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Evaluating the effects of certain probiotics on mycotic contamination in oriental sausage

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ABSTRACT

Probiotics are natural antimicrobial agents. It has great beneficial effects to human. In this study, Probiotics as *Lactobacillus casei, Lactobacillus delbrueckii* & *Bifidobacteriumlactis* were used in oriental sausage to reduce total mould and yeast counts as well as *Aspergillus flavus* count. Two oriental sausage groups were used. The 1st group was used to evaluate reduction % of total mould and yeast count. It was subdivided to 4 groups 3 were inoculated with different Probiotic strains, the 4th was control. Results showed that total mould and yeast count (cfu/g) were reduced to $4.73 x 10^3 \pm 2.33 x 10^2$, $6.97 x 10^3 \pm 2.73 x 10^2$ and$8.80 x 10^3 \pm 2.08 x 10^2$ using *Lactobacillus casei, Lactobacillus delbrueckii* and *Bifidobacterium lactis* after 12 days inducing reduction % of 91.66, 87.71 and 84.48, respectively. The 2nd group was used to evaluate reduction % of *Aspergillus flavus* count. It was inoculated with *Aspergillus flavus* then subdivided to 4 groups 3 were inoculated with different probiotic strains, the 4th was control. Results showed that *Aspergillus flavus* count (cfu/g) were reduced to $2.97 x 10^4 \pm 1.86 x 10^3$, $5.37 x 10^4 \pm 1.45 x 10^3$and $6.34 x 10^4 \pm 1.45 x 10^3$ using *Lactobacillus casei, Lactobacillus delbrueckii* and *Bifidobacterium lactis* after 12days inducing reduction % of 92.25, 85.98 and 83.45, respectively. Moreover; it was found that *Lactobacillus casei* was the most effective probiotics causing the highest reduction% in total mould count and *Aspergillus flavus* counts followed by *lactobacillus delbrueckii* then *Bifidobacterium lactis*.

1. INTRODUCTION

Red meat is considered as a major source for important micronutrients and protein of high biological value needed for good human health. Meat also contains a range of fats; including polyunsaturated fats and essential omega-3. Also, meat products are highly demanded for agreeable taste, high biological value, good price; and easily serving. These meat products also represent quick easily prepared meat meals; they solve the problem of fresh and expensive meat shortage.

The food industry may be exposed to extensive losses due to microbial spoilage. Fungi are filamentous in nature, they are considered as one of the major categories of spoilage microorganisms that involved in considerable economic losses and human health hazards (Maurya et al., 2015).

Contamination of meat products with mould may occur at several stages at which the products were prepared or when using contaminated water, equipment and utensils as well as during processing by adding of contaminated meat additives with mould spores or during packing, handling, transportation and storage. The formation of mycotoxins is considered as the most important aspect about mould spoilage of food (Morshdy et al., 2015).

Sausage is considered as a prepared food made from ground meat, animal fat, spices and salt, typically packed in a casing, Sausage manufacturing is a traditional food preservation technique. Traditionally, casings were made of animal intestine, but synthetic casings were recently used (Quasem- Jihad et al., 2009).

Using food additives was regarded as unsafe and unnatural. On other hand, additives are needed to improve the organoleptic properties and to preserve food products from spoilage; therefore, using functional protective cultures in the food fermentation industry is being explored. Accordingly, functional protective cultures are considered microorganisms that possess one inherent functional property at least. They can provide food safety and/or offer one or more organoleptic, nutritional, technological or health advantages to the food (Sarika et al., 2010).

Lactic acid bacteria that type of probiotics are considered as natural preservatives and antimicrobial agents. Antimicrobial activities of lactic acid result from the providing unfavorable conditions that reduce the growth rate of undesirable microorganisms (Bassi et al., 2015).

Probiotics can be used singly or as mixture of bacteria which can inhibit the growth of spoilage and pathogenic bacteria is of growing interest for research to improve the shelf life and safety of the meat products (Ertürkmen et al., 2016).

