary cereal application is in the bread manufacture (Valdez et al., 2010). The industrial use of cereal coarse-grains (e.g. corn, barley, oat, sorghum) have experienced continuous growth (USDA, 2013), mainly due to their economic importance for malt production (in the case of barley) and for development of other novel food products (in the case of oat).

Table 1
Cereals' world production, consumption, and trade, in million metric tonnes, since 2008/09 (USDA, 2013).

Cereal	World	2008/09	2009/10	2010/11	2011/12	2012/13
Barley	Production	155	151	123	134	130
	Consumption	144	145	136	136	133
	Trade	18	17	15	21	19
Maize	Production	801	824	832	883	857
	Consumption	785	826	850	879	864
	Trade	84	93	92	104	98
Oat	Production	26	23	20	22	21
	Consumption	24	24	21	22	22
	Trade	2	2	2	2	2
Rice	Production	449	441	449	466	470
	Consumption	437	438	446	459	470
	Trade	29	32	36	39	39
Rye	Production	17	18	11	12	14
	Consumption	16	17	13	13	14
	Trade	266	409	472	548	520
Sorghum	Production	65	54	62	54	57
	Consumption	64	57	61	56	57
	Trade	6	6	7	5	7
Wheat	Production	684	687	652	697	656
	Consumption	644	654	655	697	675
	Trade	144	136	134	154	144

3. Current challenges

3.1. Fungal infections

Agricultural crops are vulnerable to infections by a wide spectrum of plant pathogens. In today's marketplace, the increasing complexity and wide distribution chain represent enormous challenges for food production. The increased fungal infection and cross-contamination hazards are associated with the globalization of cereal trade (Waage et al., 2006). Also, agricultural crops that spread outside their original environment lack ecologic balancing factors, which expose them to foreign pathogens and thus, being vulnerable to other diseases (Gamliel et al., 2008). As such, controlling pathogenic microorganisms in the food production chain is a continuous challenge. Despite new food safety management strategy implementations certain challenges remain unidentified, which may lead to potential contamination or widespread illness (Olewnik, 2012).

Indigenous microbiota in cereal grains consists of virus, bacteria, filamentous fungi, yeast, slime moulds and protozoa. Over 10 million bacteria per gram and more than 150 different mould species can be found in grains. Additionally, cereal contamination and climate change are intimately related (Santini et al., 2012). Depending on geographic locations and climate conditions, saprophytic and parasitic organisms, either mesophilic or psychotrophic microbes are dominant. These cause external and internal plant and grain damage through colonization and nutrient depletion (Laitila, 2007; Noots et al., 1999). Fungal infections cause several plant diseases, reduce yield, cause discolouration, shrivelling of the grains reducing quantity and quality of grains (Osborne and Stein, 2007; Schwarz et al., 2001). In addition, pathogens can be transmitted along the food chain and become a source of human illness (Gaggia et al., 2011).

Cereal grains are exposed to contaminations in the field from several sources (water, composted manure, soil, etc.), during cultivation, harvest, storage, and transport. Common phytogenic microorganisms include bacteria (e.g. *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae* and *Bacillaceae*), yeasts (e.g. *Candida*, *Cryptococcus*, *Pichia*, *Sporobolomyces*, *Rhodotorula*, *Trichosporon*) and filamentous fungi (e.g. *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Claviceps*). Additionally, potential secondary infections can occur post-harvest. Grains can be contaminated during cleaning, milling, grading or packaging processes (from residues in containers, equipment, screw-conveyors, etc). Common microorganisms infecting grains in storage include xerophilic *Aspergillus glaucus* group, and *Penicillium* spp., where the most important parameter for mould germination is the minimum a_w of 0.68 (14% moisture) (Laca et al., 2006; Laitila, 2007; Noots et al., 1999).

After processing, the main spoilage fungi affecting cereal products belong to the genera *Aspergillus*, *Penicillium*, and *Fusarium*.

Currently, there are several fungal human pathogens and spoilage moulds able to adapt to the presence of food preservatives due to its frequent use in industry.

3.2. Mycotoxins

The prevalence of more adaptable mycotoxin producing chemotypes of pathogenic fungal biota in the field is a major problem (Jennings et al., 2004; Ward et al., 2008). Filamentous fungi are a main safety concern due to the production of mycotoxins accumulated in grains pre- and post-harvest, which are associated with severe health problems. Mycotoxins can be carcinogenic, mutagenic, genotoxic, teratogenic, neurotoxic, and oestrogenic, including reproductive and developmental toxicity (Fung and Clark, 2004; Jestoi, 2008; Köppen et al., 2010).

Classes of mycotoxins frequently encountered in different food systems are aflatoxins, fumonisins, ochratoxins, patulin, tricothecenes and zearalenone (ZEA) (Codex Alimentarius, 2011; Dalié et al., 2010). High incidence of mycotoxin infections in cereals have been observed worldwide (Placinta et al., 1999), in different crops and regions (Manthey et al., 2004; Warzecha et al., 2011). It is estimated that 25% of the world's agricultural commodities are contaminated with mycotoxins (FAO, 2010). Mycotoxins, such as *Fusarium* toxins, *Alternaria* toxins, and the ergot alkaloid groups, are common contaminants of cereal grains (Pleadin et al., 2012; Roscoe et al., 2008; Santos et al., 2012). Table 2 shows the most common mycotoxins detected in cereals and its health effects for humans and animals. Over the last two years, contaminations in cereals and bakery products by aflatoxins (48%) and ochratoxin A (OTA) (14%), by *Aspergillus* species, and deoxynivalenol (DON) (21%) and fumonisins (13%), by *Fusarium* species, were record (RASFF, 2012). From these, 48% had its origin in Europe. Estimated cereal economic losses attributed to mould infection, mycotoxin contamination and associated prevention costs, infected waste disposal and quality control equate to billions of euro annually.

The mycotoxin content in processed cereal-based products is dependent on the pattern of fungal infection in the grains as well as the processing steps (Laca et al., 2006). The main sources of mycotoxins in foods and feeds are usually grains and grain-based products. Due to their high chemical stability, mycotoxins are potentially transferable from grains to malt and other processed foods (Champeil et al., 2004; Lancova et al., 2008; Schwarz et al., 1995; Wolf-Hall and Schwarz, 2002). The presence of mycotoxin in grains cannot be confirmed based on the visual appearance alone (Oliveira et al., 2012b), instead, detection is dependent on chemistry-based methods (Capriotti et al., 2012).

There are rapid and continuous technological developments in detection methodology to allow adherence of foods to the maximum permitted levels of mycotoxins in cereals in EU (EC, 2006; EC, 2007) and worldwide. However, a multitude of mycotoxins is often produced within a single food matrix and their additive effects are also considered a health risk factor, even if present below their maximum individual tolerance dose (Eskola et al., 2001; García-Cela et al., 2012; Speijers and Speijers, 2004; Tanaka et al., 2010). The interactions between mycotoxins can act synergistically, such as DON with aflatoxin B1 (AFB1) or Nivalenol (NIV) or Sterigmatocystin (ST) (Sobrova et al., 2010); BEA with T-2 toxin (Ruiz et al., 2011b); OTA with citrinin (CTN) (Bouslimi et al., 2008), or antagonistically (Ruiz et al., 2011a, 2011b). Yet, further studies on mycotoxin accumulation and combined toxicity are needed for more comprehensive explanations (Capriotti et al., 2012; García-Cela et al., 2012; Tammer et al., 2007).

Moreover, emerging mycotoxins such as fusaproliferin (FUS), beauvericin (BEA), enniatins (ENNs), and moniliformin (MON) (Jestoi, 2008; Santini et al., 2012), which have been detected in cereals throughout Europe (Malachova et al., 2011), remain without legislation or legal limits (Vaclavikova et al., 2012). The occurrence and distribution of mycotoxins in foods is of extreme importance due to the ill effects and/or unknown results of prolonged exposure to these agents, which can be vectors of chronic disease (Jestoi, 2008). This is particularly important to specific population groups, such as children and vegans/macrobiotics, that can be exposed to excess levels of tolerable intake (Asam and Rychlik, 2013; Beretta et al., 2002; Leblanc et al., 2005; Lombaert et al., 2003). This was reported for T-2 and HT-2 toxins by the European Commission (EC, 2003). The risk assessment of exposure and effects of mycotoxins on children's health was recently reviewed (Sherif et al., 2009).

Another challenge is the masked effect on mycotoxins. Masked mycotoxins are those which are hidden from standard detection due to their conjugation to polar substances (e.g. sugars, amino acids and sulphate) by plants during protective detoxification, with subsequent incorporation into plant cell compartments. The polarity of these derivates will be altered and, thus, are harder to extract and detect (Malachova et al., 2011). Several analytical limitations were reported by Köppen et al. (2010) including mycotoxin modified through technological treatments where the conjugates and masked forms (Lattanzio et al., 2012) were lost during extraction. There are thousands of potential toxic metabolites produced by fungi (over 400 identified) with a broad chemical diversity. This poses analytical difficulties for techniques in terms of detection limits, recovery, or reproducibility. Due to the high variability among test results, mycotoxin concentrations in lots may not be determined with accuracy.

Health risk factors concerning the impact that the type of agriculture has in mycotoxins are still controversial. Fewer cereal rotations and less problems with lodged fields related to farming practices seem to restrict *Fusarium* infestations and mycotoxins (Bernhoft et al., 2012). Conversely, occurrence of mycotoxins have been reported to increase applying organic approaches (Ok et al., 2011; Rubert et al., 2013b; Serrano et al., 2012). It is apparent that organic practices have to implement rigorous preventive measures to maintain contaminations at a low level (Lairon, 2011).

