Journal of Microbiology (2014) Vol. 52, No. 10, pp. 807–818
DOI 10.1007/s12275-014-4294-7

MINIREVIEW

The Potential Hazards of *Aspergillus* sp. in Foods and Feeds, and the Role of Biological Treatment: A Review

Sheikh Imranudin Sheikh-Ali[1], Akil Ahmad[2], Siti-Hamidah Mohd-Setapar[1,2*], Zainul Akmal Zakaria[1], Norfahana Abdul-Talib[1], Aidee Kamal Khamis[1], and Md Enamul Hoque[3]

[1]*Institute of Bio Product Development, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia*
[2]*Center of Lipid Engineering & Applied Research (CLEAR), Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[3]*Bioengineering Research Group, Department of Mechanical, Materials and Manufacturing Engineering; University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia*

(Received May 19, 2014 / Revised Aug 4, 2014 / Accepted Aug 5, 2014)

The contamination of food and feed by *Aspergillus* has become a global issue with a significant worldwide economic impact. The growth of *Aspergillus* is unfavourable to the development of food and feed industries, where the problems happen mostly due to the presence of mycotoxins, which is a toxic metabolite secreted by most *Aspergillus* groups. Moreover, fungi can produce spores that cause diseases, such as allergies and asthma, especially to human beings. High temperature, high moisture, retarded crops, and poor food storage conditions encourage the growth of mold, as well as the development of mycotoxins. A variety of chemical, biological, and physical strategies have been developed to control the production of mycotoxins. A biological approach, using a mixed culture comprised of *Saccharomyces cerevisiae* and *Lactobacillus rhamnosus* resulted in the inhibition of the growth of fungi when inoculated into fermented food. The results reveal that the mixed culture has a higher potential (37.08%) to inhibit the growth of *Aspergillus flavus* (producer of Aflatoxin) compared to either single culture, *L. rhamnosus NRRL B-442* and *S. cerevisiae,* which inhibit the growth by 63.07% and 64.24%, respectively.

Keywords: *Aspergillus*, mycotoxins, *Lactobacillus*, *Saccharomyces cerevisiae*, biological treatment

Introduction

Aspergillus has been considered unique among the kingdom Fungi, as it has more than 180 accepted anamorphic species (Pitt *et al.*, 2000; Rodrigues *et al.*, 2007) and can only reproduce asexually (Bennett *et al.*, 2010). These are commonly found in air, water, soil, plant debris, rotten vegetation, manure, sawdust litter, bagasse litter, and animal feed, on animals and in indoor air environments (Pattron, 2006; Bennett *et al.*, 2010). High temperature, high moisture, retarded crops, and poor food storage conditions enhance mold growth and mycotoxin development. *Aspergillus* causes disease (mycotoxicoses) in human beings and animals as well. It is a subgenus in the *Circumdati* section and *Flavi* section, where the genus includes species usually with biseriate conidial heads, in shades of yellow-green to brown and dark sclerotia (Varga *et al.*, 2011). Several species assigned to this section are either important producers of mycotoxins, such as aflatoxins, cyclopiazonic acid, ochratoxins and kojic acid, or are used in oriental food fermentation processes and as hosts for heterologous gene expression. These particular mycotoxins are produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius*, *A. pseudotamarii*, *A. bombycis*, *A. toxicarius*, *A. minisclerotigenes*, *A. parvisclerotigenus*, and *A. arachidicola* in *Aspergillus* section *Flavi* (Samson *et al.*, 2006; Pildain *et al.*, 2008; Riba *et al.*, 2008, 2010; Gandomi *et al.*, 2009; Selouane *et al.*, 2009; Sindhu *et al.*, 2011; Hong *et al.*, 2013). *Aspergillus* is a fascinating group of fungi exhibiting immense ecological and metabolic diversity: *A. flavus*, accompanied by *A. fumigatus*, can cause harmful effects, such as invasive aspergillosis, which is the most common cause of superficial infection [Clinical Microbiology Proficiency testing (CMPT), 2008]. *Aspergillus flavus* colonies actively grow at 37°C and can be colored yellow to dark yellowish-green with a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface. The colour of *Aspergillus* colonies can be influenced by additives in the culture medium, such as yeast extract medium; the colonies commonly appear as having woolly, cottony to granular textures when grown with in the Potato Dextrose Agar (PDA) [Verweij and Brandt, 2007; Clinical Microbiology Proficiency testing (CMPT), 2008]. *A. flavus*, which is similar to *A. parasiticus*, can produce very rough conidia, as well as aflatoxin. *A. flavus* includes 23 species or varieties, including bisexual species, such as Petromyces alliaceus and Petromyces albertensis [Clinical Microbiology Proficiency testing (CMPT), 2008].

Some studies report that an *Aspergillus* sp. (*Aspergillus lentulus* FJ172995) has the potential to remove dye when cultured with two additives, namely glucose, and urea (Kaushik

*For correspondence. E-mail: sitihamidah@cheme.utm.my; Tel.: +607-5535496; Fax: +607-5588166

and Malik, 2011). The results indicate that the initial dye concentration has a depreciative effect on dye removal, while urea is the main factor influencing dye removal and glucose plays a major role in biomass production. Pradeep and Benjamin (2012) indicated that fungi utilize the plasticizer, di(2-ethylhexyl) phthalate (DEHP) blended in PVC blood storage bags (BB). Three types of fungi, *A. parasiticus*, *Fusarium subglutinans*, and *Penicillium funiculosum*, which were isolated from plastics-contaminated soil show the consumption of intact DEHP physically bound to BB by static submerged growth (28°C) in simple basal salt medium (BSM). Ye *et al.* (2011) reported that *A. fumigatus* has a high potential to degrade anthracene. The results show that 60% degradation occurs at optimal conditions (pH 5–7.5 at 30°C). *A. niger* can produce cellobiase, which is used as a thermal enzymatic pretreatment method in a 3:1 mixture of Cellucast 1.5 L and Novozyme 188 (cellobiase from *A. niger*) to improve the enzyme concentrations during pretreatment (Antonopoulou and Lyberatos, 2012). The results indicate the improvement in the process of saccharification and the yield of methane on pretreatment of sweet sorghum biomass. Fakhrul-Razi and Molla (2007) reported that mycelia can be used as biosolid entrapped raw wastewater sludge. This result indicates that fungal entrapped biosolids offered 98% removal of total suspended solids (TSS) in supplemented sludge treatment on the sixth day without any nutrient (wheat flour, WF) supply. *A. versicolor* biomass (AVB) can also be used as a binding mechanism and thus offers the possibility to address environmental pollution (Bairagi *et al.*, 2011). This study concludes that AVB, being the most potent of all fungal biomasses, has been successfully employed for reducing the lead content of the effluents of battery factories up to the permissible limit (1.0 mg/L) before discharging into bodies of water.