Probiotics also have other beneficial effects, one of those different beneficial effects of lactic acid bacteria consumption includes: improving intestinal tract health; synthesizing and enhancing the bioavailability of nutrients, enhancing the immune system; reducing symptoms of

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lactose intolerance, decreasing the prevalence of allergy in susceptible individuals; and reducing risk of certain cancers (Parvez et al., 2006). In recent years, probiotic foods have received special attention. Probiotics also have been used as food bio protectors due to their importance for consumer health. Efforts have been made to develop meat-based functional foods using different, modern strategies related to increasing the presence of beneficial compounds and also limiting those with negative health implications (Carlos et al., 2015).
Therefore, the current study was applied to enhance the mycological quality of oriental sausage using probiotics with special reference to *Aspergillus flavus*.

2. MATERIAL AND METHODS

2.1. Probiotic strains
Lactobacillus casei, Lactobacillus delbrueckii and Bifidobacterium lactis with adjusted concentration (10^7 CFU/g) were obtained ready to use from Faculty Of Agriculture, Ain Shams University.

2.2. Fungal strain
Aspergillus flavus reference strain with adjusted concentration (10^5 CFU/g) as recorded by (Chang and Kim, 2007) used in this study, were obtained ready to use from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.

2.3. Oriental Sausage manufacturing (Mohan, 2014)
Fresh minced beef used in this study was prepared in butcher shops in El Menofiya Governorate, for each kg of minced beef (150g chopped fat,40g salt,150g fine chopped onion,10g fine chopped garlic,3g fine crushed cinnamon,3g fine crushed clove,3g fine paprika,150g vinegar) were added and natural casing was used to manufacture the oriental sausage.

2.4. Preparation of Oriental Sausage sample
Freshly prepared oriental sausage was transported directly and aseptically to the laboratory in an ice box. The collected sample was divided into 2 parts.
Part 1 was used to evaluate total mould and yeast count, so it was subdivided into four groups:
- The 1st group was as control group (control group).
- The 2nd group was inoculated with *Lactobacillus casei* (10^7CFU/g).
- The 3rd group was inoculated with *Lactobacillus delbrueckii* (10^7 CFU/g).
- The 4th group was inoculated with *Bifidobacterium lactis* (10^7 CFU/g).

Each sample was packed in polyethylene bag, labeled and stored at 4 ºC in refrigerator till examination.
The trials were performed in triplicate.

2.5. Mycological examination
Ten-fold serial dilution was prepared from each sample then inoculated into sabaroud dextrose agar to calculate total mold and yeast count/gm, and *Aspergillus flavus* count were calculated and recorded according to APHA (2001)

Part 2 manufactured oriental sausage as prepared before was inoculated with *Aspergillus flavus* with adjusted concentration (10^5 CFU/g) to be used to evaluate total *Aspergillus flavus* count then subdivided into four groups:
- The 1st group was as control group (control group).
- The 2nd group was inoculated with *Lactobacillus casei* (10^7CFU/g).
- The 3rd group was inoculated with *Lactobacillus delbrueckii* (10^7 CFU/g).
- The 4th group was inoculated with *Bifidobacterium lactis* (10^7 CFU/g).

Each sample was packed in polyethylene bag, labeled and stored at 4 ºC in refrigerator till examination.
The trials were performed in triplicate.
Ten-fold serial dilution was prepared from each sample then inoculated into Sabouraud dextrose agar to calculate *Aspergillus flavus* count were calculated and recorded.

2.6. Statistical analysis
The data was statistically treated by one-way ANOVA using SPSS program (Steel and Torrie, 1980).

3. RESULTS

Mould and yeast counts were reduced from ($5.67x10^4 \pm 8.09x10^3$, $3.20x10^4 \pm 5.51x10^3$, $4.30x10^4 \pm 5.57x10^3$and $4.87x10^4 \pm 5.21x10^3$) at zero day to ($4.37x10^5 \pm 5.55x10^4$, $4.73x10^3 \pm 2.33x10^2$, $6.97x10^3 \pm 2.73x10^2$ and $8.80x10^3 \pm 2.08x10^2$) with in 12th days in control group, *Lactobacillus casei, Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups respectively (Table 1 & Fig. 1) with a reduction % (91.66, 87.71 and 84.48) for *Lactobacillus casei, Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups respectively (Table 2 & Fig. 2). The highest reduction % caused by *Lactobacillus casei* followed by *Lactobacillus delbrueckii* then *Bifidobacterium lactis.*