The complete elimination of mycotoxin contaminated commodities is not achievable (Codex Alimentarius, 2003), and prevention strategies post-harvest are only effective for mycotoxins formed at this stage (Magan and Aldred, 2007). This represents a challenge for plant breed management programs and for the food processing industry.

3.3. Barley Fusarium head blight (FHB)

One example of fungal infections in cereals crop cultivation with serious agricultural repercussions is the case of *Fusarium* Head Blight (FHB) (Clark et al., 2012). FHB, commonly known as scab, is a devastating fungal disease that occurs in barley, wheat and other small cereal grain crops (Desjardins, 2006; Miedaner et al., 2010; Parry et al., 1995).

FHB is of growing international importance in recent years leading to significant economic losses across the value chain by reducing grain yield and quality of barley and wheat in cultivation sites worldwide (Gilbert and Tekauz, 2000), particularly in warm, humid and temperate climate regions over the past 25 years (Pirgozliev et al., 2003). Indeed, high temperature and humidity levels (e.g. heavy dew) favour fungal attack and disease development (Doohan et al., 2003; Xu, 2003), which can justify the presence of more pathogen species detected in the UK and Ireland in comparison to other European countries (Xu, 2010). Favourable conditions for fungal infection are long periods (over 2–3 days) of high humidity (over 90%), with heavy rainfall (over 500 mm), and

Table 2
Mycotoxins found in cereal crops and their fungal source, with the health effects for humans and animals and the lethal dose in 50% of sample values of mycotoxins per body weight (LD_{50}). References: (Burmeister et al., 1980; Cardona et al., 1991; EFSA, 2005; EFSA, 2011; Finnegan, 2010; Frisvad et al., 2007; Makun et al., 2011a; Pitt, 2002; Streit et al., 2013; Sumalan et al., 2011; Tess and Saul, 2012; Visconti, 2001; Wijnands and Leusden van, 2000).

Mycotoxin	Fungi source	Cereal crops	Health effects in humans and animals	LD_{50} (mg kg^{-1})
Aflatoxins (B_1, B_2, G_1, G_2)	*Aspergillus* (*flavus*, *bombycis*, *nomius*, *ochraceoroseus*, *parasiticus*, *parvisclerotigenus*, *pseudotamarii*, *rambellii*, *toxicarius*); *Emericella* (*astellata*, *olivícola*)	Maize	Potent carcinogens, neurotoxins and immunosuppressants. Aflatoxicosis: death due to consumption of contaminated food; liver disease and cancer in humans and animals; hydroxylated aflatoxin metabolites (M1 and M2) found in milk.	AFB_1 Mice: $9.0^{p.o.}$ Rabbit: $0.3^{p.o.}$ Dog: $0.5–1.0^{p.o.}$
Alternaria alternata lycopersici (AALs)	*Alternaria* (*alternata*, *triticina*, *arborescens*, *cucumerina*, *dauci*, *kikuchiana*, *solani*)	Wheat, barley, oat	Carcinogenic; might be responsible for oesophageal cancer. Experiments with rodents indicate the following mycotoxin acute toxicity: altenuene (ALT) > (tenuazonic acid) TeA > (alternariol monomethyl ether) AME > (alternariol) AOH.	AME, AOH Mice: $400^{i.v.}$ TeA Mice: $162–115^{i.v.}$, $225^{p.o.}$ ALT Mice: $50^{i.v.}$
Avenacein Y	*Fusarium* (*avenaceum*, *chlamydosporum*, *lateritium*, *tricinctum*)	Wheat	Significant antibiotic properties against phytopathogenic bacteria with low cell toxicity.	Not available
Butenolide	*Fusarium* (*avenaceum*, *crookwellense*, *culmorum*, *graminearum*, *poae*, *sambucinum*, *sporotrichioides*, *tricinctum*, *venenatum*)	Broad	Associated with cattle diseases, synergistic effects with enniatins B.	Mice: $44^{i.p.}$, $275^{p.o.}$
Citreoviridin	*Aspergillus terreus*; *Eupenicillium cinnamopurpureum*; *Penicillium* (*citreonigrum*, *manginii*, *miczynskii*, *smithii*)	Rice	Possibly involved in acute cardiac beriberi, sporadically associated with yellow rice disease.	Mice: $7.5^{i.p.}$, $20–29^{p.o.}$, $11^{s.c.}$
Citrinin (CTN)	*Aspergillus* (*terreus chemotype II*, *carneus*, *niveus*); *Blennoria* sp.; *Clavariopsis aquatic*; *Monascus ruber*; *Penicillium* (*manginii*, *chrzaszii*, *citrinum*, *expansum*, *odoratum*, *radicícola*, *verrucosum*, *westlingii*)	Broad	Potent nephrotoxin.	Mice: $35–58^{i.p.}$, $110^{p.o.}$ Rat: $50^{p.o.}$ Rabbit: $19^{i.p.}$
Culmorin and derivates	*Fusarium* (*crookwellense*, *culmorum*, *graminearum*, *langsethiae*, *poae*, *sporotrichioides*)	Broad	Synergistic effect with DON towards caterpillars.	Low toxicity in *in vitro* assays
Cyclochlorotine	*Penicillium islandicum*	Rice	Chlorine containing cyclic peptides associated with yellowed rice toxicosis.	Mice: $0.3^{i.p.,i.v.}$, $6.5^{p.o.}$, $0.48^{s.c.}$ Rat: $50^{i.p.}$, $5^{p.o.}$, $0.4^{s.c.}$
Cyclopiazonic acid	*Aspergillus* (*flavus*, *lentulus*, *oryzae*, *parvisclerotigenus*, *pseudotamarii*, *tamarii*); *Penicillium* (*camemberti*, *commune*, *dipodomyicola*, *griseofulvum*, *palitans*)	Broad	Potent organ damaging calcium chelating mycotoxin; produces focal necrosis in most vertebrate inner organs.	Rat: $2.3^{i.p.}$, $36–63^{p.o.}$
Deoxynivalenol (DON) and derivatives	*Fusarium* (*culmorum*, *graminearum*, *pseudograminearum*)	Broad	Nausea, vomiting and stomach pains; chronic and fatal toxic effects. At the cellular level, the main toxic effect is the inhibition of protein synthesis via binding to ribosome.	Mice: $49–70^{i.p.}$, $46–78^{p.o.}$ Duckling: $27^{s.c.}$ Chicks: $140^{p.o.}$
Diacetoxyscirpenol (DAS)	*Fusarium* (*venenatum*, *poae*, *equiseti*, *sporotrichioides*, *langsethiae*, *sambucinum*)	Broad	Effects in immune system, inhibits initiation of protein synthesis, killing rapidly proliferating cells.	Mice: $23^{i.p.}$ Rabbit: $1.0^{i.v.}$ Swine: $0.37^{i.v.}$
Enniatins (ENNs) (A, A1, B, B1) and cyclic peptides	*Fusarium* (*acuminatum*, *avenaceum*, *langsethiae*, *lateritium*, *poae*, *sambucinum*, *sporotrichioides*); *Halosarpeia* sp.; *Verticillium hemipterigenum*	Broad	Antibiotic and ionophoric activity. Induction of apoptosis. Enniatin B often occurs together with enniatin B1 and A.	Mice: $10–40^{i.p.}$ (death within 2–5 days)
Ergot alkaloids (ergolines)	*Claviceps* (*fusiformis*, *paspali*, *purpurea*)	Rye	Ergotism in human and animals, ergot alkaloids cause vasoconstriction and neurotoxicity including hallucinations.	Ergometrine Mice: $160^{i.v.}$, $448^{p.o.}$ Rabbit: $3.2^{i.v.}$ Ergotamine Mice: $265^{i.v.}$ Rabbit: $3^{i.v.}$, $550^{p.o.}$
Fumonisins (B_1, B_2, B_3)	*Fusarium* (*anthophilum*, *dlamini*, *napiforme*, *nygamai*, *proliferatum*, *thapsinum*, *verticillioides*)	Maize, millet, sorghum, rice	Interfere with some steps that contribute to cell growth. Weak link with increased risk of throat cancer. Affect nervous system of horses.	*F. verticillioides* extract Mice: $45.4–51.7^{i.p.}$, $>1000^{p.o.}$ Chicks: $81–88^{i.p.}$
Fusaproliferin (FUS)	*Fusarium* (*globosum*, *guttiform*, *proliferatum*, *pseudocircinatum*, *pseudonygamai*, *subglutinans*, *verticillioides*)	Maize	Recent mycotoxin which shows teratogenic and pathological effects in cell assays. Toxic in *in vitro* trials to brine shrimp and mammalian cells.	Not available

(*continued on next page*)

Table 2 (*continued*)