Isolation of *Aspergillus* around the world

Earlier studies on various substrates targeted to different degrees by a consortia of organisms from different kingdoms in the ecosystem (Bennett *et al.*, 2010) have produced varying results. Many countries around the world have been doing research on *Aspergillus* groups (Varga *et al.*, 2011), as given in Fig. 1, which demonstrates the phylogenetic trees of isolates in many countries.

Aspergillus as a dominating host through specific vectors

Aspergillus and other molds play an important role in these consortia because they are adapted for growth on recycling starches, hemicelluloses, celluloses, pectins, and other sugar polymers. *Aspergillus* spores are common components of

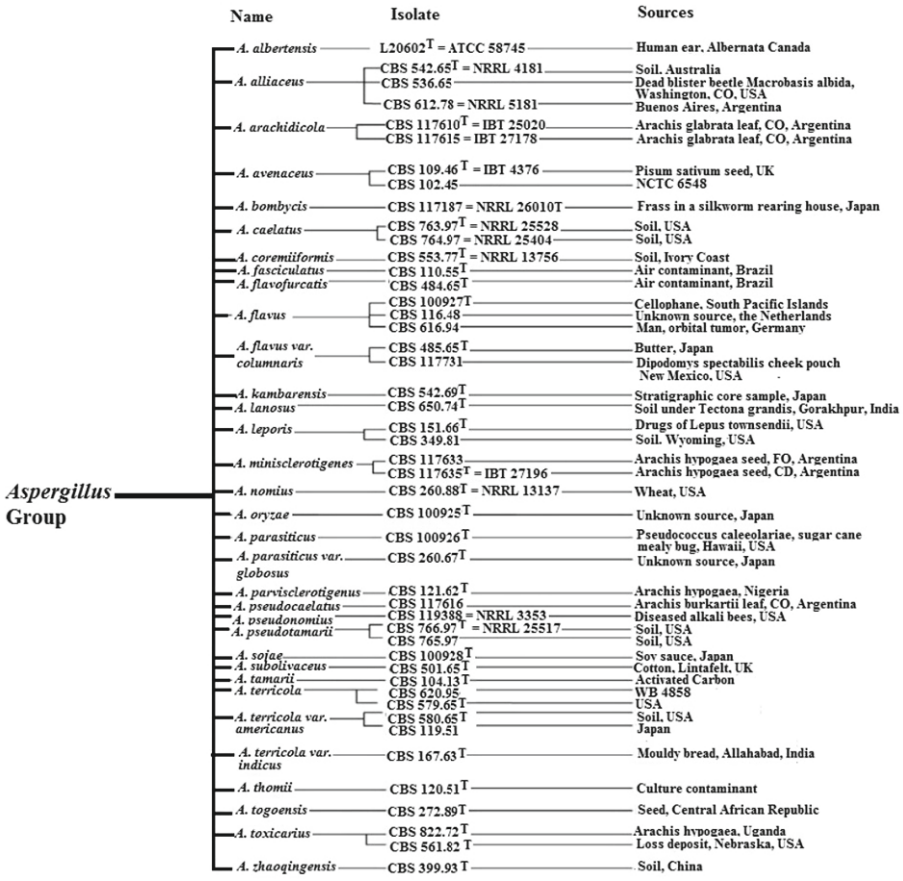

Fig. 1. ***Aspergillus*** **isolation in several countries (Vagra *et al.*, 2011).**

aerosols, where they drift on air currents, dispersing themselves over both short and long distances, depending on the environmental conditions. These include notorious pathogens, such as *A. flavus*, that produces aflatoxin, one of the most potent, naturally occurring compounds known (Bennett *et al.*, 2010). *A. flavus* can produce aflatoxins to contaminate crops, such as maize, cotton seeds, almonds, and others (Rudramurthy *et al.*, 2011). *A. flavus* accumulates when the spores come in contact with solid or liquid surfaces under suitable moisture conditions for germination. It is capable of dispersing in air and growing anywhere as long as appropriate food and water sources are available (Bennett *et al.*, 2010). On the contrary, the population is genetically diversified (Chang *et al.*, 2006).

Advantages of *Aspergillus* in food production

Some of the *Aspergillus* groups are advantageous for the industrial food production; for example, *A. oryzae*, *A. sojae*, and *A. tamari* are used in oriental food fermentation processes and as hosts for heterologous gene expression (Varga *et al.*, 2011; Chancharoonpong *et al.*, 2012). *A. oryzae* is used as genetic modifier to produce enzymes, such as lactase, pectin esterase, lipase, protease, and xylanase (Varga *et al.*, 2011). In bakeries (Sander *et al.*, 1998), *Aspergillus*-derived enzymes are used in dough to improve the quality of baked goods. Recent research indicated that *A. sydowii* MG 49 can produce exosplitting xylobiohydrolase during its growth on xylan (Gosh and Nanda, 1994).

Aspergillus is commonly associated with biomass degradation in addition to the production of a wide range of secreted hydrolases, including native endo- and exo-acting enzymes, which are involved in the degradation of plant cell walls. Parenicova *et al.* (2000) indicated that there are certain type of strains associated with this phenomenon, particularly, the black aspergilli (viz., *A. niger* group is associated with the well-known industrial strains of *A. niger*, *A. aculeatus*, and *A. awamori*). These strains have been used successfully as industrial hosts for the production of various plant cell wall degrading enzymes with cost-effective applications in the food and beverage, animal feed, and paper-and-pulp industries (De Vries *et al.*, 2003). With regard to the production of enzymes, *Aspergillus* has been established as an efficient host for the production of a variety of enzymes, including proteases, lipases, phytases, and numerous glycosyl hydrolases, as well as pharmaceuticals products (Punt *et al.*, 2002).

