



**BIOLOGICAL CONTROL OF MOLDS AND MYCOTOXINS CONTAMINATION
IN FOOD AND DAIRY PRODUCTS****WAFIK S. RAGAB^{1,2*}, NANIS H. GOMAH^{3,4} AND MOHAMED A. ABDEIN^{5*}**¹Dept. of Biology, College of Sci., Univ. of Hafr Al Batin, P.O. Box 1803, Saudi Arabia²Food Sci. & Technol. Dept., College of Agric., Univ. of Assiut, Egypt³College of Sci., Univ. of Hafr Al Batin, P.O. Box 1803, Saudi Arabia⁴Dairy Department, Faculty of Agriculture, Assiut University, Egypt⁵Dept. of Biology, Faculty of Arts and Science, Northern Border University, Rafha, Saudi Arabia***Corresponding Author: Mohamed A. Abdein, E Mail address: abdeingene@yahoo.com**

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ABSTRACT

Huge amounts of food and dairy products wasted worldwide because of their contamination by the harmful fungi that cause spoilage. In order to avoid this huge economic loss, some technological treatments were applied to preserve foods from fungal contamination; the most common ways at the industrial level are using of physical and chemical preservation methods. Recently, there is a growing societal demand supported by public authorities for less processed and preservative-free foods and the use of natural alternative to keep it from fungal contamination. This review presented to give an overview on the biological preservation of food and dairy products against the contaminant fungi and their toxins. Many strategies for control of mold growth and mycotoxins production demonstrated based on researches indicate that antagonistic microorganisms or their antimicrobial metabolites have some potential as bio preservatives to control undesirable fungi. Recent researches discussed with respect to underlying mechanisms of inhibition by protective bacterial cultures, and special reference made to lactic acid bacteria (LAB) and their 'food-grade' safety. Many other types of bacteria showed different effects in inhibition of fungal growth. In addition, yeasts possess many properties that make them useful in controlling fungal growth, eliminate or reduce the mycotoxin levels by degradation or detoxification them in food and dairy

products. Non-mycotoxigenic fungi belonging to various genera and species developed to create bio control formulations by outcompeting the toxigenic strains. However, the best way for avoiding mycotoxin contamination in food and dairy products is to prevent mold growth since there are limitations of degradation or detoxifications.

Keywords: Biological, control, fungi, food, dairy, mycotoxin.

INTRODUCTION

Food products are a rich nutrient source attracts both fungal and bacterial colonization. Food products could be contaminating with a wide range of microorganism during harvesting, processing and handling operations. The exact statistic of the total economic loss by food spoilage is unknown. It is estimated that one-fourth of the global food supply is wasted because of post-harvest microbial spoilage (Gram, et al., 2002). The susceptibility does depend on factors such as, a nature of food, chemical composition, physical and biological properties, management during harvesting of fruits, storage conditions and technological processes applied during manufacture. These factors usually lead to the selection or dominance of fungal on food product (Abualreish and Abdein (2014), Abigail & Randy, 2018).

Fungi colonization could be accompanying by the production of toxic secondary metabolites called mycotoxins, which is result in grave medical problem. Spoilage of foods by fungi is a major threat. Which makes it a very relevant research area with

respect to the demand for foods. Knowledge concerning the mechanisms might generate novel insight that results in increased food and not to an increase in the land (Dijkstrhuis et al., 2013).

The preservation strategies of food applied in the industry involve chemical and physical techniques. The strategies methods are decreasing fungal infection and contamination elimination. Some chemical preservatives could use for the control of mycotoxins. The sorbate salts level during cheese manufacture under the Federal Standard of Identity is 0.2% to 0.3%. This level can inhibit the mold growth that contaminates the cheese (Liewen and Marth, 1985). High sodium chloride affects the required level of water for toxin and growth. Sodium and ion transport interferes in the organism (Thanaboripat et al., 1992). To avoid the addition of different chemical preservatives. The presence of competing microorganisms deals to restricts mycotoxin and fungal (Bullerman et al., 1984). Bio-preservation technologies are favored to nutrition, safety, and food organoleptic properties due

to some consumer demands (Bourdichon et al., 2011).

This review is to report the advances on the use of antifungal microorganisms as biological control agents against the spoiler fungi and their mycotoxins in food and dairy products.