Mycotoxin	Fungi source	Cereal crops	Health effects in humans and animals	LD_{50} (mg kg^{-1})
Fusarenon-X (FUS-X)	*Fusarium* (*culmorum*, *graminearum*, *cookwellense*, *poae*, *nivale*, *equiseti*, *tricinctum*)	Rice, wheat	It is toxic to murine thymocytes, lymphocytes and gastric epithelial cells and to human hepatoblastoma cells, acute toxic effects on gastric epithelial cells in animals such as vomiting.	Mice: 4.5[p.o.] Rat: 4.4[p.o.]
Moniliformin (MON)	*Fusarium* (*avenaceum*, *napiforme*, *nygamai*, *oxysporum*, *proliferatum*, *subglutinans*, *tricinctum*, *thapsinum*, *verticillioides*)	Corn, sorghum, millet, rice	Cytotoxic, inhibits protein synthesis and enzymes, chromosome damages, induce heart failure in mammals and poultry.	Mice: 21–29[i.p.] Rat: 42–50[i.p.] Chicks: 5.4[p.o.]
Nivalenol (NIV)	*Fusarium* (*graminearum*, *poae*, *culmorum*, *venenatum*, *equiseti*, *crookwellense*)	Broad	Hormone (oestrogen) mimic, limited evidence of genotoxicity. Oestrogenic toxin affects reproduction. Inhibition of protein synthesis.	Mice: 4.1[i.p.]
Ochratoxin A (OTA)	*Aspergillus* (*carbonarius*, *cretensis*, *flocculosus*, *lacticoffeatus*, *niger*, *ochraceus*, *pseudoelegans*, *roseoglobulosum*, *sclerotioniger*, *sclerotiorum*, *steynii*, *sulphureus*, *westerdijkiae*); *Neopetromyces muricatus*; *Penicillium* (*nordicum*, *verrucosum*); *Petromyces* (*albertensis*, *alliaceus*)	Rice, wheat	Toxic to the kidneys (nephrotoxic) and the immune system, it is classified as a probable human carcinogen. Neurotoxins and immunosuppressants.	Mice: 22–40[i.p.], 26–34[i.v.], 46–58[p.o.] Rat: 12.6[i.p.], 20–30[p.o.] Chicken/swine: 2.1–4.7[p.o.]
Patulin	*Aspergillus* (*clavatonanica*, *clavatus*, *giganteus*, *longivesica*, *terreus*); *Byssochlamys nivea;* *Penicillium* (*carneum*, *clavigerum*, *concentricum*, *coprobium*, *dipodomyicola*, *expansum*, *formosanum*, *gladioli*, *glandicola*, *griseofulvum*, *marinum*, *paneum*, *sclerotigenum*, *vulpinum*)	Rye, rice	Very toxic with various toxic effects; can harm the immune system and gastrointestinal tract.	Rat: 5–15[i.p.], 15–25[i.v.], 25–46[p.o.] Mice: 7.6[i.p.]
Penitrem A	*Penicillium* (*clavigerum*, *crustosum*, *glandicola*, *janczewskii*, *melanoconidium*, *tulipae*)	Broad	Mycotoxic indol-terpene with tremorgenic properties, implicated with mycotoxicoses of animals, suspected to be implicated in tremors in humans.	Mice: 1[i.p.]
T-2 toxin and HT-2 toxin	*Fusarium* (*sporotrichioides*, *langsethiae*, *poae*, *sambucinum*)	Broad	It is the most toxic of the *Fusarium* trichothecenes. Interferes with protein synthesis and DNA/RNA synthesis (HT-2 toxin derivate is less toxic).	Mice: 5.2[i.p.], 5.2–10.5[p.o.] Rat: 5.2[p.o.] Swine: 1.2[i.v.]
Trichodermin	*Trichoderma viride*	Wheat, maize	Potent inhibitor of plant growth with several phytotoxic effects. It inhibits wheat coleoptile growth. Inhibits protein synthesis by binding to ribosomes, proposed as antifungal and antineoplastic, used as tool in cellular biochemistry.	Mice: 500[s.c.]
Zearalenone (ZEA)	*Fusarium* (*graminearum*, *culmorum*, *equiseti*, *crookwellense*)	Broad	Oestrogenic activity in farm animals and it is implicated in hyperestrogenic syndromes in humans.	Mice: >500[i.p.,p.o.]
Xanthomegnin	*Aspergillus* (*auricomus*, *bridgeri*, *elegans*, *flocculosus*, *insulicola*, *melleus*, *neobridgeri*, *ochraceus*, *ostianus*, *persii*, *petrakii*, *roseoglobulosus*, *sclerotiorum*, *steynii*, *sulphureus*, *westerdijkiae*); *Microsporon cookie;* *Neopetromyces muricatus*; *Penicillium* (*cyclopium*, *freii*, *janthinellum*, *mariaecrucis*, *melanoconidium*, *tricolour*, *viridicatum*); *Trichophyton* (*megninii*, *mentagrophytes*, *rubum*, *violaceum*)	Broad	Mycotoxicosis in animals, toxic to liver and kidneys in mammals.	Mice: 450[p.o.]

[i.p.] Intraperitoneal administration.
[i.v.] Intravenous administration.
[p.o.] Oral administration.
[s.c.] Subcutaneous administration.

temperatures between 23 and 29 °C, in particular during flowering (anthesis) and early grain-filling crop stages (McMullen and Stack, 2011). However, infection also occurs at cooler temperatures when high humidity persists for longer than 72 h (McMullen and Stack, 2011). In addition, the global warming phenomenon can alter the physiology and morphology of both the crop and pathogen and is, therefore, recognized as a serious global environmental problem (Brennan et al., 2005). Currently, FHB is presently the most damaging disease of barley in Canada and it cost millions of dollars per annum in the USA alone (Nganje et al., 2004).

FHB rapidly destroys a crop within few weeks with disease symptoms including premature necrosis and a brown/grey discolouration of spike tissue (Parry et al., 1995). Physical damage

from scab is multifold encompassing reduced yields, discolouration, shrivelled kernels, contamination with mycotoxins, and overall reduction in seed quality. Yield losses in FHB-infected barley (scabby or tombstone kernels) occur through sterile blighting florets and shrivelled kernels (disruption of grain filling and reduced size) (Subedi et al., 2007). The earliest infections generally kill the florets compromising the kernel development. FHB infection also causes the accumulation of trichothecene mycotoxins produced in the mature grain (Jansen et al., 2005), which is the primary cause of reduced grain quality (Desjardins, 2006).

FHB infection of field-grown cereal plant develops when the phytopathogen *Fusarium* fungi infects the crop spikes tissue after they emerge in the late-milk to soft-dough stages of seed development (Bushnell et al., 2003). *Fusarium* fungi can enter in cereal florets either passively through natural openings, such as stomata, or actively by direct penetration (Bushnell, 2001; Lewandowski et al., 2006). The first symptom of this disease tends to occur around the middle of the head (Bushnell et al., 2003), the region where flowering begins (Kirby, 2002). Once inside the floret, the fungus quickly penetrates the highly susceptible interior surfaces, causing yellow and brown lesions. Hyphae grow subcuticularly and intercellularly (Jansen et al., 2005; Kang and Buchenauer, 2000) during the first two days of infection (Jansen et al., 2005; Pritsch et al., 2000). Barley is also susceptible to fungal infection for prolonged period after anthesis, and FHB can develop more rapidly in plant tissues nearing natural senescence (Scanlan and Dill-Macky, 2010). FHB incidence (number of diseased spikes/total) and severity (number of diseased spikelets/total) are highly correlated (Xu, 2010).

Fusarium species, responsible for FHB, can grow on a variety of substrates, can tolerate diverse environmental conditions, and also have high levels of intraspecific genetic and genotypic diversity (Kerenyi et al., 2004). Most frequently detected isolates include *Fusarium graminearum* (teleomorph: *Gibberella zeae* (Schwein.) Petch); *Fusarium cerealis* (synonyms *Fusarium crookwellense*), *Fusarium culmorum* Wm. G. Sm., *Fusarium poae* (Peck) Wollenw, and *Fusarium avenaceum* (Fr.) Sacc. (McMullen et al., 1997; Walter et al., 2010). In parts of Northern Europe, *F. culmorum* and *F. avenaceum* are the prevalent species (Parry et al., 1995). Nonetheless, *F. graminearum*, has caused most of the recent outbreaks of FHB in the USA and Canada, as well as in South America, Southern and Central Europe, China, and Japan (Osborne and Stein, 2007; Speijers and Speijers, 2004). *F. graminearum* higher tolerance to environmental conditions variability and capacity to produce ascospores are two key factors for its competitive advantage.

These filamentous ascomycete fungi typically produce trichothecene DON, its derivates (3- acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON)), NIV, and ZEA (Desjardins, 2006; Puri and Zhong, 2010). These mycotoxins pose serious hazards to humans and animals. They cause neurological disorders and immunosuppression due to inhibition of protein biosynthesis (Choo, 2006; Goswami and Kistler, 2004; Parry et al., 1995; Rocha et al., 2005; Walter et al., 2010). However, trichothecene mycotoxins play a predominant role in establishment of FHB and have been implicated in pathogen virulence. DON is the most common mycotoxin produced by *Fusarium* spp. (Desjardins, 2006; Placinta et al., 1999).

It is highly challenging to control FHB due to poor understanding of the mechanisms of plant resistance and lack of available Mendelian resistance genes (Boyd et al., 2010). Resistance from exotic landraces has proven to be difficult to incorporate and, as such, no approved resistant cultivars exist (Geddes et al., 2008; Jordahl et al., 2010). Additionally, resistance to FHB is difficult to evaluate due to low-heritability traits which are strongly influenced by environmental conditions. These cause difficulties in the development of efficient management tactics and recommendations.

Trichothecenes produced by various species of *Fusarium* are increasingly contaminating cereal crops worldwide. Thus, improving FHB resistance remains a high priority in wheat and barley breeding programs throughout the world (Dill-Macky et al., 2009). Agronomic and crop management strategies aiming to control FHB include foliar fungicide application, crop rotation, and tillage practices; however these are generally not highly effective (Martin et al., 1991; McMullen et al., 1997; Parry et al., 1995). At present, FHB is managed primarily through combined approach of moderately resistance cultivars and a triazole fungicide application. While not completely effective, fungicides usually reduce both disease and DON (Jordahl et al., 2010; Ransom et al., 2010). The application of fungicide is most effective if sprayed prior to infection. However, when conditions for disease development are favourable, infection and DON contamination cannot be avoided, even when integrated management practices are implemented. Recent research work is focused on the identification of plant genes which enhance trichothecene resistance and ultimately, FHB resistance in barley. Moreover, efforts to develop transgenic barley carrying these genes are ongoing (Boyd et al., 2010; Sallam et al., 2010).