Meyer *et al.* (2011) reported that an impressive set of promoters are available for filamentous fungi, thus allowing the overexpression of a gene of interest. The promoters are either constitutively active and growth-related, such as the *A. niger* glyceraldehyde-3-phosphate dehydrogenase promoter, P_{gpdA}, or dependent on the carbon or nitrogen source (e.g., *A. niger* P_{glaA} and P_{inuE}, *A. nidulans* P_{alcA}, *Neurospora crassa* $P_{qa\text{-}2}$, *Ustilago maydis* P_{crg1} and P_{nar1}, and *Trichoderma reesei* P_{cbh1}) and some are also leaky (e.g., P_{inuE} and $P_{qa\text{-}2}$). Among the gene promoters that are tight, tuneable, and metabolism-independent, three systems have recently been tested for application in filamentous fungi, such as the thiamine promoter system (P_{thiA}) in *A. oryzae* (Shoji *et al.*, 2005). In a certain study, as reported by Kang *et al.* (2004), cellulases and hemicellulases, which are produced by *A. niger* KK2, were involved in solid state fermentation (SSF) of mixtures of rice straw and wheat bran combined at various ratios (Contesini *et al.*, 2009). The result shows that *A. niger* KK2 that was grown on rice straw, served as the lone solid support in SSF with the maximum FPase activity of 19.5 IU/g in 4 days. The CMCase (129 IU/g) beta-glucosidase (100 IU/g), xylanase (5,070 IU/g), and beta-xylosidase (193 IU/g) activities were concurrently obtained after 5–6 days of fermentation. The higher enzyme activities produced by *A. niger* KK2 is a significant advantage from the perspective of using a practical saccharification reaction. Cellulases and hemicellulases, produced by *A. niger* KK2, might be applied to the pulp and paper industry, animal feed industry, and chemical industry.

Potential hazards of the *Aspergillus* strain and its impact

Morphology of *Aspergillus* and type of mycotoxins secreted

The variations of *A. flavus* species can be characterized into several subgroups. A profound characteristic of *A. flavus* is its sclerotial size where the larger strain (L strain) holds a bigger size compared to the smaller strain (S strain). *A. flavus* L strain produces abundant conidiospores and sclerotia that are usually larger than 400 µm in diameter, while the S strain produces fewer conidiospores and the sclerotia size is usually less than 400 µm in diameter (Horn and Dorner, 1999; Chang *et al.*, 2006). On the other hand, the S strain can produce aflatoxin on an even keel, while L strain cannot produce aflatoxins (Vaamonde *et al.*, 2003; Chang *et al.*, 2006; Pildain *et al.*, 2008). Some other *Aspergillus* species that produce aflatoxins (B_1, B_2, G_1, and G_2) include *A. parasiticus* and *A. nomius* (Hesseltine *et al.*, 1970; Scott, 1987; Abbas *et al.*, 2005). Depending on the type culture of *A. flavus* var. *parvisclerotigenus* produces only B aflatoxins, but other S strain isolates produce both B and G aflatoxins (Novas and Cabral, 2002; Abbas *et al.*, 2005).

Geiser *et al.* reports that the molecular characteristics of *A. flavus* are non-monophyletic and can be categorized into two genetic groups that cannot be readily determined morphologically: group I consists of both L and S strains and produces only B aflatoxins and group II comprises S strains that produce B or B+G aflatoxins (Geiser *et al.*, 1998, 2000; Abbas *et al.*, 2005). According to Chang *et al.* (2006), *A. oryzae* is closely related to *A. flavus*. This is associated with the fact that *A. oryzae* isolates arise from the domestication of *A. flavus* subgroups (Chang *et al.*, 2006). *A. oryzae* possesses the GRAS (Generally Regarded as Safe) status in the food industry, and efforts have been made to develop molecular methods to unambiguously distinguish *A. oryzae* from *A. flavus* using restriction fragment length polymorphisms (Klich and Mullaney, 1987; Chang *et al.*, 2006). The importance of aflatoxin in foods and feeds, coupled with the large variations in toxigenicity, has prompted considerable interest in the intraspecific variability within the species (Abbas *et al.*, 2005).

Effects of mycotoxins on foods and feeds industry

Previous researchers have conducted a study where a total of 2,753 analyses were performed on 1,507 samples sourced from the European and Mediterranean markets, and 6,391 analyses were undertaken on 1,291 samples originated from the Asian-Pacific region (Binder *et al*., 2007). It showed that more than half of the materials sampled in Europe were contaminated at the level above the permitted limit of quantification method applied, and one-third of the tests on Asian-Pacific sourced samples were positive. The study was conducted with *Fusarium* mycotoxins, which shows high impact on feed industry and animal husbandry. These mycotoxins included deoxynivalenol (DON), T-2 toxin, and zearalenone (ZON), as well as, fumonisins B_1, B_2, and B_3. Additionally, mycotoxins that are not produced by *Fusarium*, such as ochratoxin A and aflatoxin B_1, were also tested. Binder indicated that the European samples had DON, ZON and T-2 toxin as major contaminants, while materials from Asia and Pacific tend to be contaminated with DON, ZON, fumonisins and aflatoxins (Binder *et al*., 2007). In addition, the findings also demonstrated that 30% of all mycotoxin tests obtained through the sample from Asian-Pacific countries showed positive tests, while 52% samples in positive ratio was evidenced in European and Mediterranean samples (Binder *et al*., 2007). In summary, the incidence of mycotoxins relevant for animal production is quite high in feeds, although an assessment of the acceptable level is quite difficult.