1- Fungal contamination in fruits and vegetables

Fruits and vegetables as well as all agricultural crops prone to fungal during harvesting, marketing, transporting, and storage under domestic conditions. A survey study conducted by Khashaba, et al. (2018) concerning contamination of various types of fruits and their products by fungi and mycotoxins revealed that fresh grape and dried grape fruits (raisins) were highly contaminated by fungi and their toxins because of its rich chemical composition. Three hundred and forty-five fungal isolates belonging to 12 fungal genera obtained from 40 samples of fresh fruits and 30 samples of dried fruits. *Aspergillus* was the most predominant genus followed by *Penicillium*, *Alternaria* and *Fusarium*, respectively. They also

reported that one sample of fresh fruits was found to be contaminated with aflatoxins B₁ and G₁. Meanwhile, seven samples of raisins were contaminating with ochratoxin A and aflatoxins B₁, B₂ and G₁.

A large number of studies was carry out to ass's fungal contamination in different categories of raw and processed foodstuffs, Frisvad, et al., (2007).

For controlling the contamination foods with mycotoxins. It is very necessary to understand the conditions and for agreed to the growth of molds and mycotoxins. Mycotoxins are dependent on a factors such fungal strain, water activity, substrate, temperature, aeration and gas composition (Bullerman et al., 1984; Varga et al., 2005). Different fungi such as *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Claviceps* and produce various types of mycotoxins (Victor Kagot et al., 2019). More than 300 different mycotoxins were identifying, with approximately 30 toxins adverse health on vertebrates upon ingestion (Alassane-Kpembé, et al., 2017 and Alshannaq, et al., 2017).

Table 1: The fungal species isolated from some raw and processed foodstuffs (Frisvad et al., 2007)

Crop	Production	Fungal
Peas and Beans	Cowpeas, Black Beans	<i>Asp. parasiticus</i> , <i>Alternaria alternata</i> , <i>Penicillium citrinum</i> , <i>Aspergillus flavus</i> , <i>Asp. Ochraceus</i> , <i>Fusarium proliferatum</i>
Cereal	Wheat, oat, barley, rye	<i>P. polonicum</i> , <i>P. aurantiogriseum</i> , <i>Asp. versicolor</i> , <i>P. discolor</i> , <i>P. verrucosum</i> , <i>P. cyclospium</i> , <i>P. nalgiovense</i> , <i>P. commune</i> , <i>P. solitum</i> , <i>P. melanoconidium</i> , <i>P. freii</i>
	Rye bread	<i>Eurotium rubrum</i> , <i>Eur. repens</i> , <i>P. roqueforti</i> , <i>P. paneum</i> , <i>F. verticillioides</i> , <i>Asp. flavus</i> , <i>P. citrinum</i> , <i>F.</i>

		<i>semitectum, Eur. Rubrum, Eur. Repens, P. carneum, Alt. alternate</i>
	Maize	<i>P. citrinum, Asp. ochraceus, Asp. flavus, Asp. niger, F. verticillioides, F. graminearum, F. proliferatum</i>
	Wheat bread	<i>Alt. tenuissima, Asp. flavus, Asp. parasiticus, F. culmorum, F. graminearum, F. avenaceum,</i>
	Rice	<i>P. citrinum, Asp. niger, Asp. flavus,</i>
Cheeses	Hard cheese	<i>Asp. Tamaritii, Asp. niger, Asp. Candidus</i>
Coffee	Coffee-monsoon	<i>Asp. westerdijkiae, P. citrinum, Asp. steynii, Asp. Carbonarius</i>
	Coffee-traditional	<i>Asp. flavus, P. digitatum, P. italicum, Asp. carbonarius, Asp. niger, Asp. Ochraceus, Alt. turkisafria, Alt. tenuissima, Alt. tangelonis</i>
Fruits	Dried fruits	<i>Asp. Tubingensis, Asp. carbonarius, P. expansum, Asp. Niger</i>
	Grapes	<i>P. olsonii, Eurotium spp., P. nalgiovensis, P. nordicum, P. chrysogenum,</i>
	Citrus	<i>Talaromyces spp., Byssochlamys nivea, Neosartorya spp., B. spectabilis Eupenicillium spp.</i>
	Fruit juice	<i>F. lateritium, P. solitum, Alt. tenuissima sp.-grp., P. expansum, P. crustosum, Alt. arborescens sp.-grp.</i>
	Pomaceous and stone	<i>P. crustosum, F. avenaceum, Asp. flavus, Asp. tamaritii, P. discolor, F. acuminatum, F. semitectum, Alt. arborescens sp.-grp., Asp. Niger</i>
Meat	Sausages	<i>Alt. alternate, Asp. versicolor, Asp. Niger, P. expansum Peanuts Asp. flavus, P. citrinum</i>
Nuts	Almonds, hazelnuts, pistachio, walnuts	<i>Asp. parasiticus, F. verticilloides, Alt. alternata, Asp. flavus, Asp. niger, F. semitectum</i>
Oil crops	Olives	<i>Petromyces alliaceus Alt. alternate, P. glabrum, P. brevicompactum P. allii,</i>
	Sunflower	<i>F. sambucinum, P. tularense, Asp. parasiticus, F. coeruleum, Alt. alternata, Alt. solani, Asp. flavus, Asp. Tamaritii</i>
Vegetables	Onion and garlic	<i>P. sclerotigenum</i>
	Ginger	<i>Asp. flavus, P. olsonii, Alt. tenuissima sp.-grp., F. verticillioides, Alt. alternata, Alt. subtropica, P. expansum, Stemphylium turmiunum, Stemphylium solani</i>
	Pepper-black	
	Pepper-bell	
	Yams	
	Tomatoes	<i>Botryosphaeria rhodina, Asp. Niger</i>
	Potatoes	