3.4. Cereal industry

Post-harvest decontamination methods to improve the microbiological safety of cereals include heat, ozone, and irradiation based methodologies. Prior to milling kernels are cleaned using screens, air currents, brushes, and magnets, which reduce the total microbial load to about 1 log (Laca et al., 2006; Rose et al., 2012) as well as decreasing other foreign and objects. Proper cleaning and milling processing can reduce the mycotoxin load (Cui et al., 2012). However, after this stage, further microbial elimination steps are limited due to potential concurrent product quality deterioration. Common methods include dry heat and steam applications, irradiation, non-ionizing radiation-like microwave, and radio frequency treatments as well as newer processes, such as, pulsed electric field and high pressure processing. Post-processing contamination can also occur during milling, packaging, shipping, or receiving. Good manufacturing practices (GMP) with an environmental monitoring plan (EMP) associated to risk zones are commonly used to assess the potential for finished product contamination (Akins-Lewenthal, 2012; Rose et al., 2012).

Microbial contaminants are found mostly on the grain surface, although, flour can still retain unsafe contaminants from the field or pre-/post-harvest stages. Even if microbiological properties of flour do not support growth of pathogens, several studies have reported the presence of contaminants in flour, such as *Penicillium* spp., *Aspergillus* spp., *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, and *Salmonella* (Deibel and Swanson, 2001; Eglezos, 2010; Lyon and Newton, 1997).

Common methodologies to minimize fungal spoilage in cereal-based products such as bakery products include modified atmosphere packaging, irradiation, pasteurization, or addition of preservatives such as propionic, sorbic, benzoic acids and their salts (Bouslimi et al., 2008; Eskola et al., 2001).

While most processed foods undergo inactivation steps, some cereal food products like cake mixes, brownie mixes and refrigerated prebaked dough require consumers to perform the baking step. Other products like cold-pressed cereal bars may not be cooked or baked (Rose et al., 2012). This represents a health hazard if the products are not microbiologically stable, as spoilage/pathogenic microorganisms can survive in a dormant state for extended periods in dry flour (Eglezos, 2010) and cause food poisoning

(Magan and Aldred, 2007). Additionally, cereal foods are also susceptible to parse baking environmental contaminations.

Vaclavikova et al. (2012) studied the fate of one of the emergent mycotoxin groups, ENNs, after malting, brewing, milling and baking using both barley and wheat. The authors found that these mycotoxins deferred, not only in physicochemical properties, but also in their incorporation and distribution within infected cereal grains. After milling wheat grains, 40% of the ENNs mycotoxins remained in the final wheat flour, with the highest concentration detected in the bran. After baking, ENNs were still detected in the final bread. By malting, there was a 30% remaining mycotoxins in barley. These mycotoxins were also detected in the spent brewing by-product, which should be taken into consideration when using for food/feed applications.

When several cereal-based products (bakery products, breakfast cereals, and snacks) from the Czech market were analysed for the contents (Malachova et al., 2011), tricothecenes B and four ENNs (A, A1, B and B1) were detected. At least one ENN was present in all of the 160 examined samples, and trichothecenes A and B and ENNs were found in all breakfast cereals tested. DON was frequently detected alone with its derivate DON-3-β-D-glucoside and ENNs, whereas NIV was rarely detected.

During malting, the accumulation of mycotoxins may render the malt useless for grist and other food/feed products. The European Union has regulations with stringent limits for mycotoxin levels of less than 750 ppb (DON) and 75 ppb (ZEA), in cereal flour used. Table 3 shows the European Commission maximum limits for commonly detected mycotoxins in cereal-based products, and examples of detected mycotoxin levels in cereal-based food products from the market.

Malting is a simple process in which a complex ecosystem evolves due to the favourable moisture and temperature conditions, thus allowing contaminating microorganisms to thrive and potentially influence negatively the malt quality (Laitila, 2007; Laitila et al., 2007; Noots et al., 1999; Raulio et al., 2009; Wolf-Hall, 2007). Soaking barley during steeping promotes the microbial biota and biofilm formation (Laitila et al., 2011; Raulio et al., 2009). During the malting process, significant increases in mycotoxin levels can occur (Oliveira et al., 2012b; Vegi et al., 2011). Microbiota competes with grain metabolism for oxygen, therefore reducing grain germination (Doran and Briggs, 1993; Noots et al., 1999). Several *Fusarium* fungal species were shown to proliferate from steeping through germination until early stages of kilning (Oliveira et al., 2012b; Sarlin et al., 2005; Vegi et al., 2011). *Fusarium* mould depletes grain nutrients, such as starch and protein (Fig. 1), and colonizes its interior by hydrolyzing exo-proteolytic and cellulolytic enzymes (Kang and Buchenauer, 2000; Oliveira et al., 2012b, 2013), which might result in significant malting losses (Oliveira et al., 2012b).

Scabby kernels are associated with primary gushing in cereal-based beverages, including beer (Ruiz et al., 2011b). Primary gushing is the sudden overfoaming on opening a cereal-based

Table 3
Mycotoxin levels detected in cereal-base food products available in the market with the respective European Commission maximum permitted levels.

Mycotoxin	Purpose of use	EU maximum permitted level (μg kg^{-1})	Food product	Maximum detected level (μg kg^{-1})	References
Aflatoxin B1	Cereals and processed cereals for direct human consumption	2	Rice	34.1	(Makun et al., 2011b)
			Snacks	23	(Rubert et al., 2013a)
	Cereal based food for infants/children	0.10	Infant cereals	3.11	(Hernández-Martínez and Navarro-Blasco, 2010)
Deoxynivalenol (DON)	Processed cereals for direct human consumption	500	Breakfast cereals	468	(Montes et al., 2012)
			Pale beer	89	(Varga et al., 2013)
			Wheat flour	976	(Škrbić et al., 2012)
	Cereal based food for infants/children	200	Porridge	87	(Pieters et al., 2004)
Enniatins (ENNs)		Not available	Pasta	106	(Juan et al., 2012)
			Multicereal baby food	1100	
			Breakfast cereals	941	(Malachova et al., 2011)
			Flours	2532	
Fumonisins	Maize-based breakfast cereals/snacks	800	Corn meal	8039	(de Castro et al., 2004)
			Corn	204.7	
			Flakes	(FB_1) + 199.9 (FB_2)	(Rubert et al., 2013b)
	Maize-based food for infants/children	200	Instant corn-base	1096	(de Castro et al., 2004)
			Infant cereal	1753	
Nivalenol (NIV)		Not available	Breakfast cereals	31	(Malachova et al., 2011)
			Breakfast cereals	56.7	(Montes et al., 2012)
			Instant-drink powder	79	(Jestoi et al., 2004)
			Bread	169	(Schollenberger et al., 1999)
Ochratoxin A (OTA)	Cereals for direct human consumption	3	Rice	188.2	(Makun et al., 2011b)
			Wheat	2.56	(Salem and Ahmad, 2010)
	Cereal based food for infants/children	0.50	Rice-based baby food	0.20	(Ozden et al., 2012)
T-2 and HT-2	Cereals (except oat)	100	Barley grains	133.2	(Mankevičienė et al., 2011)
			Malting barley	40 (T-2) + 47 (HT-2)	(Barthel et al., 2012)
			Pasta	259.6 (T-2)	(González-Osnaya et al., 2011)
	Cereal products derived from oat	200	Oat flakes	159	(Pettersson et al., 2011)
	Maize-based food for infants/children	50	Cereal-based food	12 (HT-2)	(Schollenberger et al., 1999)
Zearalenone (ZEA)	Processed cereals for direct human consumption	50	Rice	8.8	(Makun et al., 2011b)
			Corn snacks	22.8	(Cano-Sancho et al., 2012)
			Sliced bread	20.9	(Ibáñez-Vea et al., 2011)
			Wheat-base breakfast	38.6	
	Cereal based food for infants/children	20	Market baby food	5.4	(Cano-Sancho et al., 2012)