Previous studies demonstrated that aflatoxins can be isolated from all major cereal crops, even from various sources ranging from peanut butter to marijuana (Bennett and Klich, 2003; Bennett *et al.*, 2010). Aflatoxins are also generally found in the food supply chain of both pet and human foods, as well as in feedstock for agricultural animals during food processing. The study also indicated that the transfer of aflatoxin products can be found in eggs, dairy products and meat when animals are fed with contaminated grains. The metabolites of aflatoxins are deposited in animal tissues, milk and eggs after consumption of contaminated feed (Kusimaningtyas *et al.*, 2006). In Kenya, IFST (Information Statement of Mycotoxin, 2009) indicated that there were up to 15 mg of aflatoxin per kg of corn contaminated by *A. flavus*, which is fatal. The IFST also reported that the largest aflatoxicosis outbreak recorded at 317 cases and 125 deaths were caused by maize contamination with aflatoxin B_1 levelled at 4,400 ppb, which was 220 times higher than the permitted limit for food in the country (Information Statement of Mycotoxin, 2009).

Effects of *Aspergillus* contamination on foods and feeds productivity

A. flavus has caused problems to the feed industry since the 1960s (Goldblatt, 1969). Aflatoxin is a high potential source for hepatotoxic and carcinogenic compounds produced by *Aspergillus*, which can cause death or the reduction of productivity in feed and poultry. Aflatoxin is associated with large scale deaths of livestock, such as chicken, trout and other species of animals around the world, which ultimately proves that the fungus poses a threat to food supplies and overshadows its ability to decompose plant materials (Goldblatt, 1969). In a recent study, in some countries, such as Gulf countries, India and Sudan, *A. flavus* is the predominant etiological agent among patients with fungal rhinosinusitis and endophthalmitis (Rudramurthy *et al.*, 2011). The study proved that *A. flavus* spores can kill animals compared to *A. fumigatus* spores, based on an observation using immunosuppressed mice.

Effects of *Aspergillus* towards organ in animals and humans body system

Previous studies indicated that the major quantitative trait loci (QTL) [major quantitative trait loci (QTL) (*p1*) identified on chromosome 1S had effects of 54.0, 42.1, and 28.3% on the phenotypic variability for concentrations of silk maysin, 3′-methoxymaysin/apimaysin, and chlorogenic acid, respectively (Widstrom *et al.*, 2003). Markers/QTLs for husk phenotypic traits and total aflatoxin concentrations have been determined, but more detailed mapping of these chromosomic regions will be necessary to locate precise markers/QTLs for husk traits and aflatoxin production. Aflatoxins are polyketide-derived secondary metabolites that are highly toxic and carcinogenic towards many animal species and are suspected carcinogens in human (Dvorockova, 1990; Prieto *et al.*, 1996). Research on aflatoxins has led to a so-called 'golden age' of mycotoxin research because there are many new fungal toxins discovered from the species of *Aspergillus* and other common molds (Bennett *et al.*, 2010). Aflatoxin has been recognized as the most important mycotoxin because it is synthesized by a few *Aspergillus* species that are the most problematic, such as *A. flavus* and *A. parasiticus*, which target certain organs in mammals, especially liver, causing hepatic diseases (Bennett *et al.*, 2010). They are weedy molds that grow on a large number of substrates, particularly under high moisture (Bennett *et al.*, 2010). Studies conducted (Kusimaningtyas *et al.*, 2006) on rats proved that Aflatoxin B_1 15 ppb can cause liver tumours (hepatic carcinomas). According to Bennett *et al.* (2010) and Williams *et al.* (2004), the expression of aflatoxin-related diseases is influenced by specific factors, such as age, nutrition, sex, species, and the possibility of concurrent exposure to other toxins. Bennett indicated that certain conditions, such as limited availability of food, environmental conditions that favour mold growth on foodstuffs, and the lack of regulatory systems for monitoring and controlling aflatoxin, increase the likelihood of aflatoxicosis in humans (Bennett *et al.*, 2010).

Aspergillus flavus is more virulent than other *Aspergillus* species, except *A. tamarri*, which has marginally higher virulence (Ford and Friedman, 1967; Anand and Tiwary, 2010). This has been proven by a study conducted by (Kaliamurthy *et al.*, 2003; Anand and Tiwary, 2010) through the immune compromising of rats and rabbits. Animals were considered to be a host of disseminated invasive pulmonary and sinus *A. flavus* infection. Chicken, geese and turkeys are also susceptible to *A. flavus*, even without immunosuppressant (Anand and Tiwary, 2010).

Cereal plants may be contaminated by mycotoxins in two ways; fungi growing as pathogens on plants or growing saprophytically on stored plants (Binder *et al.*, 2007; Glenn,

2007). Other important *Aspergillus* mycotoxins include ochratoxin, patulin and fumagillin (Cole and Cox, 1981; Bennett and Klich, 2003; Bennett *et al.*, 2010). Direct consequences of consuming mycotoxin-contaminated feeds include reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression), and reduced reproductive capacities (Binder *et al.*, 2007). At the same time, it causes economic losses (Wu, 2004, 2006; Binder *et al.*, 2007).

Several situations highlighted with an attributed outbreak of aflatoxicosis had been investigated in Kenya (Anonymous, 2004). Similar cases also happened on impaired child growth in Benin West Africa, believed to be caused by post-weaning exposure to aflatoxins (Gong *et al.*, 2004). In developing countries, aflatoxin exposure leads to overall health disorders that can result in reduced life expectancy. However, exposure to mycotoxin continues in developing countries due to compromised food security, poverty and malnutrition (Shetty and Jespersen, 2006).

Aspergillus flavus can cause a broad spectrum of diseases, ranging from hypersensitive reactions to invasive infections associated with angio invasion (Anand and Tiwary, 2010). *Aspergillus flavus* is the second leading cause of invasive and non-invasive aspergillosis (Morgan *et al.*, 2005; Anand and Tiwary, 2010). The primary route of infection is via inhalation of fungal spores (Anand and Tiwary, 2010). For example, allergic bronchopulmonary aspergillosis (ABPA) is the hypersensitive reaction of the immune system to *A. flavus* (Chakrabarti *et al.*, 2002; Anand and Tiwary, 2010). In addition, 10 cases of chronic cavitary pulmonary aspergillosis (CCPA) and aspergilloma had been previously reported in the hot and dry demotic regions (Liao *et al.*, 1998; Anand and Tiwary, 2010). *Aspergillus flavus* causes harm from the upper respiratory track faster than any other *Aspergillus* species (Kennedy *et al.*, 1997; Panda *et al.*, 1998; Anand and Tiwary, 2010).