Fungi and mycotoxins in Dairy products

Many types of cheese are excellent materials for the proliferation and growth of mold. The common fungi are isolated from cheese belong to the Cladosporium, Aspergillus, Mucor, Geotrichum, genera Penicillium, and Trichoderma (Taniwakiet *et al.*, 2001). In the cheese's production should be select carefully fungal cultures that have low toxigenic whereas the mold cheeses should not be consumed. A

mycotoxin is found in dairy products: (1) indirect contamination (aflatoxin M1), (2) direct contamination (intentional or accidental growth). The mycotoxin is can be stabled in the cheeses. It is *citrinin, roquefortine C, sterigmatocystin, penitrem A, and aflatoxins* (Sengun, *et al.* 2008).

Cheese is susceptible to fungal (Van, 1983). Dairy product with undesirable molds, like *Penicillium* and *Aspergillus* is a disturbing problem because their growth is

caused alteration with health hazard mycotoxin (Filtenborg *et al.*, 1999). Aflatoxins are both acute and chronically toxic to the animal, humans, liver damage, and tumors (Stoloff, 1977). They are frequently produce of *A. parasiticus*, *Aspergillus flavus*, and described species *A. nomius* (Klich and Pitt, 1988). The genera growth on cheese leads to get some

changes effects on the products. Development of mold in the cheese means the sign of spoilage (Bullerman, 1981). Mold species have toxigenic used to products that have organoleptic (mold ripen cheeses). This cheese is ripening by *Penicillium*, which grow by molds or in cheeses and it produce using starter mold culture (Beresford, 2001).

Table 2: Mycotoxins isolated from cheeses (Sengun, *et al.*, 2008)

Cheese	Mold	Type of mycotoxins produced
Blue	<i>P. roqueforti</i>	PR toxin, isofumigaclavine A, Roquefortine
Blue moldy Tulum cheese	<i>P. roqueforti</i>	PR toxin, Roquefortine, Patulin, penicillic acid
Blue-molded	<i>P. roqueforti</i>	Mycophenolic acid
Blue-veined	<i>A. fumigates</i>	Kojic acid
	<i>P. roqueforti</i>	undefined toxic metabolites, Penicillic acid
	<i>A. versicolor</i>	Sterigmatocystin
Camembert	<i>P. camemberti</i>	Cyclopiazonic acid
Kashar	<i>Penicillium spp.</i>	Citrinin, penicillic acid, cyclopiazonic acid, patulin
Cheddar	<i>P. roqueforti</i>	Patulin, Penicillic acid
Taleggio	<i>Penicillium spp.</i>	Patulin, Citrinin
	<i>Penicillium spp.</i>	Cyclopiazonic acid
Manchego	<i>Aspergillus spp.</i>	Aflatoxin M1
	<i>P. roqueforti</i>	Mycophenolic acid Prato and Parmesan, Roquefortine

Restriction of fungal growth and mycotoxins production by some Antagonistic microorganism

A microorganisms were test for antifungal against food spoilers. The microorganisms were tested as bio protective tools in food.