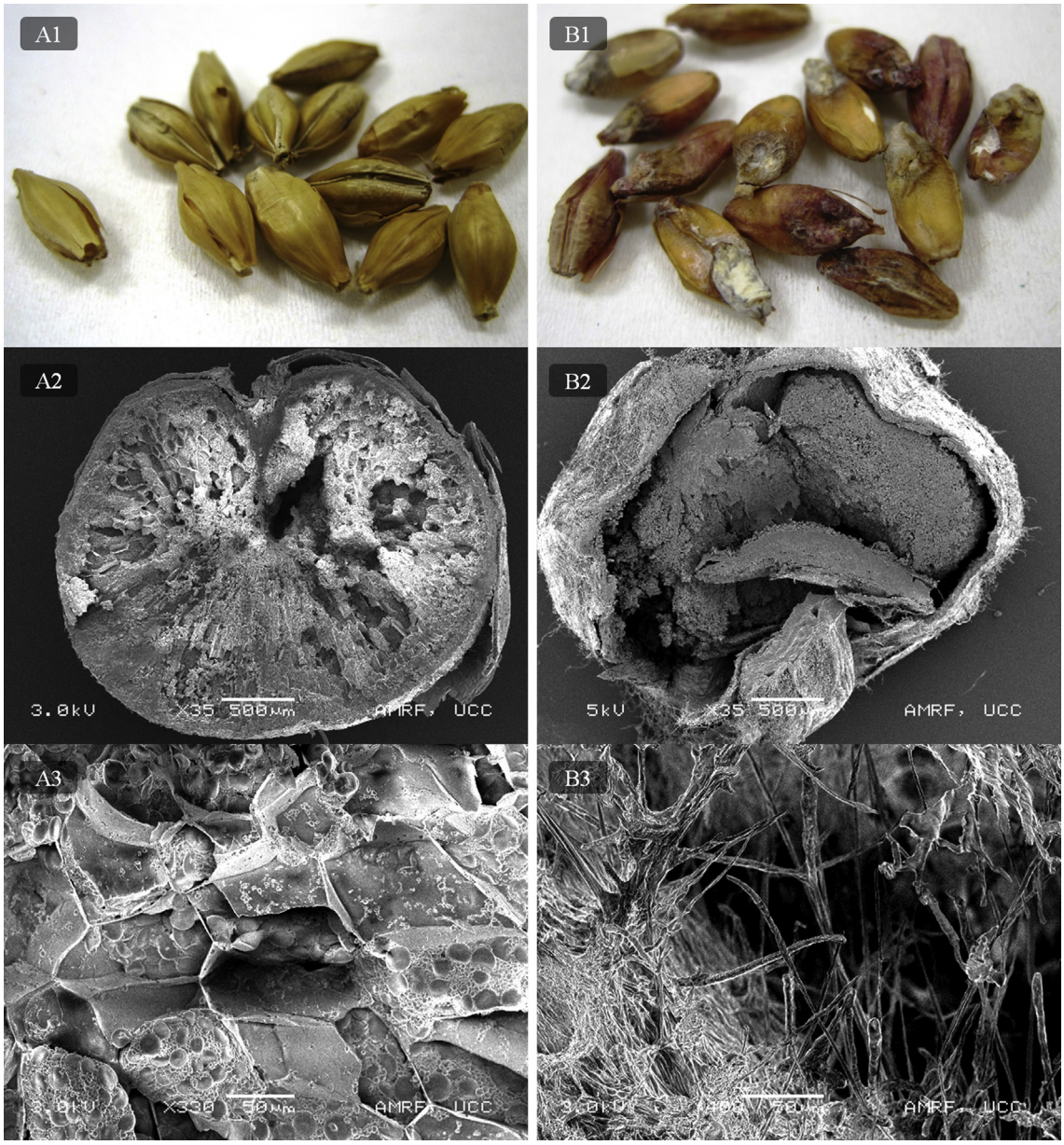

Fig. 1. A. Visual aspect of standard barley malt grains (1), and scanning electron microscope representation with a transversal cut (A2), zoomed (A3), showing an organized ultrastructure. B. Visual aspect of infected barley malt grains (1), and scanning electron microscope representation with a transversal cut (A2), zoomed (A3), showing overgrowth fungal mycelia and damaged ultrastructure.

beverage package that results from fungal infections in the cereal grains (Ruiz et al., 2011b; Sarlin et al., 2005; Shokribousjein et al., 2011). The fungi produce small polypeptide molecules with high hydrophobicity (hydrophobins) that originates nucleation centres and growth of bubbles (Ruiz et al., 2011a; Shokribousjein et al., 2011). *Fusarium* infections are the most problematic and are directly correlated with an increased propensity to produce gushing inducing factors (Haikara, 1983; Sarlin et al., 2007; Tammer et al., 2007). Over the last 10 years, barley and beer contributed with 24% and 8%, of total DON dietary intake in the world, respectively. These amounts have been shown to be region dependent (JECFA, 2010). Infected malt grains (Fig. 1) have a significant impact on wort and beer qualities (Oliveira et al., 2012a). Malt infected with *Fusarium* mould cause premature yeast flocculation, an increased beverage staling character, and mycotoxins, such as DON, accumulate in beer (Oliveira et al., 2012a).

4. Bioprotection

4.1. Fermentation

Earliest records of fermented cereal products appear in the Fertile Crescent (Middle East) dating 6000 BC. Fermentation is a simple and economical way to improve the nutritional value, microbial safety, sensory properties and functional qualities of food. Several indigenous cereal fermented foods and beverages produced worldwide include those which are rice-based (*Idli*, *Dosa*, *Dhokla*); wheat-based (soy sauce, Kishk, Tarhana); maize-based (Ogi, Kenkey, Pozol); sorghum-based (Ingera, Kiska), as well as fermented beverages (beers, sake, Bouza, Bushera, Togwa, Chicha, Mahewu, Boza) (Blandino et al., 2003; Prado et al., 2008).

The fermentation microbiota can be indigenous (autochthonous) or are added as starter cultures (allochthonous). The

fermentation process can be driven by bacteria belonging to the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococus*, *Micrococcus*, and *Bacillus*, in addition to fungi belonging to the genera *Aspergillus*, *Paecilomyces*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Trichothecium*, and common yeasts such as *Saccharomyces* (Blandino et al., 2003).

4.2. Lactic acid bacteria (LAB)

The bioprotective potential of lactic acid bacteria (LAB) has been studied since Louis Pasteur (in 1857) first described the lactic acid fermentation and Lister (in 1873) developed the first pure bacterial culture ("*Bacterium lactis*," Syn.: *Lactococcus lactis*).

LAB are classified as Gram-positive microorganisms which include low GC content as well as being acid tolerant, non-motile, non-spore forming and are rod- or coccus-shaped. LAB ferment carbohydrates to produce various end-products and include homofermenters: *Enterococcus*, *Pediococcus*, *Streptococcus*, *Lactococcus*, *Streptococcus* and some *Lactobacillus* spp. that produce lactic acid as a major end product; as well as heterofermenters such as: *Weissella*, *Leuconostoc* and some *Lactobacillus* that produce equimolar amounts of lactic acid, CO_2 and ethanol. Some strains of *Lactobacillus* and *Streptococcus* can also convert complex sugars (starch) into lactic acid. LAB are microaerophilic and their growth is strictly dependent on the sugars available. They have complex metabolic requirements including amino acids, vitamins, purines, and pyrimidines (Collins et al., 2010; Reis et al., 2012; Valdez et al., 2010). Most plant surfaces in nature are occupied by lactic acid producing microbiota with a tendency for *Lactobacillus* species to become predominant in the fermentation, likely, due to its acid tolerance (Rathore et al., 2012).

LAB have received the GRAS (USA) and the QPS (EU) status, although some species of *Enterococcus* and *Streptococcus* are pathogenic in nature (Collins et al., 2010). Nevertheless, the use of LAB in foods is not regulated by harmonized legislation of the EU level (except in Denmark and France) with regards to their use as starter cultures or as protective culture or food supplements (Franz et al., 2010).

LAB has a large impact on the food industry. It is estimated that over 3400 tonnes of pure LAB cells are consumed every year in Europe alone (Franz et al., 2010). Lactic acid fermentation is considered a simple and safe biotechnology to keep and/or enhance the properties of food. Cereal-based lactic acid fermentations are long-established methods for the production of beverages, gruels, and porridge to improve their nutritional value and digestibility (Kalui et al., 2012).

LAB represents the microbial group most commonly used as protective cultures (Gaggia et al., 2011). These microorganisms enjoy such widespread popularity and acceptance as they play an important role in the manufacture and storage processes by enhancing the shelf-life, microbial safety, texture, sensory characteristics, nutritional value, and overall quality of the fermented products offering beneficial health outcomes to consumers (Di Cagno et al., 2012; Pawlowska et al., 2012; Peres et al., 2012; Ravyts et al., 2012; Vignolo et al., 2012). Additionally, limitations in chemical preservatives and their acceptance give an advantage to LAB-based biopreservatives (Pawlowska et al., 2012; Schnürer and Magnusson, 2005).

Biological preservation refers to the food's shelf-life extension and improvement of their microbial safety by inoculating protective cultures in the food matrix (*in situ* production of antimicrobial compounds), or incorporation of purified microbial metabolites (Gaggia et al., 2011).

LAB antimicrobial activity is primarily attributed to a wide variety of active antagonistic metabolites that include: organic acids (lactic, acetic, formic, propionic, butyric, hydroxyl-phenyllactic acid, and phenyllactic acid (PLA)), or antagonistic compounds (carbon dioxide, ethanol, hydrogen peroxide, fatty acids, acetoin, diacetyl, antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, cyclic dipeptides and 3-hydroxy fatty acids, PLA), bacteriocins (nisin, reuterin, reutericyclin, pediocin, lacticin, enterocin, etc.)), or bacteriocin-like inhibitory substances (Muhialdin et al., 2011a; Reis et al., 2012; Schnürer and Magnusson, 2005). The production of organic acids reduces the pH to below 4.0 which counteracts the growth of spoilage organisms present in cereals (Schnürer and Magnusson, 2005). Generally, inhibitory compounds are most likely LAB secondary metabolites which are produced after 48 h of fermentation (Rouse et al., 2008). Even so, following LAB stationary phase, there's a possibility of cell lyses to contribute to fungal toxicity. Other mechanisms that might explain the inhibitory effect of LAB on fungal infections are the competition of LAB for nutrients, space and exclusion of the pathogen from entry sites in the matrix, and alteration of spore membrane, viscosity and permeability. In addition, the LAB growth range and antifungal spectrum is wide, thus allowing a broad application in food under different conditions (Pawlowska et al., 2012).