In the Middle East and India, *A. flavus* causes Allergic Fungal Sinusitis (AFS) (Taj-Aldeen *et al.*, 2004; Thankar *et al.*, 2004; Saravanan *et al.*, 2006; Anand and Tiwary, 2010). *Aspergillus flavus* acts as an aetiological agent in keratitis, cutaneous aspergillosis endocarditis, wound infections, craniocerebral aspergillosis, osteomyelitis, and nosocomial infection and such cases are mostly reported in countries, such as Pakistan, India, Saudi Arabia, Sudan, and other African countries (Anand and Tiwary, 2010).

Since 1959, aspergillosis has been reported in all species of domestic animals and many wild species. For example, previous studies have indicated that birds show a particularly high susceptibility. Historically, aspergillosis was the first recognised avian disease (Ainsworth and Austwick, 1959). Studies from the 1960s also revealed that, originally, *Aspergillus* was considered a serious problem largely because of its prevalence in climates that are favourable to deterioration of all types of stored products. This was further combined with the fact that there was a lack of knowledge and necessary facilities to combat the problem (Christensen and Kaufman, 1969). Animal diseases caused by *Aspergillus* infection are all included under the term 'aspergillosis'. *Aspergillus* can cause animal diseases, through the production of mycotoxins, induction of allergic responses, and localised or systemic infections (Bennett *et al.*, 2010).

Allergies and asthma are known to be triggered by an active host immune responses to the presence of fungal spores or hyphae (Bennett *et al.*, 2010). *Aspergillus* can cause allergic

Table 1. **Worldwide basis observation for mycotoxin regulation during December 31, 2003 (Hans *et al.*, 2003)**

	Africa	Asia/Oceania	Europe	Latin America	North America
No. of countries	15 countries with known regulation (59% of inhibitants of the region)	26 countries with known regulation (59% of inhibitants of the region)	39 countries with known regulation (99% of inhibitants of the region)	19 countries with known regulation (91% of inhibitants of the region)	2 countries with known regulation (100% of inhibitants of the region)
Regulation country	Most detail regulations in Morocco Majority of countries stated non-existent regulations Some of country should be developed the regulations	Most detail regulations in China and Iran Harmonized regulations exist for Austrilia and New Zealand	Most detail regulations in several candidate-EU countries	Most detail regulations in Uruguay	Fully detail regulations in Canada and USA
Types of mycotoxins which had existing regulation	Most regulations exist for aflatoxin	Regulations exist for total aflatoxin which dominate in food and aflatoxin B_1 which dominate in feed Regulation exist in Austrilia and New Zealand for Agaric acid and phomopsins	EU harmonized regulations exist for aflatoxins, ochratoxin A and patulin EU harmonized regulation recommendations exist for deoxynivanol EU harmonized regulations are develop for Fusarium in food, baby food and feed	Harmonized regulations of aflatoxin exist in MERCOSUR member state	Regulations are set by total aflatoxins, Canada: detail tolerances for *Fusarium* damaged kernels (% by FDK) and ergot (% by weight); HT-2 toxin regulated in feed USA: detailed tolerances for total fumonisins (B_1, B_2, B_3) regulated in foods and feeds

responses, and colonising and invasive diseases as well as becoming an immune competent host (Widstrom *et al.*, 2003). Atopy is a type of genetic predisposition that causes a person to develop certain hypersensitive reactions, such as asthma, hay fever (allergic rhinitis) and food allergies. An allergic reaction to *Aspergillus* in atopic individuals can be triggered by fungal spores in the air and from fungi ingested in food. Airborne spores are readily inhaled when we breathe or when they come into contact with our eyes and other exposed body parts. Molds are involved in the initiation and exacerbation of the lower airway diseases, such as asthma, although the specific aetiology is poorly understood (Bush *et al.*, 2006; Bennett *et al.*, 2010). The inhalation of spores varies enormously according to the local environmental conditions. Some forms of these ill-defined human diseases have been particularly associated with exposure to high concentrations of *Aspergillus* spores. Predominantly it is for the lungs of farmers, malt workers, compost workers and bird fanciers (Bennett *et al.*, 2010).

Worldwide established regulation responsible for foods and feeds safety

In order to solve the problem of worldwide food and feed contamination, several advisory bodies had been enacted. Table 1 shows the worldwide basis observation for mycotoxin regulation (Hans *et al.*, 2003). As per the public health point of view and laws of legislation of any country, the contamination of food is unacceptable, and if the food is contaminated up to a certain amount of level and in particular, at a toxicological level, it cannot be marketed in that country. Contaminant levels are required to be reserved as low as can reasonably be achieved by good practice. The European Food Safety Authority (EFSA) has carried out risk assessments on certain mycotoxins found in animal feed, that are considered to pose a potential risk to human and as well as animal health: aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A, fumonisins, T-2 and HT-2. In each case, EFSA has issued an opinion that provides an assessment of the potential risk to animal, as well as human, health. Each opinion has been used as a basis for the current legislative controls on these mycotoxins [Laboratory for Food and Residue Analyses (ARO), 2003; Zinedine and Manes, 2009; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011]. The effects of mycotoxin are a major concern in both developed and developing countries for food and feed supply. Mycotoxin contamination in the food supply chain is a primary concern for human, as well as animal, health in developing countries, and production is the second concern. On the other hand, mycotoxin contamination in the food and feed chains is tightly regulated to reduce human and animal exposure; thus for the producer and/or the consumer the additional costs and the economic burden of regulating the food and feed supply in developed countries is the major mycotoxin concern (Bryden, 2012; Marin *et al.*, 2013).

Biological control of *Aspergillus* and mycotoxin in food

The growth of mold and its mycotoxin production are greatly influenced by the environment. It has been demonstrated that lactic acid bacteria (LAB), *Bacillus* species and sourdough bread cultures can inhibit mold growth as they compete for space and nutrients required for mycotoxin production but not for the growth and production of antimycotic and antimycotoxigenic metabolites (Biachini and Bullerman, 2010). LAB has been reported to be capable of binding mycotoxins, thus demonstrating the potential use of these organisms as sequestering agents in fermented and other processed foods, as well as in the gut. Molds share a common habitat with other microorganisms, which can naturally influence mold growth and mycotoxin production. Studies on the use of biological control methods began in the 1960s when Ciegler *et al.* (1996) screened over 1,000 microorganisms to test their ability to degrade aflatoxins, and research was also performed on the ability of *Flavobacterium aurantiacum* to irreversibly remove aflatoxin from solutions.