1- Bactria

Nesciet *et al.* (2005) found that several strains of *Bacillus subtilis* and *Pseudomonas solanacearum* were able to inhibit aflatoxin. Palumbo *et al.* (2006) showed a number of *Pseudomonas*, *Bacillus*, *Ralstonia* and *Burkholderia* could completely inhibit *A. flavus*. Bluma and Etcheverry (2008) reported a significantly reduction in Aflatoxin B₁ when *A.*

flavus grew with *B. amyloliquefaciens*. Pereira *et al.* (2007) reported that *B. amyloliquefaciens* was shown to effectively reduce *Fusarium verticillioides* propagation and fumonis in production in maize kernels at harvest when applied as seed coating. Bacon *et al.* (2001) reported that *B. subtilis* proved to be promise for reducing mycotoxin contamination by *F. verticillioides*. Three bacterial strains namely *Bacillus amyloliquefaciens* BICS 08b, *B. subtilis* AUMC 63 and *Pseudomonas fluorescence* BICS 0149b were tested by Marwa Abdel-Karim (2014) to inhibit mycotoxins by 11 toxigenic fungi's. Results showed that

Pseudomonas fluorescence BICS 0149b completely inhibited growth of nine fungi and completely inhibited toxin production by the 11 tested fungi. *Bacillus subtilis* AUMC 63 reduced fungal growth of the 11 fungal isolates by 40-100%, and reduced their toxins formation by 20- 100%. *Bacillus amyloliquefaciens* BICS 08b completely inhibited toxins formation by all the studied fungi although their growth was not highly affected. **Hikal, (2018)** studied the antibacterial activity of piperine and black pepper oil.

Lactic acid bacteria (LAB)

In few decades, there was a growing interest in bio-preservation, like the use of metabolites and microorganisms to inhibit spoilage and increasing the foods. LAB is of special interest as bio-preservative microorganisms. Their preserving effect is related to the formation of lactic and acetic acids; competition for nutrients; and bacitracin (**Lindgren and Dobrogosz, 2001 and Stiels, 1999**). **Chelkowski (1989)** explained that lactic acid bacteria have the ability as natural food-grade bio-control agents of mycotoxin and mold. **Florianowicz (2001)** reported the ability of *Lactococcus cremoris* to control mycotoxinogenic mold growth. **Haskard et al. (2001)** showed that viable cell of *L. casei* and *L. lactis* remove 21.8% and 59% of Aflatoxin B₁, respectively. **El-Nezami**

et al., (2002) showed that strains of *L. rhamnosus* have the ability to remove 55.0 % of Zearalenone and its derivative α -zearalenol with a rapid reaction instantly after mix it with bacteria. **Nanis and Zohri (2014)** examined Lactobacilli (*L. paracasei* subsp. *Paracasei* and *L. rhamnosus*) for their ability to stop mycotoxins by *F. proliferation*, *F. culmorum*, and *F. graminearum*,. Which are the producers of Fumonisin B₁, Zearalenone, and mycotoxins Deoxynivalenol, respectively. *L. paracasei* subsp. *paracasei* stopped the Deoxynivalenol, Zearalenone, and Fumonisin B₁ by 56.8%, 73.0%, and 76.5%, respectively. *L. rhamnosus* showed the highest inhibitory against fungal and mycotoxins.

Nanis et al., (2009) studied the antifungal of *L. plantarum* ATCC 4008, *Lactobacillus plantarum* 299V, *L. plantarum* 12006, *L. rhamnosus* VTI, and *L. paracasei* subsp. *Paracasei* LMG 13552 against cheese (*A. parasiticus* NRRL2999, *A. flavus*, *A. versicolor*, *P. communi*, and *Penicillium roqueforti*). The Aflatoxin B₁ created by *Aspergillus flavus* in the presence of these *Lactobacilli* reduced between 85% to 98.3% compared with the control treatment.

Nanis(2012) examined the ability of three different strains of *Lactobacillus plantarum* to inhibit production and growth

and of mycotoxins by *Fusarium*. The target molds were *F. graminearum*, the main producer of *F. proliferatum*, Deoxynivalenol, the main producer of Fumonisin B₁ and *F. culmorum*, which produce Zearalenone. The tested bacterial strains showed varying degrees of growth inhibition as well as mycotoxins production. *L. plantarum* ATCC 4008 and 299V strains were totally suppressed the production of Deoxynivalenol and reduced the amount of zearalenone and Fumonisin

B₁(95%). *L. plantarum* 12006 reduced the Deoxynivalenol, Fumonisin B₁ and Zearalenone by about 84.8%, 70% and 92 %, respectively compared with the control treatment. **Hikal, (2015)** studied the importance of eggplant peels anthocyanins on edible oils as natural antioxidants.