LAB are found in many different types of habitats, and exhibit a broad and complex antifungal activity spectrum (Table 4) (Magnusson and Schnürer, 2001; Magnusson et al., 2003). Antifungal LAB have been to that isolated from cereal grains, flours, sourdoughs (Valdez et al., 2010; Wakil and Osamwonyi, 2012), as well as fruit and vegetables. In addition to the studies reported in Table 4, also, one strain of *Pediococcus acidilactici*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus arizonensis*, *Lactobacillus alimentarius*, *Lactobacillus rossiae*, *Leuconostoc mesenteroides*, and *Pediococcus parvulus* were found to express antifungal activity (Florianowicz, 2001; Guo et al., 2011; Lavermicocca et al., 2000; Magnusson et al., 2003; Mandal et al., 2007; Stiles et al., 2002; Valerio et al., 2009). LAB cultures screened and isolated from several sources have shown promising antifungal potential *in vitro* (De Muynck et al., 2004; Florianowicz, 2001; Gerez et al., 2012; Guo et al., 2011; Magnusson et al., 2003; Mauch et al., 2010; Valerio et al., 2009) and *in situ* (Garcha and Natt, 2012; Lan et al., 2012; Rouse et al., 2008; Trias et al., 2008). The antifungal and detoxification potential of LAB has been reviewed by Dalié et al. (2010). LAB are known to inhibit spoilage microorganisms and mycotoxigenic fungal growth because of their acidification and a complex production of low molecular weight compounds during fermentation (Table 4). Several antifungal compounds have been fully or partially characterized (Brosnan et al., 2012; Lavermicocca et al., 2000; Sjögren et al., 2003; Ström et al., 2002; Yang and Chang, 2010). Brosnan et al. (2012) detected 16 antifungal compounds, including five with significantly higher concentrations (Table 4) from *Lactobacillus amylovorus* DSM19280, which had been fermented for 48 h in synthetic media. Organic acids have been shown to be antifungal in several studies (Table 4). Lactic acid is the major LAB metabolite, and other acids like acetic, propionic, formic, benzoic, and PLA acids, are also produced. Organic acids defuse through the membrane of the fungi and subsequently dissociate, thereby releasing hydrogen ions and causing a pH drop. Additionally, organic acids increase the plasma membrane permeability and neutralize the electrochemical proton gradient, thus killing the microorganism. The production of organic acids alone does not explain the antifungal activity and the synergistic effect of antifungal compounds still remains unclear. Several studies assume some kind of positive interaction, although this has not been proven for many metabolites (Ström et al., 2002). PLA (MIC: 50 mg mL^{-1}) has been the subject of many LAB antifungal trials (Table 4) as well as proteinaceous compounds with low molecular weights (especially 2,5-diketopiperazines (cyclic dipeptides))

Table 4
Lactic acid bacteria studied with antifungal activity, their source, antifungal compounds, and spectral inhibitory activity, over the last 10 years.

LAB species (number of strains)	Source	Antifungal compounds	Mould and yeast activity spectrum	Reference
Lactobacillus acidophilus (4)	Chicken intestine, ensilage	Organic acids	*Aspergillus* sp., *Fusarium* sp., *Alternaria alternata*, *Penicillium* sp.	(De Muynck et al., 2004; Garcha and Natt, 2012; Gerez et al., 2012; Magnusson et al., 2003)
Lactobacillus amylovorus (2)	Gluten-free sourdough	DL-ρ-Hydroxyphenyllactic acid, (S)-(−)-2-Hydroxyisocaproic acid, PLA, 3-Hydroxydecanoic acid, 2-Hydroxydodecanoic acid, 3-phenylpropanoic acid, p-coumaric, (E)-2-methylcinnamic acid, 3-phenyllactic acid, 3-(4-hydroxyphenyl)lactic acid, lactic acid, acetic acid, D-glucuronic acid, salicyclic acid	*Penicillium paneum*, *Cerinosterus* sp., *Cladosporium* sp., *Rhizopus oryzae*, *Endomyces fibuliger*, *Aspergillus* sp., *Fusarium culmorum*	(Belz et al., 2012a; De Muynck et al., 2004; Ryan et al., 2011)
Lactobacillus brevis (8)	Brewing barley, sourdough	Proteinaceous, organic acids	*Aspergillus flavus*, *Fusarium culmorum*, *Penicillium sp.*, *Rhizopus oryzae*, *Eurotium repens*, *Trichophyton tonsurans*	(De Muynck et al., 2004; Gerez et al., 2009; Guo et al., 2011; Mauch et al., 2010)
Lactobacillus casei (12)	Dairy products, cheese	ND[a]	*Penicillium* sp., *Trichophyton tonsurans*, *Aspergillus niger*, *Fusarium graminearum*	(Florianowicz, 2001; Gerez et al., 2012; Guo et al., 2011)
Lactobacillus coryniformis (17)	Grass silage, flowers, sourdough	cyclo(L-Phe-L-Pro), cyclo(L-Phe-*trans*-4-OH-L-Pro), PLA, proteinaceous (3 kDa)	*Aspergillus* sp., *Penicillium paneum*, *Cladosporium* sp., *Cerinosterus* sp., *Fusarium* sp., *Rhodotorula* sp., *Mucor hiemalis*, *Talaromyces flavus*, *Debaromyces* sp., *Kluyveromyces* sp.	(De Muynck et al., 2004; Magnusson and Schnürer, 2001; Magnusson et al., 2003; Ström et al., 2002)
Lactobacillus fermentum (2)	Fermented food, dairy products	Proteinaceous (<10 kDa)	*Aspergillus niger*, *Penicillium* sp., *Fusarium graminearum*	(Gerez et al., 2012; Muhialdin et al., 2011b)
Lactobacillus paracasei (2)	Cheese, kefir	Proteinaceous (43 kDa)	*Fusarium* sp., *Saccharomyces cerevisiae*, *Candida* sp.	(Atanassova et al., 2003; Franco et al., 2011)
Lactobacillus paracollinoides (2)	Fresh vegetables	ND[a]	*F. graminearum*, *Rhizopus stolonifer*, *Sclerotium oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia minor*	(Sathe et al., 2007)
Lactobacillus pentosus (2)	Sourdough, fermented food	ND[a]	*Fusarium* sp., *Aspergillus* sp.	(Franco et al., 2011; Muhialdin et al., 2011b)
Lactobacillus plantarum (30)	Flowers, sourdough, grass silage, sorghum, wheat, dairy products, sausages, wheat semolina, *kimchi* (Korean pickles), malted barley, fresh vegetables	Organic acids, PLA, 4-hydroxyphenyllactic acid, cyclo(L-Phe-L-Pro), cyclo(L-Phe-*trans*-4-OH-L-Pro), 3-phenyllactic acid, proteinaceous, ethanol, ethyl acetate, 3-hydroxy fatty acids, cyclo(Leu-Leu), cyclo(L-Leu-L-Pro)	*Penicillium* sp., *Monilia* sp., *Aspergillus* sp., *Fusarium* sp., *Eurotium sp.*, *Talaromyces* sp., *Epicoccum* sp., *Cladosporium* sp., *Rhizopus stolonifer*, *Sclerotium oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia minor*, *Endomyces fibuliger*, *Rhodotorula* sp., *Candida albicans*, *Debaryomyces hansenii*, *Kluyveromyces marxians*, *Saccharomyces* sp., *Phichia* sp.	(Coda et al., 2011; Dal Bello et al., 2007; De Muynck et al., 2004; Franco et al., 2011; Gerez et al., 2012, 2009; Lavermicocca et al., 2000; Magnusson and Schnürer, 2001; Magnusson et al., 2003; Rouse et al., 2008; Sathe et al., 2007; Sjögren et al., 2003; Ström et al., 2002; Valerio et al., 2009; Yang and Chang, 2008, 2010)
Lactobacillus reuteri (3)	Sourdough, porcine and murine gut	Acetic acid, PLA, organic acids	*Penicillium* sp., *Trichophyton tonsurans*, *Fusarium graminearum*, *Aspergillus niger*	(Gerez et al., 2012, 2009; Guo et al., 2011)
Lactobacillus sakei (2)	Dandelion flour and leaves	ND[a]	*A. fumigatus*, *F. sporotrichioides*	(Magnusson et al., 2003)
Lactobacillus salivarius (2)	Chicken intestine	ND[a]	*A. nidulans*, *F. sporotrichioides*, *P. commune*	(Magnusson et al., 2003)
Lactococcus lactis (4)	Sourdough, wheat semolina	ND[a]	*Penicillium* sp., *Eurotium sp.*, *Monilia sp.*, *Aspergillus sp.*, *Endomyces fibuliger*	(Florianowicz, 2001; Lavermicocca et al., 2000; Valerio et al., 2009)
Leuconostoc citreum (2)	Sourdough, wheat semolina	ND[a]	*Aspergillus niger*, *Eurotium* sp., *Penicillium roqueforti*, *Monilia* sp., *Endomyces fibuliger*	(Lavermicocca et al., 2000; Valerio et al., 2009)
Pediococcus pentosaceus (19)	Sorghum, fermented food, fresh vegetables, malted cereals	Proteinaceous, possibly cyclic acids	*Penicillium* sp., *Aspergillus sp.*, *Fusarium* sp., *Rhizopus stolonifer*, *Sclerotium oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia minor*, *Rhodotorula* sp.	(Magnusson et al., 2003; Muhialdin et al., 2011b; Rouse et al., 2008; Sathe et al., 2007)
Weissella cibaria (16)	Brewing barley, sorghum, wheat semolina, fermented wax gourd, fruit and vegetables	Proteinaceous, organic acids	*Fusarium culmorum*, *Penicillium* sp., *Aspergillus* sp., *Rhodotorula* sp., *Endomyces fibuliger*	(Lan et al., 2012; Mauch et al., 2010; Rouse et al., 2008; Trias et al., 2008; Valerio et al., 2009)
Weissella confusa (2)	Sorghum, wheat semolina	Proteinaceous, organic acids	*Penicillium* sp., *Aspergillus nidulans*, *Rhodotorula* sp., *Endomyces fibuliger*	(Rouse et al., 2008; Valerio et al., 2009)
Weissella paramesenteroides (8)	Fermented wax gourd	Organic acids	*Penicillium* sp., *Fusarium graminearum*, *Rhizopus stolonifer*, *Sclerotium oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotinia minor*	(Lan et al., 2012; Sathe et al., 2007)

[a] ND is not determined.