Usage of *Bacillus pumilus* as a fungal growth inhibitor

Bacillus pumilus is one of the mold growth inhibitors other than the genera of *Aspergillus*, *Penicillium*, and *Fusarium.* It also inhibits the production of aflatoxins, cyclopiazonic acid, ochratoxin A, and patulin. It was reported that the cell-free supernatant of *B. pumilus* inhibited more than 99% of aflatoxin production by *A. parasiticus* and up to 53% of mold growth (Munimbazi and Bullerman, 1998; Biachini and Bullerman, 2010). Previous studies described the production of a small thermoresistant peptide (B-TL2) by a *Bacillus* strain, isolated from tobacco stems, that was able to act as a strong inhibitor of *A. niger* mycelial growth (Zhang *et al.*, 2008; Biachini and Bullerman, 2010).

Usage of propanoic acid bacteria as a fungal growth inhibitor

Propionibacterium is a type of bacteria that secretes propionic acid by generating energy through the fermentation of lactate and sugars to propionate, acetate, and carbon dioxide (Piveteau, 1999; Biachini and Bullerman, 2010). This finding was established through research conducted on the effect of inhibition by pH reduction and production of propionic and acetic acids, which stimulates an effect on the inhibition of fungal growth. A previous study conducted with variety of molds showed that the minimum inhibitory concentration of propionic and acetic acids is about 10–120 mM at pH 5.0 (Lind *et al.*, 2005; Biachini and Bullerman, 2010).

Usage of LAB as a fungal growth inhibitor

LAB consist of four main genera: *Lactococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, which are used conventionally and traditionally as starter cultures for the fermentation of dairy products, vegetables, and meats because of their contributions to flavor development and preservative ions (Buckenhuskes, 2006; Olson, 2006; Biachini and Bullerman, 2010).

Food-borne and food grade LAB have been studied more extensively than *Bacillus* species or propionic bacteria, and their antifungal activities have been reported by several authors throughout the years (Plockova *et al.*, 2001; Stiles *et al.*, 2002; Biachini and Bullerman, 2010). LAB are multipurpose microorganisms that are useful in the food and livestock industries. Table 2 shows some of the studies that have demonstrated the antifungal activities of LAB, as well

Table 2. Example studies on the antifungal activity

LAB	Activity spectrum
Streptococcus lactis C10	*A. parasiticus*
Lactobacillus casei var rhamnosus	Broad spectrum
Lactobacillus reuteri	Broad spectrum
Streptococcus lactis subsp. *diacetilactis*	*A. fumigatus*
DRC1 and *S. thermophilus* 489	*A. parasiticus, Rhizopus stolonifer*
Lactobacillus spp.	*A. flavus* subsp. *parasiticus*
Lactobacillus casei subsp. *pseudoplantarum*	*A. flavus*
Lactococcus lactis subsp. *lactis*	*A. flavus* *A. parasiticus*
Lactobacillus casei	*Fusarium* spp. *Penicillium* spp.
Lactobacillus sanfrancisco CB1	*Penicillium* spp. *Aspergillus* spp. *Monilia* spp.
Lactobacillus plantarum	Broad spectrum

as their usage in other applications, which range from broad to very specific among and within species.

Advantages of using *Lactobacillus rhamnosus* strains to reduce mycotoxin and *Aspergillus*

Some studies reported that *Lactobacillus rhamnosus* can act as a medium for mycotoxin binders. A previous study by Gratz *et al.* (2007), using more than 250 strains of LAB isolated from either dairy products or healthy human microbiota concluded that the efficacy of aflatoxin binding varies widely, depending on the genus and strain of bacteria. Probiotic *L. rhamnosus* GG may contain hepatocarcinogen aflatoxin B_1 (AFB_1) and thus can potentially restrict its rapid absorption by the intestine. Two of the *L. rhamnosus* strains, namely GG and LC-705, were found to be the most efficient in binding a range of mycotoxins, including aflatoxins (Pierides *et al.*, 2000; Haskard *et al.*, 2001; Peltonen *et al.*, 2001; Gratz *et al.*, 2007). Carbohydrates and proteins of the bacterial surface components are important binding agents of aflatoxin B_1 (Haskard *et al.*, 2001; Gratz *et al.*, 2007). It should be highlighted that heat treatment cannot reduce the consistency of the binding agent (Lee *et al.*, 2003; Gratz *et al.*, 2007).

Gratz *et al.* (2007) indicated that *L. rhamnosus* GG is currently used in various dairy products, including yogurt, and is therefore a good candidate for assessing protective effects due to its usefulness to human. The studies showed that aflatoxin B_1, bonded by probiotic bacteria, can successfully reduce its tissue uptake in the duodenum of chicks. The reduction of aflatoxin B_1 from 11.1% ± 1.9% to 6.4% ± 2.5% (P = 0.019) and to 3.3% ± 1.8% (P = 0.002) within the first hour in the monolayer co-incubated with *L. rhamnosus* GG (1.0×10^{10} and 5×10^{10} ml, respectively) was bounded to 40.1% ±8.3% and 61.0% ± 6.0% of added aflatoxin B_1 after 1 h, respectively. Aflatoxin B_1 also caused significant reductions at 30.1% ($P = 0.01$), 49.4% ($P = 0.004$), and 64.4% ($P < 0.001$) in transepithelial resistance after 24, 48, and 72 h, respectively (Gratz *et al.*, 2007).

