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Appraisal of lactic acid bacteria as protective cultures

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ABSTRACT

Food industry employs various physical and chemical methods to control fungal contamination of food products. However, many fungal strains are defiant to these techniques as a result of various resistance mechanisms they acquired with time. The synergistic actions of various antimicrobial compounds produced by lactic acid bacteria (LAB) prevent the growth of food spoilage bacteria and fungi. Antimicrobial peptides and phenolic compounds from LAB have been successfully applied in wheat grain preservation against fungi. Bread made from sourdoughs fermented with various LAB strains is found to prevent or delay fungal attack. Apart from the mycotoxin removal, some of these bioactive from LAB have antioxidant and anti-cancer potential and it further enhances nutritional value and safety of food products. This review focuses on recent research developments on the bioactive potential of compounds from LAB as well as the commercially available protective cultures and biopreservatives based on LAB and thus tried to substantiate its status as protective culture.

1. Introduction

Food, by its very nature is a rich nutrient source and attracts microbial colonization as a suitable habitat. Nutritional properties of the food alter successful colonization of the microbes and when the nutritional value, structure, and taste of the product are harmfully affected, it is called food spoilage. Many strains of food spoilage fungi including *Aspergillus*, *Penicillium* and *Fusarium* species are capable of producing mycotoxins which cause serious health problems once consumed. These are often produced when the fungus is under stress, in particular, when the temperature, water activity or amount of oxygen becomes less. Mycotoxins of health and agro-economic impact include aflatoxins, trichothecenes, zearalenone, ochratoxins, fumonisins, tremorgenic toxins, and ergot alkaloids (Hussein & Brasel, 2001). Aflatoxin B_1, the potent hepatocarcinogenic toxin, has been recently proven to be genotoxic also. Ochratoxin A is nephrotoxic and nephrocarcinogenic and zearalenone, produced by different strains of *Fusarium* has an osteogenous property and is toxic to the reproductive system of animals (Milicevic, Skrinjar, & Baltic, 2010; Zain, 2011). Since filamentous molds and yeasts are common spoilage organisms of food products like fermented milk products, cheese, bread as well as stored crops, and feed such as hay and silage, the risk of mycotoxins incorporate into food and feed are very high. Cereals go through the processes of cultivation, harvesting, drying, preparation, and storage under natural conditions before being consumed as food, and therefore, often involve microbiological contamination and infection. Between 5 and 10% of the world's food production is estimated to be lost due to fungal attack (Oerke & Dehne, 2004; Pitt & Hocking, 2009). Fisher et al., (2012) reported the destruction caused by fungi to wheat, rice and maize costs global agriculture $60 billion per year. The effects are devastating for those in the developing world, where people rely most on these food products.

Lactic acid bacteria (LAB) produce a variety of antifungal compounds, the synergistic action of which prevent the growth of a broad range of fungi and can be used as protective culture for improving microbiological safety of food products without changing their sensory characteristics (Florou-Paneri, Christaki, & Bonos, 2013). LAB occur naturally in different food sources and have been used for centuries in food fermentation and became a part of human diet without any adverse health effects which procured them the 'GRAS' (generally recognized as safe) status. The long tradition of using LAB in food and feed substantiated with recent scientific understanding on its antifungal efficacy and enhanced health effects (Divya, Varsha & Nampoothiri, 2012; Divya, Varsha, Nampoothiri, Ismail & Pandey 2012) suggest them as perfect alternatives to chemical preservatives.

Abbreviations: 2, 4 DTBP, 2, 4-di-*tert*-butyl phenol; AMP, Antimicrobial peptide; CFS, Cell free supernatant; LAB, Lactic acid bacteria; GRAS, Generally recognized as safe.

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2. Control of food spoilage fungi and their mechanism of resistance

Control of spoilage fungi has been achieved through techniques like dehydration (freeze and heat drying), cold storage, heat and microwave treatment as well as ultrasound and irradiation which come under physical methods of food preservation (Farkas, Doyle, & Beuchat, 2007). The organic acids such as acetic, lactic, propionic, sorbic and benzoic acid are used as chemical food preservatives to manage fungal growth. Despite of the application of these methods to control fungal growth on food, different species of fungi and yeast prevail over those conditions leading to colonization and deterioration of food. The resistant mechanisms of some of these strains are identified to genetic level. Many strains of *Zygosaccharomyces bailii* are resistant to weak acid preservatives such as sorbic acid and acetic acid due to the low intracellular pH of certain proportion of cells (Stratford et al., 2013). In *Saccharomyces cerevisiae*, resistance to acetic acid is acquired by loss of plasma membrane aquagleceroporin that facilitates passive diffusional entry of undissociated acid into cells through activation of the high-osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) signaling cascades that activate multifunctional Hog1 MAPK. This Hog1 then phosphorylates the plasma membrane aquaglyceroporin, Fps1 (fdp1 suppressor) and results in degradation (Mollapour & Piper, 2007). Carboxylate preservatives propionate, sorbate or benzoate, once inside the cell activates War1p, a transcription factor that activates the gene for the Pdr12p plasma membrane ATP-binding cassette transporter. Pdr12p decreases the intracellular levels of propionate, sorbate or benzoate by catalyzing the active efflux of the preservative anion from the cell (Hatzixanthis et al., 2003). In *Candida glabrata* HOG pathway is activated by sorbic acid and confers resistance along with the drug: H^+ antiporter CgAqr1 (ORF*CAGL0J09944g*) that is identified as the determinant of resistance to acetic acid as well as the antifungal agents flucytosine and clotrimazole (Costa et al., 2013; Jandric, Gregori, Klopf, Radolf, & Schuller, 2013). *Aspergillus niger* decarboxylate weak acid preservatives and cause spoilage. The decarboxylation mechanism was carried out by the Pad-decarboxylation system encoded by a gene cluster in germinating spores of *A. niger* and involves activity by two decarboxylases, PadA1 and OhbA1 along with a regulator, SdrA (Stratford et al., 2012).