(Broberg et al., 2007; Ryan et al., 2009b). Specific examples of cyclic dipeptides isolated from LAB broth include: cyclo(Gly-Leu), cyclo(Phe-Pro), cyclo(Phe-OH-Pro), and cyclo(Leu-Leu). Ryan et al. (2009b) presents an extensive list of antifungal cyclic dipeptides with confirmation that heat and acidification are initiators of 2,5-diketopiperazines formation. Also, to a lesser extent, phenolic compounds and hydroxy fatty acids (MIC: 10/100 μg mL^{-1}) have shown potential antifungal activity. Hydrogen peroxide (MIC: 0.025%), in the presence of oxygen, exhibits antifungal activity with oxidizing potential on the fungal membrane and proteins. Reuterin can be anaerobically produced from glycerol in starving cells and subsequently inhibit the fungal growth.

The LAB-fungal interactions tend to be complex in nature and this, combined with matrix intricacies, provide the hurdle relating to study and isolation of certain antifungal compounds (Schnürer and Magnusson, 2005). The antifungal activity of LAB depends on the growth media, the temperature and incubation time, the pH, nutritional factors, antifungal substance and production levels, mode of action, and MIC (Dalié et al., 2010; Muhialdin et al., 2011b). Sodium acetate, which can be present in media, was seen to have inhibitory synergetic effects on fungal growth (Stiles et al., 2002). Previous studies have reported the thermostable antifungal activity of LAB, such as: *Lactobacillus coryniformis*, *Leuconostoc citreum*, *L. mesenteroides*, *Lactobacillus plantarum*, *L. rossiae*, *Lactobacillus pentosus*, and *Lactobacillus fermentum* (Magnusson and Schnürer, 2001; Muhialdin et al., 2011b; Okkers et al., 1999; Valerio et al., 2009; Yang and Chang, 2008).

4.2.1. LAB in plant protection

Annual fungal-associated crop losses in the US alone exceed $1 billion per year. These diseases, such as FHB, are difficult to control as they rapidly decimate cereal crops in a brief period, during flowering, and under certain environmental conditions, which are outside farming control. There are many ways to manage plant diseases including: genetics, crop rotation, tilling fields, and biological control which involve the use of antifungal microorganisms (Hell and Mutegi, 2011). It is estimated that the agricultural chemical industry produces over 45,000 different artificial pesticides/fertilizers worldwide. Even if chemicals show attractive applications in crop protection (Lamberth, 2009), they acidify the soil, and thus decrease beneficial organism populations and interfere with plant growth. The pressure to reduce the use of insecticides, fungicides and herbicides paves the way for agricultural systems with greater levels of sustainability (Chandler et al., 2008). Very few biological controls are available, and permitted chemical disease management products (e.g. copper, elemental sulphur, vinegar, silica) have a high risk of phytotoxicity, often with a very small margin of error (MAFRI, 2012). However, effective, ecological disease management programs require a high level of knowledge and management.

In Western countries, agricultural practices lie between sustainable (permaculture or organic), and intensive (industrial) farming approaches. Organic farming tends to produce lower crop yields than conventional agriculture, however, it has many advantages in terms of its emphasis on renewable resources, ecology, and biodiversity (Chandler et al., 2008). Furthermore, organic farming aims to reduce mould (and mycotoxins) present in the field (Bernhoft et al., 2012; Tsitsigiannis et al., 2012), though this is an arguable subject matter, as previously discussed in section 3.3.2.

Microorganisms that control plant diseases operate through one or more mechanisms including the production of antimicrobial compounds, direct antagonism of pathogens, competition with pathogens for space and nutrients and the induction of host resistance to disease (Compant et al., 2005). Microbial interactions and cooperation in the rhizosphere can contribute to plant crop biocontrol (Barea et al., 2005; Whipps, 2001).

LAB show great potential to be applied in plant protection programs, and although explored to a lesser extent, it has been shown that LAB can have critical effects on fungal pathogenicity (Frey-Klett et al., 2011). The LAB acidification can reduce the post-harvest decay caused by pathogens (Prusky et al., 2006) and inhibit the production of mycotoxins (Tsitsigiannis et al., 2012). A useful LAB application concept for plant protection has been developed as a product called EM (Effective microorganisms) (Higa and Parr, 1994). Spraying diluted solutions of LAB onto the plant and soil are hypothesized to assist plant health and growth. EM is an example of a naturally fermented microbial cocktail using microorganisms which include lactic acid bacteria, yeast, and phototrophic bacteria. These are neither harmful, nor pathogenic or genetically engineered/modified. Species used in products, such as EM, are often dominated by photosynthetic bacteria, lactic acid bacteria, and yeasts: *Bacillus subtilis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus buchneri*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *L. delbrueckii*, *L. fermentum*, *L. plantarum*, *Lc. diacetylactis*, *Lc. lactis*, *Rhodopseudomonas palustris*, *R. sphaeroides*, *Saccharomyces cerevisiae*, and *Streptococcus thermophilus* (Capriotti et al., 2012; EM, 2009; García-Cela et al., 2012). A coculture has the advantage of providing different metabolites. EM represents a low-cost microbial technology which, when optimized for a specific geographical and ecosystem, can be beneficial in agriculture (Ya and Partap, 1996). Its application in the field promotes crop growth and yields, increase photosynthesis, increase plant disease's resistance, promotes soil and crop detoxification, improves water treatment, suppresses soil borne pathogens, promotes the growth of naturally occurring beneficial microbes in agricultural environment; improves composting, and improve the technological recycling of all kind of materials (Asam and Rychlik, 2013; Capriotti et al., 2012; EC, 2003; García-Cela et al., 2012; Sherif et al., 2009). EM can be applied at the different plant growth stages with specific dosages to counteract specific fungal infections. This kind of approaches can be used as an alternative to agricultural chemicals, as a processing tool to manufacture organic fertilizers, to improve soil's microbiota, and promote a healthy environment for plants (EM, 2009; Gourlay, 2012; Serrano et al., 2012; Sobrova et al., 2010). Nonetheless, effective application measures in field still need further optimization (Leblanc et al., 2005).

One of the major beneficial indigenous microorganisms used in natural farming are *Lactobacillus*. When applied to the soil or the leaves, LAB aid in the composition process, thus allowing more food to be assimilated by the plant. LAB producing enzymes and natural antibiotics also helps effective digestion with antibacterial properties, including control of microbial pathogens (e.g. *Salmonella* or *E. coli*) (Carandang, 2006). Some LAB strains inhibit more than one phytopathogen, which is an advantage when managing a wide range of plant protection.

Biocontrol agents have been investigated as control agents for cereal diseases caused by *Fusarium* species, including FHB (Khan and Doohan, 2009; Khan et al., 2006). The application of microbial starter cultures in the field by spray has proven to be efficient in reducing *Fusarium* contamination, decrease water sensitivity with increases in extract, FAN and alpha-amylase activity (Lowe and Arendt, 2004; Reinikainen et al., 1999).

LAB is also used to preserve cereal and grass silage inhibiting detrimental bacteria and fungi (Broberg et al., 2007; Kung and Ranjit, 2001), and feed biopreservation (Melin et al., 2007). Broberg et al. (2007) found several antifungal metabolites present in silage including lactic acid, 2,3-butanediol, 3-hydrohydecanoic acid, 3-phenyllactic acid, which were already seen to be produced by LAB. LAB enhanced the production of hydrocinnamic acids and cinnamic acids. The compounds azeleic acid and (*trans*,*trans*)-3,4-

dihydroxycyclohexane-1-carboxylic acid were dependent of LAB presence. Diketopiperazines were also present in both inoculated and non-inoculated silage with LAB. It was proposed by the authors that LAB strains in grass silage might promote the production of antifungal substances.

Several microorganisms have been subject of study in bioprotection of cereal plants (de Souza et al., 2012; Santoyo et al., 2012; Turan et al., 2012). Even if belonging to species other than LAB, the antifungal potential can be attributed to the production of antifungal substances such as organic acids and peptides (Wang et al., 2012). Symbiosis is common in nature. Therefore, it is worth exploring the potential of endophytes in cereal disease suppression (O'Hanlon et al., 2012; Rodriguez et al., 2009). Also interesting are the plant natural resistance elicitors, such as β-amino butyric acid (BABA), benzothiadiazole (BTH), 2,6-dichloroisonicotinic acid (INA), and methyl jasmonate (MeJA), with potential to neutralize fungal infections (Lyon and Newton, 1997; Small et al., 2012). Resistance elicitors can be inoculated into crops to induce a faster response against phytopathogenic microorganisms and thus, contribute in favour of disease resistance (Lyon and Newton, 1997; Small et al., 2012).

4.2.2. LAB in malting

The application of antimicrobial LAB during malting and brewing can be successfully applied as a hurdle to spoilage microorganism growth (Rouse and van Sinderen, 2008; Wolf-Hall, 2007). Rouse and van Sinderen (2008) proposed that LAB with an antifungal bioprotective capacity can be applied in the early stages of malting, or in wort production, or for bioacidification purposes (Lowe and Arendt, 2004; Vaughan et al., 2005).

Malt is a product of industrial relevance with a growing market outlet, where the majority is used in the brewing and distillation industry. LAB is present in grains ($\geq 10^8$ CFU g^{-1}) during steeping. *Leuconostoc* species and *Lactobacillus* tend to dominate during steeping and germination, respectively (Booysen et al., 2002; Justé et al., 2011).