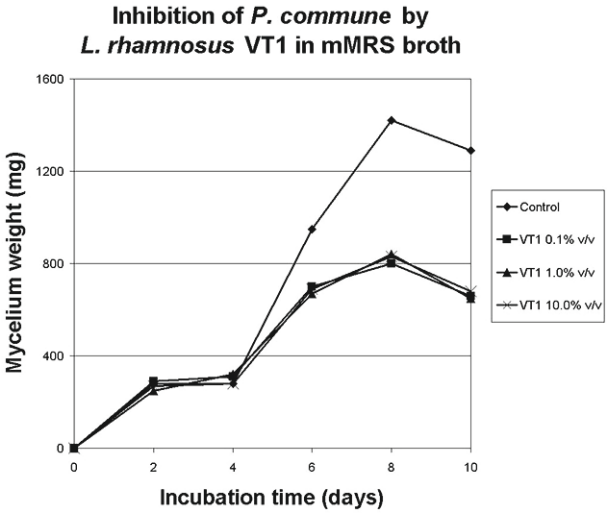

Fig. 2. Inhibition of *P. commune* NRRL 1889 by *L. rhamnosus* VTI (0.1, 1.0, and 10% v/v) in mMRS broth (Stiles *et al.*, 2002; Biachini and Bullerman, 2010).

A study conducted by Stiles *et al.* (2002) demonstrated the inhibitory activity of *L. rhamnosus* isolated from a Czech tartar sauce. The study indicated that in a simultaneous antagonistic assay, *L. rhamnosus* showed the ability to inhibit mycelial growth of *Penicillium commune* and *A. niger* when both organisms (mold and bacteria) were cultivated together for 10 days at 30°C. Figures 2 and 3 show the inhibition of *P. commune* NRRL 1889 and *A. niger* NRRL 326 by using *L. rhamnosus* VTI (Stiles *et al.*, 2002; Biachini and Bullerman, 2010), through the addition of live bacterial cells to cheese that was simultaneously inoculated with *P. commune* and *A. niger*. After 10 days of incubation, the growth of *A. niger* was inhibited by more than 70% and the growth of *P. commune* was inhibited by more than 40%.

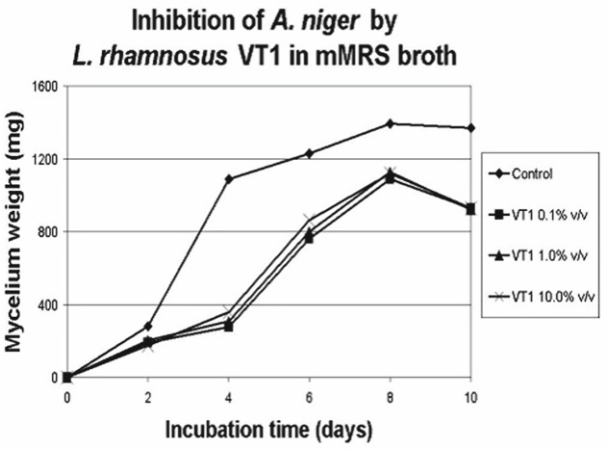

Fig. 3. Inhibition of *A. niger* NRRL 326 by *L. rhamnosus* VTI (0.1, 1.0, and 10% v/v) in mMRS broth (Stiles *et al.*, 2002; Biachini and Bullerman, 2010).

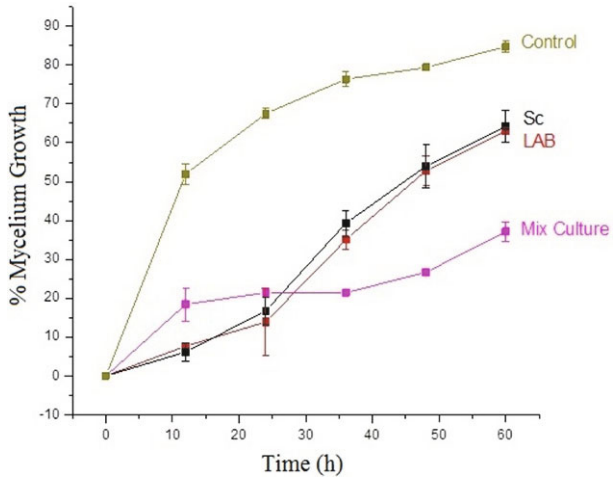

Fig. 4. Inhibition of *A. flavus* CFFC F0070 by using single culture and mixed culture of *L. rhamnosus* NRRL B-442 and *S. cerevisiae*.

Stile *et al.* (2002) also studied the effect of *L. rhamnosus* on the growth and production of mycotoxin by *Fusarium* species, including *F. proliferatum*, *F. verticillioides*, and *F. graminearum*. The results showed a reduction of up to 63.2% for production of fumonisin B_1 by *F. proliferatum*, 43.4% for the production of fumonisin B_2 by *F. verticillioides*, 92% for the production of deoxynivalenol by *F. graminearum*, and finally 87.5% for the production of zearalenone.

Potential of mix culture *L. rhamnosus* and *S. cerevisiae* application to increase the antifungal activity

Probiotics are living microorganisms that help to maintain the bacterial balance in the digestive tract of mammals when ingested, and which may also be included in the treatment of pathological conditions, such as diarrhea, candidiasis, urinary infections, immune disorders, lactose intolerance, hypercholesterolemia, and food allergies (Nada *et al.*, 2010). It also has antigenotoxic effects, especially among the species of *Lactobacillus* and *Streptococcus*.

Some studies discovered that *Lactococcus* and *Bifidobacterium* have antimutagenicity in the Ames test and successfully decreased DNA damage in colon cells treated with *N*-methyl-*N*-nitro-*N*-nitroso-guanidine in an *in vitro* study. *Saccharomyces cerevisiae* (Sc), in particular, was proven to be beneficial to health in several ways through stimulation of intestinal microflora growth in mammals; pH modulation in ruminants (which gives rise to an increase in the rate of cellulitic bacteria); improvement of reproductive parameters in milk cows and fowl (fertility and foetal development); and reduction in the number of pathogenic microorganisms in monogastric animals. *S. cerevisiae* and lactic acid bacteria (*L. rhamnosus* GG and *L. rhamnosus* LC705), on the other hand, are potentially inhibited (Pool-Zoobel *et al.*, 1996; Nada *et al.*, 2010).