Because of such resistance mechanisms by these food pathogens, there is need to find out a new strategy to control their occurrence in food and feed. Since LAB are normal inhabitants of various food systems, their potential to prevent the growth of fungi can be envisaged.

3. Role of LAB as protective culture

LAB have been reported to produce wide range of fungal growth inhibiting substances such as organic acids including hydroxyl fatty acids, low molecular weight bioactive compounds and proteinaceous compounds. Selected LAB strains (as in fermentation) or the bioactives purified from the culture medium can be exploited as efficient alternatives for food preservation. Oranusi, Wesley, and Oguoma (2013) reported the antifungal activity of *Lactococcus lactis* against various *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. Growth inhibitory action of *Lactobacillus*, *Enterococcus* and *Leuconostoc* cultures were reported against varying fungal groups such as *Debaryomyces hansenii*, *Saccharomyces cerevisiae* and *Penicillium* sp. (Voulgari et al., 2010). Application of *L. plantarum* IMAU10014 against *Botrytis cinerea*, *Glomerella cingulate*, *Phytophthora drechsleri Tucker*, *P. citrinum* and *Fusarium oxysporum* (Wang, Yan, Wang, Zhang, & Qi, 2012) as well as the use of *Lactobacillus* and *Weisella* cultures against *A. niger* MUCL 28699, *Candida albicans* MUCL 30112, *Aspergillus tubingensis* MP1 and *P. crustosum* MY1. Ndagano, Lamoureux, Dortu, Vandermoten, and Thonart (2011) have been reported. *Lactobacillus* also reported to have strong inhibitory activity against the human pathogenic fungi *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum* (Guo et al., 2013). Le Lay et al., (2016) recently reported the *in situ* antifungal activity of LAB and propionibacterium against bakery product contaminating molds. LAB are inhibitory to many fungal pathogens and at the same time they coexist with various yeast strains during fermentations. Lattanzi et al., (2013) reported the coexistence of different LAB strains along with *S. cerevisiae* and *Candida humilis* in various sourdoughs such as Pandoro and Mbriagotto. Strong aggregation between LAB and the yeasts *Geotrichum candidum*, *Pichia galeiformis* and *Candida sorbosa* were observed on fermented olive epidermis (Arroyo-Lopez et al., 2012). This explains LAB can be used in food products along with yeast strains of industrial importance to increase the product quality.

3.1. Aflatoxin removal

Several LAB species are reported to be able to bind aflatoxin B_1 which is classified by the International Agency for Research in Cancer as a class 1 human carcinogen and reduced bioavailability in the medium (Gratz, Mykkanen, & El-Nezami, 2005). *Aspergillus flavus* growth inhibition and aflatoxin B_1 (AFB_1) degradation in kutukutu, fermented maize-based dough was achieved with various strains of LAB (Roger & Leopold, 2015). Corassin, Bovo, Rosim, and Oliveira (2014) showed that pool of LAB strains in combination with *S. cerevisiae* cells bound (100%) and reduced the concentration of aflatoxin M_1 (AFM_1) in milk. Kachouri, Ksontini, and Hamdi (2014) studied the effect of application of *L. plantarum* on AFB_1 during storage of olives and observed that the amount of AFB_1 was reduced to 5.9 μg/kg from 11 μg/kg along with 86.3% decrease in the amount of molds. Results also showed an increase of amount of total phenolic compounds and antioxidant activity of olives, respectively, by 8.6 and 24%. Elsanhoty, Ramadan, El-Gohery, Abol-Ela, and Azeke (2013) demonstrated that fermentation with a mixture of yeast and *L. rhamnosus* removed AFs from contaminated wheat flour during baking process and the authors conclude that LAB and bifidobacteria can be used for aflatoxin detoxification. Pizzolitto, Salvano, and Dalcero (2012) removed fumonisin B_1 (FB_1) from liquid medium using a combination of *S. cerevisiae* CECT 1891 and *Lactobacillus acidophilus* 24.

3.2. LAB and biopreservation

Fermentation is a long age process used to improve the quality and shelf life of food products and the secret of their extended shelf life is the presence of antimicrobial LAB, which are natural inhabitants of fermented foods. The presence and function of different LAB strains in fermented foods like rice-wine, beer-takju, tapuy; acid leavened bread, noodle-idli, puto, khanomjeen; fermented vegetable-kimchi, dhamuoi, burong; fermented fish and meat-sikhae, narezushi, nham are well described. The biologically active compounds in kimchi include benzyl isothiocyanate, indol compounds, thiocyanate and sistosterol which are antibiotic, anticarcinogenic, immune-stimulant and cholesterol reducing in their activity respectively (Rhee, Lee, & Lee, 2011). The cell free supernatant (CFS) of *Lactobacillus fermentum*, *Pediococcus pentosaceus*, *Lactobacillus pentosus* and *Lactobacillus paracasei* delayed the growth of fungi for 23–40 days at 4 °C and 5–6 days at 20 and 30 °C in tomato puree, 19–29 days at 4 °C and 6–12 days at 20 and 30 °C in processed cheese and 27–30 days at 4 °C and 12–24 days at 20 and 30 °C in commercial bread (Muhialdin, Hassan, & Sadon, 2011).

Studies have been conducted regarding the application of LAB as