LAB can act as food-grade biocontrol agents contributing with beneficial effects to malting like the production of antimicrobials, hydrolytic enzymes and hormones, thus improving malt modification (Laitila, 2007; Laitila et al., 2011).

Several LAB species including *Streptococcus alactolyticus*, *L. sanfranciscensis*, *Lactobacillus salivarius*, *Lactobacillus reuteri,* and *Weissella paramesenteroides,* have been shown to substantially reduce *F. culmorum* contamination in malt (Liske et al., 2000). *L. plantarum* and *Pediococcus pentosaceus* (10^7 CFU g^{-1}) were added as starter cultures in barley grain steeping water (Haikara and Laitila, 1995; Laitila et al., 2006) increasing the lactic acid production, thereby inhibiting the growth of spoilage bacteria and *Fusarium* fungi. *Weissella confusa* FST 1.31, *L. plantarum* TMW 1.460, and *L. amylovorus* FST 1.1 also showed promise as a *Fusarium* spore proliferation inhibitor (Lowe et al., 2006). Furthermore, Laitila et al. (2002) used cell-free extract of *L. plantarum* species (E76 and E98) to inhibit *Fusarium* mould metabolism. In addition, *Fusarium* proliferation was successfully restricted during malting of naturally infected two-row barley grains, applying *L. plantarum* E76 cell-free-extract in steeping water (Laitila et al., 2002).

In addition to the inhibition of mould growth, LAB can have an active role in detoxifying infected grains. LAB-mediated detoxification in grains is through absorbing mycotoxins by the bacterial cell structure or metabolic biodegradation (Dalié et al., 2010). This topic was reviewed by Shetty and Jespersen (2006). The authors show that LAB pre-incubation with intestinal mucus results in reduced aflatoxins binding the gastro-intestinal tract. LAB, including *L. rhamnosus* and *Propionibacterium freudenreichii* have been shown to bind different *Fusarium* mycotoxins (e.g. DON, NIV, fusarenon-X (FUS-X), T-2 toxin, HT-2 toxin), and *Aspergillus* mycotoxins (e.g. aflatoxin B1, B2, G1, G2) (Shetty and Jespersen, 2006). Franco et al. (2011) isolated several LAB with antifungal activity and capacity to remove DON from products or habitats associated with *F. graminearum*. The authors found that, using viable LAB cells, DON reduction ranged from 16 to 56%, while dead cells reduced DON by 35–67%, respectively. Inactivated cells showed the greatest potential for DON reduction, which proves that the most likely mechanism of detoxification was by absorption of mycotoxins by the cell walls of the LAB. This is supported by previous studies where peptidoglycan and polysaccharides present in the cell walls of LAB, after heat or acid treatments, increase their pore sizes, thus improving the capacity to accumulate the toxins (Niderkorn et al., 2006, 2009)

In addition to antimicrobial benefits of LAB application, it is widely accepted that a reduced pH in mashing is beneficial for the brewing process. LAB can be added to green malt for bioacidification of mash and wort, holding germinated barley under anaerobic conditions to produce lactic acid. Kilning the grains will concentrate the acid, thus, acidifying the mash. Kunze (2010) used successfully *L. delbrueckii* for mash acidification. The resulting acidification also serves to reduce rootlets growth and, thus prevent high malting losses (Mauch et al., 2011). LAB such as *L. amylovorus*, *L. amylolyticus*, *L. plantarum*, and *P. pentosaceus* can enhance the technological performance and malt characteristics, contributing to grain's enzymatic activity (Laitila et al., 2006; Lowe and Arendt, 2004; Lowe et al., 2005; Malfliet et al., 2010).

4.2.3. LAB in bakery products (sourdough)

Spontaneous sourdough fermentation is one of the oldest cereal fermentations and paradoxically is an important modern biotechnological process. Sourdough bread is prepared from a mixture of flour and water which is fermented by lactic acid bacteria (10^9 CFU g^{-1}), and yeast into natural ratio of 100:1. LAB sourdough typically includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, or *Weissella* genera. Mainly heterofermentative strains, producing lactic acid and acetic acid in the mixture, give pleasant sour flavour to bread (Chavan and Chavan, 2011). *L. plantarum* dominates in the European sourdoughs (Chavan and Chavan, 2011; Gobbetti et al., 2005).

Sourdough has many functionalities and advantages as the metabolic activity of LAB during sourdough fermentation improves several dough properties, thus, bread quality (Arendt et al., 2007; Corsetti and Settanni, 2007; Ravyts et al., 2012; Zannini et al., 2009).

Additionally, sourdough also retards the bread staling and microbial spoilage processes. Mould is the most common contaminant responsible for spoilage of bakery products. *Penicillium* spp. are responsible for the majority of bread spoilage followed by *Fusarium*, *Clodosporium*, *Rhizopus*, and *Aspergillus* (Pateras, 2007; Smith et al., 2004). Several sourdough LAB produce inhibitory substances (Messens and De Vuyst, 2002), as previously discussed in Section 4.2.2 PLA has been reported as one of the most abundant acids found in sourdough products (Ryan et al., 2009a). A PLA producing LAB (*L. plantarum* 21B) delayed *Aspergillus niger* growth for an extra 7 days in bread, in comparison to the controls (mould after 2 days) (Lavermicocca et al., 2000). In a separate study, PLA produced by *Propionibacterium freudenreichii* and two strains of *L. plantarum* showed a broad spectrum of activity with concentrations ranging from 3.75 to 7.5 mg mL^{-1} (Table 3) (Lavermicocca et al., 2003). The bread shelf-life was extended by more than two days for most strains and at pH 4.0; PLA inhibited more than 50% of fungal growth (7.5 mg mL^{-1}). In addition, this is non-toxic and odourless, thus giving it potential for use in food industry (Lavermicocca et al., 2003).

Due to its antifungal characteristics, many sourdough LAB are effective replacements for chemical preservatives (Arendt et al., 2007; Chavan and Chavan, 2011; Gänzle et al., 2007; Hansen and Schieberle, 2005; Liu et al., 2008; Ravyts et al., 2012). The inclusion of several antifungal LAB strains enabled the reduction of the chemical additive calcium propionate (CP) by 50% in wheat dough, whilst maintaining the same bread shelf-life (Dal Bello et al., 2007; Gerez et al., 2009). *L. plantarum* 1A7 sourdough was seen to inhibit fungal contamination for up to 28 days of storage under normal bakery conditions which compares well to a 0.3% CP formulation (Coda et al., 2011). In this study, nine novel antifungal peptides were identified. Ryan et al. (2011) used 20% sourdough bread fermented with *L. amylovorus* DSM19280, which inhibited the growth of *F. culmorum*, *A. niger*, *Penicillium expansum* and *Penicillium roqueforti* more effectively than CP in wheat bread systems. Antifungal LAB sourdough has inhibitory activity against mould and still produces bread of good quality (Dal Bello et al., 2007; Ryan et al., 2011).

Bread contributes significantly to daily salt intake, and the consumption of low-salt bread represents an emerging market in the food business. However, salt acts as a preservative agent in bread and reducing salt levels also reduces bread shelf-life. Additionally, bread is a high moisture product with an $a_w > 0.95$ (Smith et al., 2004), and, therefore, is easily infected by mould (Belz et al., 2012b; Quilez and Salas-Salvado, 2012). In a recent study, Belz et al. (2012a) used sourdough fermented with antifungal *L. amylovorus* DSM19280 for the production of low-salt breads. This increased bread shelf-life even longer than by using 0.3% CP formulations. The trials were conducted under normal bakery conditions and the breads challenged against typical bread spoilage fungi. In this study, 23.8% sourdough in breads, without salt, challenged against *P. expansum*, had five days of shelf-life, in comparison to the control wheat bread (4 days). *F. culmorum* was completely inhibited over the 14 days of the trial, with an extra 11 shelf-life days in comparison to the control breads (3 days), and *A. niger* was inhibited for an extra five shelf-life days, in comparison to the control (3 days) (Belz et al., 2012a).

5. Conclusions

Food safety is of fundamental importance to both the consumer and food industry for health and economical reasons. New fungal threats continue to arise with more aggressive pathogens adapting to the environment and producing emergent mycotoxins. Infections in cereal-crops are one of the primary vectors for food chain fungal infections. Developing natural, safe and healthy food products represents a challenge, where lactic acid fermentation plays an important biopreservative role. LAB and its metabolites represent a viable solution to implement biocontrol technology in agricultural management programs and as biopreservatives in food systems. Due to its broad antifungal spectrum, and the multifold nature of antifungal metabolites produced, LAB can be applied from crop farming to cereal food products, as well as in other food matrices. Furthermore, the bioprotection can be applied as an additional hurdle technology to already established GMP guidelines.

6. Future trends

LAB species are reliable candidates to develop stabilizing agents for application as useful biostrategies in food preservation systems. In particular, advanced molecular approaches will increase data acquisition on a genetic level allowing further understanding of pathogen-host and pathogen-biocontrol microbe interactions. Additionally, the comprehension of the *in situ* behaviour of starter cultures by means of proteomics and metabolic interspecies quorum sensing research will elucidate the underlying action mechanisms on fermentation processes and microbial adaptation strategies. This will allow us to obtain bioprotective cultures with improved capabilities. Furthermore, LAB protective cultures will find further application in food systems as the use of chemical agents will continuously decrease with cheaper processing at industrial scale. The food industry will focus on work implementation according to a clean label technology, which forces manufactures to further manage and explore autochthonous microbial sources to counteract fungal infections.

The scientific methodology and developing control of LAB-containing bioprotection systems have to be employed through pathogen-specific biocontrol targets. This is vital having into account the huge external variability's arising in agricultural systems. As such, future focus must be placed on producing biocontrol tools which can maintain functionality in a dynamic microbial environment.

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