The over mentioned studies indicated that many Lactic acid bacteria have potential as food - grade bio-control agents of mycotoxins and molds contamination of foods.

Table 3: Antifungal metabolites of lactic acid bacteria, their sources, and spectral inhibitory activity, (Pedro Oliveira et al., 2014)

LAB	Source	Antifungal	Mold and Yeast
<i>Lactobacillus amylovorus</i> (2)	Gluten-free sourdough	DL-r-Hydroxyphenyllactic acid, PLA, 3-Hydroxydecanoic acid, 2-Hydroxyisocaproic acid, 2-Hydroxydodecanoic acid, 3-phenylpropanoic acid, p-coumaric, 2methylcinnamic acid, lactic acid, 3-phenyllactic acid, 3-(4-hydroxyphenyl)lactic acid, acetic acid, salicylic acid, D-glucuronic acid	<i>Endomycesfibuliger, Penicilliumpaneum, Fusarium culmorum, Cerinosterus sp., Cladosporium sp., Aspergillus sp., Rhizopus oryzae</i>
<i>Lactobacillus coryniformis</i> (17)	Sourdough, Grass silage, Flowers	PLA, proteinaceous (3 kDa), cyclo(L-Phe-trans-4-OH-L-Pro), cyclo(L-Phe-L-Pro)	<i>Cladosporium sp., Debaromyces sp., Cerinosterus sp., Fusarium sp., Rhodotorula sp., Mucorhiemalis, Talaromycesflavus, Kluyveromyces sp., Aspergillus sp., Penicilliumpaneum</i>
<i>Lactobacillus brevis</i> (8)	Sourdough, Brewing barley	Proteinaceous compounds, Organic acids	<i>Rhizopus oryzae, Trichophyton tonsurans, Aspergillus flavus, Eurotiumrepens, Fusarium culmorum, Penicillium sp.</i>
<i>Lactobacillus acidophilus</i> (4)	Silage, Chicken intestine	Organic acids	<i>Penicillium sp., Fusarium sp., Alternaria alternate, Aspergillus sp.</i>
<i>Lactobacillus casei</i> (12)	Cheese, Dairy products	ND	<i>Aspergillus niger, Penicillium sp., Fusarium graminearum, Trichophyton tonsurans</i>
<i>Lactobacillus paracollinoides</i> (2)	Fresh vegetables	ND	<i>Aspergillus sp., Fusarium sp.</i>
<i>Lactobacillus fermentum</i> (2)	Dairy products, Fermented food	Proteinaceous (10 kDa)	<i>Candida sp., Fusarium graminearum, Fusarium sp., Saccharomyces cerevisiae, Penicillium sp., Aspergillus niger</i>
<i>Lactobacillus pentosus</i> (2)	Fermented food, Sourdough	ND	<i>Penicillium sp., Monilia sp., Phichia sp., Aspergillus sp., Eurotium sp., Talaromyces sp., Epicoccum sp., Cladosporium sp., Saccharomyces sp., Rhizopusstolonifer, Rhizoctonia solani, Candida</i>