A. flavus growth and aflatoxins production had been studied under *in vitro* and *in vivo* conditions. Nada *et al.* (2010) conducted a study on its effectiveness in mammalian histopathological through the examination of liver and kidney in rats treated with aflatoxin B_1. It demonstrated necrosis, vascular degeneration and fatty changes in hepatocytes, cellular swelling, and convoluted tubules in renal tissue by pyknotic nuclei. The results also indicated a significant decrease in DNA content in liver and kidney tissues with aflatoxin B_1 administration. These findings were ameliorated by treatment with probiotic bacteria and *S. cerevisiae*, which showed the ability to inhibit the growth of *A. flavus* and the production of mycotoxins (Nada *et al.*, 2010).

Figures 4 and 5 present the results that demonstrate the potential of a mixed culture compared with a single culture of *L. rhamnosus* NRRL B-442 and *S. cerevisiae*, which was intended to inhibit the growth of *A. flavus*. The result indicated a significant decrease in the optimal mycelium growth of *A. flavus* (37.08% in a mixed culture vs. 63.24% and 64.07% in single cultures of *Lactobacillus* and *S. cerevisiae*, respectively). The result suggests that the use of mixed cultures have higher potential to inhibit the mycelium growth than that of single cultures.

Future suggestions about the usage of mix culture to develop food and feed industry

According to the European Food Safety Authority EFSA, (2011), *L. rhamnosus* is a technological additive intended to improve the ensiling process at a proposed dose of 1.0×10^8 CFU/kg for fresh materials. EFSA considers *L. rhamnosus* to be a qualified safety presumption. The additive can potentially improve the production of silage from all forages by consistently increasing the lactic acid content, preserving dry matter and reducing the pH and also moderates the loss of protein, as determined by ammonia-nitrogen. Coeuret *et al.* (2004) indicated that there are many *Lactobacillus* strains and yeasts (*Sacchromyces cerevisiae* var. *bourardii*) that have probiotic activity. Based on research by Celyk *et al.* (2003), yeast has been used in commercial feed for more than a century. Yeasts and *Lactobacillus* are potential tools to solve the problem of mycotoxins in cereal-based foods and in feed (Shetty and Jespersen, 2006). Some

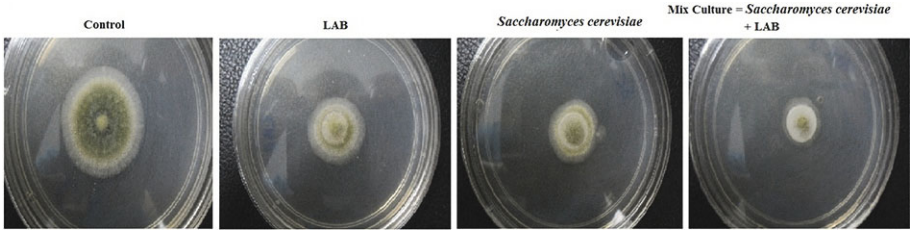

Fig. 5. Inhibition of *A. flavus* CFFC F0070 by using single culture and mixed culture of *L. rhamnosus* NRRL B-442 and *S. cerevisiae* in 60 h.

Table 3. Some commodities of mycotoxin produced by *Aspergillus* that will affect animals and human

Mycotoxin	Producers fungus	References	Commodities found contaminated	Effect of mycotoxins		
				Affected species References	Pathological effects	
Aflatoxin (B_1, B_2, G_1, G_2, M_1, M_2)	AFB_1: *A. flavus* AFB_2: *A. flavus* AFG_1 and AFG_2: *A. arachidicola* AFM_1: *A. flavus, A. parasiticus* (convert normally from AFB_1) AFM_2: *A. flavus, A. parasiticus* (convert normally from AFB_2, consider less toxic compare with AFM_1)	Wallin *et al.* (1991) Schroeder *et al.* (1972) Pildain *et al.* (2004) IFIS (2009) IFIS (2009)	Peanuts, corn, wheat, rice, cottonseed copra, nuts, various foods, milk, eggs, cheese, figs	Birds: Duckling, turkey, poultry, pheasant chick, mature chicken, quail Mammals: Young pigs, pregnant sows, dog, calf, mature cattle, sheep, cat, monkey, human Fish Laboratory animals	Hepatotoxicity (liver damage), Bile duct hyperplasia, Hemorrhage, Intestinal tract, Kidneys, Carcinogenesis (liver tumors)	CAST (2003)
Citrinin	*A. oryzae*	Sakai *et al.* (2012)	Cereal grains (wheat, barley, corn, rice)	Swine, dog, laboratory animals	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy	CAST (2003)
Cyclopiazonic acid	*A. flavus* and *A. parvisclerotigenus*	Pildain *et al.* (2004)	Corn, peanuts, cheese, kodo millet	Chicken, turkey, swine, rat, guinea pig, human	Muscle necrosis. Intestinal hemorrhage and edema Oral lesions	CAST (2003)
Ochratoxin A	*A. alutaceus*	Bruinink *et al.* (1997)	Cereal grains (wheat, barley, oats, corn), dry beans, moldy peanuts, cheese, tissues of swine, coffee, raisins, grapes, dried fruits, wine	Swine, dog, duckling, chicken, rat, human	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy Mild liver damage Enteritis Teratogenesis Carcinogenesis (kidney tumors) Urinary tract tumors	CAST (2003)
Sterigmatocystin	*A. nidulans*	Yu and Leonard (1995)	Green coffee, moldy wheat, grains, hard cheeses, peas, cottonseed	Mouse, rat	Carcinogenesis, Hepatotoxin	CAST (2003)

commodities of mycotoxin produced by *Aspergillus* that will affect animals and human are shown in Table 3.

This review concludes the advantage of mixed cultures of *Lactobacillus* and *S. cerevisiae*, which counteract the effects of mycotoxigenic fungi and prevent the production of the associated mycotoxins in human and animal food chains. In addition to inhibiting the growth of fungus, mixed cultures (*Lactobacillus* and *S. cerevisiae*) are proposed to detoxify existing mycotoxin in raw materials through anaerobic fermentation during the ensiling process.

Acknowledgements

This study was funded in part by the research grant under Research University Grant's project entitled "Production of Bio Organic Animal Feed from Rice Straw by Using Lactic Acid Bacteria" from Universiti Teknologi Malaysia.

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