			<i>albicans, Botrytis cinerea, Sclerotinia minor, Endomycesfibuliger, Rhodotorula sp., Debaryomyceshanseni, Kluyveromycesmarxians, Fusarium sp., Sclerotium oryzae</i>
<i>Lactobacillus paracasei</i> (2)	Kefir, Cheese	Proteinaceous (43 kDa)	<i>Sclerotium oryzae, F. graminearum, Sclerotinia minor, Rhizopusstolonifer, Rhizoctonia solani, Botrytis cinerea</i>
<i>Lactobacillus plantarum</i> (30)	Sourdough, malted barley, grass silage, sorghum, wheat, sausages, Wheat semolina Flowers, fresh vegetables, dairy products, kimchi (Korean pickles)	Organic acids, cyclo(LPhe-L-Pro), PLA, 4-hydroxyphenyllactic acid, cyclo(L-Phe-trans-4-OH-LPro), 3-phenyllactic acid, ethanol, proteinaceous, ethyl acetate, 3-hydroxy fatty acids, cyclo(L-Leu-L-Pro), cyclo(Leu-Leu)	<i>Aspergillus niger, Trichophyton tonsurans, Fusarium graminearum, Penicillium sp.</i>
<i>Lactobacillus salivarius</i> (2)	Chicken intestine	ND	<i>Penicillium sp., Endomycesfibuliger, Eurotium sp., Aspergillus sp., Monilia sp.</i>
<i>Lactobacillus reuteri</i> (3)	Porcine and murine gut, Sourdough,	Acetic acid, organic acids, PLA	<i>F. sporotrichioides, A. fumigatus</i>
<i>Lactobacillus sakei</i> (2)	Flour and leaves, Dandelion	ND	<i>P. commune, A. nidulans, F. sporotrichioides</i>
<i>Leuconostocitreum</i> (2)	Wheat semolina, Sourdough	ND	<i>Rhizoctonia solani, Penicillium sp., Rhodotorula sp., Fusarium sp., Rhizopusstolonifer, Sclerotinia minor, Sclerotium oryzae, Botrytis cinerea, Aspergillus sp.</i>
<i>Lactococcus lactis</i> (4)	Wheat semolina, Sourdough,	ND	<i>Monilia sp., Penicilliumroqueforti, Aspergillus niger, Endomycesfibuliger, Eurotium sp.</i>
<i>Weissellacibaria</i> (16)	Brewing barley, sorghum, fermented wax gourd, fruit and vegetables, wheat semolina,	Organic acids, Proteinaceous,	<i>Endomycesfibuliger, Aspergillus nidulans, Rhodotorula sp., Penicillium sp.</i>
<i>Pediococcus pentosaceus</i> (19)	Sorghum, malted cereals, fermented food, fresh vegetables	Possibly cyclic acids, Proteinaceous	<i>Fusarium culmorum, Endomycesfibuliger, Penicillium sp., Rhodotorula sp., Aspergillus sp.</i>
<i>Weissellaparamesenteroides</i> (8)	Fermented wax gourd	Organic acids	<i>Rhizoctonia solani, Sclerotinia minor, Botrytis cinerea</i>
<i>Weissellaconfusa</i> (2)	Wheat semolina Sorghum	Organic acids, Proteinaceous	<i>Sclerotium oryzae, Fusarium graminearum, Rhizopusstolonifera, Penicillium sp.</i>

ND: Not detected

2- Yeasts

Yeast is considering one of the potent bio-control agents due to non-toxic and biology properties. It (specially, *S. cerevisiae*) has properties make it useful for control purposes. It generally recognized as safe (GRAS). It does not produce allergenic or mycotoxins or antibiotic metabolites

such as generate by bacterial antagonist (Droby and Chalutz, 1994). It has β -glucan in their cell wall as immune-stimulator and in activator for mycotoxins. Yeasts have a nutritional requirement and eligible to colonize dry surfaces in a long time (El-Tarabily and Sivasithamparam, 2006). It can make on inexpensive

substrates in fermenters in large quantities (Druvefors, 2004).

Stinson et al. (1978) showed degradation of patulin during fermentation of apple juice by *S. cerevisiae*. Polonelli and Morace, (1986) and Magliani et al., (1997) showed that *Saccharomyces cerevisiae* strains were killer toxin effective against other yeasts, bacteria and filamentous fungi. Roostita et al., (2011) reported that yeasts have potential antimicrobial inhibit the molds. Masoud et al., (2005) showed that *P. kluyveri* and *Pichia anomala* inhibited the ochratoxin by *A. ochraceus*. Armando et al., (2013) explain that *S. cerevisiae* RC016 and RC008 were stopped *F. graminearum* and *A. carbonarius*.

Four yeast species namely, *Candida krusei* AUMC 8161, *Pichia guilliermondii* AUMC 2663, *Pichia anomala* AUMC 2674, and *Saccharomyces cerevisiae* AUMC 3875 were examined by Marwa Abdel-Karim, (2014) against production and growth of mycotoxins of 11 toxigenic fungal.

Nanis et al. (2017) Examined the antifungal activity of seven selected yeast strains namely *Saccharomyces cerevisiae*, *Candida sake*, *Candida tropicalis*, *Candida utilis*, *Pichia anomala*, *Pichia membranifaciens* and *Pichia guilliermondii* against five species of fungi (*Aspergillus niger*, *Aspergillus ustus*, *Aspergillus flavus*,

Aspergillus fumigates and *Penicillium sp.*) which were previously isolated from the surface of commercial Egyptian Ras Cheese. Each yeast strain was separately applied as life cells and as heat-killed cells on the surface of laboratory manufactured Ras Cheese discs before covering with wax to investigate their inhibitory effect on growth of cheese contaminant fungi during storage for six months under ripening conditions. The obtained results indicated that all the examined yeast strains showed inhibition against all molds. Adherence of life yeast cells on the surface of Ras Cheese discs were more effective in reducing or preventing growth of the contaminant molds than killed yeast cells. Most of the studied yeasts were not able to produce hydrolytic enzymes and the sensory evaluation test of the treated cheese samples showed good acceptability after six months of storage.

Several modes of the bio-control activity of yeast were suggested such as competition for space and nutrients and production of cell wall-degrading enzyme (Pimenta et al., 2009). Mechanisms were reported to play a significant role of bio-control include production of cell wall-degrading enzyme such as β -1, 3-glucanase and chitinase, antifungal diffusible, and volatile metabolites (Castoria, et al. 2001, Höfte et al., 2004).

The extracellular proteases and antimicrobial found in yeasts, enabling them to be exploited as novel agents in the bio-control of food (Fleet, 2003). Yeasts ability in producing sulphur dioxide gives interesting inhibitory of microorganisms (Fleet, 2006). The antimicrobial is one of the methods to maintain microbiological safety and prolong the life of food (Blngöl and Bostan, 2007). The yeasts in such studies included the genera *Saccharomyces*, *Zygosaccharomyces* (Suzziet al., 1995), *Pichia* (Masih and Paul 2002), *Debaryomyces* (Santos et al., 2004), *Candida* (Gamagae et al., 2004) and *Rhodotorula* (Sansone et al., 2005) and *Alternaria solani* (Alhaithloul et al., 2019). Stanley et al. (1993) and Newman (1994) reported that *S. cerevisiae* has the ability to produce enzymes such as proteases and phosphatases that interacts with aflatoxins and stimulate the immune response. Basmacioglu et al., (2005) reported that the glucomannan and β -glucan of yeast cell wall showed considerably high binding ability (80 -97%) with aflatoxins, T-2 toxins, ochratoxins and zearalenone. Baptista et al., (2008) stated removal of mycotoxins by adhesion to cell rather than covalent binding and metabolism. While the dead cells do not lose the binding ability.

2- Fungi

Non-mycotoxigenic fungi belonging to various genera and species have to create bio control formulations by outcompeting the toxigenic. Amaike and Keller (2011) demonstrated that large-scale studies led to the make of biocontrol agents in the application based on the non-aflatoxigenic ability to reduce aflatoxin of agricultural crops. Non-aflatoxigenic and aflatoxigenic strains are chosen by vegetative incompatibility. Which it limits sexual reproduction and exchange of genetic material through the parasexual cycle, ensuring the stability of the bio-control formulations (Ehrlich, 2014). However, Cotty, (1990) explain that the aflatoxin not reduce when aflatoxigenic was inoculated before their non-aflatoxigenic counterpart. Marcia Salas, et al. (2017) stated that not all filamentous fungi are involved in food and post-harvest diseases of the agricultural crops. Some of them are contribute to the favor formation and developing of the visual characteristics of fermented food like surface –ripened cheese, ham and sausage (Banjara et al., 2015; Lozano, et al., 2015 and Alia, et al., 2016). In this respect, both *Penicillium nalgioence* and *P. chrysogenum* were tested against some mycotoxins- producing strains of *Aspergillus* and *Penicillium*. *P. nalgioence* limits the growth and ochratoxin production of *P. verrucosum* (Bernaldez,

et al., 2013). Meanwhile, *P. chrysogenum* inhibited growth of spoilers *P. restrictum* and *A. flavus* in vitro by production of an antifungal protein (Delgado, et al., 2015).

CONCLUSIONS

Bacteria, yeasts and filamentous fungi have been widely studied and generally been shown to act synergistically. Several species of these microorganisms as well as their metabolites have found to be natural alternatives of interest for use in food and dairy products as bio-preservative tools to prevent fungal spoilage and to answer the increasing consumer demand for less processed and more natural food products. However, this approach alone may be insufficient but should be used in conjunction with good agricultural practices coupled with good postharvest management and within the HACCP context as one of the hurdle technology to prevent fungal spoilage.

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