Table 1 The effects of different of probiotics on total mould count (cfu/g) in the examined oriental sausage samples

Groups	Control	*L. Casei*	*L. delbrueckii*	Bifidobacterium
Zero day	$5.67x10^4 \pm 8.09x10^{3a}$	$3.20x10^4 \pm 5.51x10^{3b}$	$4.30x10^4 \pm 5.57x10^{3ab}$	$4.87x10^4 \pm 5.21x10^{3ab}$
3rd day	$7.53x10^4 \pm 7.31x10^{3a}$	$2.07x10^4 \pm 1.45x10^{3c}$	$2.70x10^4 \pm 3.22x10^{3bc}$	$3.57x10^4 \pm 2.91x10^{3b}$
6th day	$8.90x10^4 \pm 2.52x10^{3a}$	$1.20x10^4 \pm 5.77x10^{3c}$	$1.97x10^4 \pm 1.76x10^{3b}$	$2.47x10^4 \pm 2.03x10^{3b}$
9th day	$1.63x10^5 \pm 1.76x10^{4a}$	$7.77x10^3 \pm 3.53x10^{2b}$	$9.17x10^3 \pm 1.76x10^{2b}$	$1.30x10^4 \pm 1.16x10^{3b}$
12th day	$4.37x10^5 \pm 5.55x10^{4a}$	$4.73x10^3 \pm 2.33x10^{2b}$	$6.97x10^3 \pm 2.73x10^{2b}$	$8.80x10^3 \pm 2.08x10^{2b}$

The values represent Mean ± SE of three experiments. Means within a row followed by different letters are highly significantly different ($P < 0.05$). The data was statistically treated by one-way ANOVA using SPSS program.

Table 2 Reduction % of different probiotics on total mould count (cfu/g) in the examined oriental sausage samples

Groups	*L. Casei*	*L. delbrueckii*	Bifidobacterium
Zero day	43.56	24.16	14.11
3rd day	63.49	52.38	37.04
6thday	78.84	65.26	56.44
9th day	86.3	83.83	77.07
12th day	91.66	87.71	84.48

The data was statistically treated by one-way ANOVA using SPSS program.

Aspergillus flavus count were reduced from ($3.83x10^{5}\pm2.85x10^{4}$, $2.57x10^{5}\pm3.28x10^{4}$, $3.13x10^{5}\pm2.85x10^{4}$ and $3.37x10^{5}\pm 2.67x10^{4}$) at zero day to ($5.13x10^{6}\pm3.38x10^{5}$, $2.97x10^{4}\pm1.86x10^{3}$, $5.37x10^{4}\pm1.45x10^{3}$ and $6.34x10^{4}\pm 1.45x10^{3}$) with in 12th days in control group, *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups respectively (Table 3 & Fig. 3) with a reduction % (92.25, 85.98 and 83.45) for *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups respectively (Table 4 & Fig. 4). The highest reduction % caused by *Lactobacillus casei* followed by *Lactobacillus delbrueckii* then *Bifidobacterium lactis.*

4. DISCUSSION

Development of functional food consider as opportunity for experts to show their innovation in meat industry in line with consumer's concerns on food nutrition, well-being and food safety. Natural preservatives as Lactic acid bacteria (LAB) currently used in fresh sausage manufacture for their antimicrobial activities against pathogens. Within 12th days of experiment total mould and yeast counts were reduced to ($4.37x10^{5}\pm5.55x10^{4}$, $4.73x10^{3}\pm 2.33x10^{2}$, $6.97x10^{3}\pm 2.73x10^{2}$ and $8.80x10^{3}\pm2.08x10^{2}$) for control group, *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups, respectively, With a reduction % (91.66, 87.71 and84.48) for *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups, respectively.

Also during12th days of experiment *Aspergillus flavus* counts were reduced to ($5.13x10^{6}\pm3.38x10^{5}$, $2.97x10^{4}\pm 1.86x10^{3}$, $5.37x10^{4}\pm 1.45x10^{3}$and $6.34x10^{4}\pm1.45x10^{3}$) for control group, *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups respectively, With a reduction %(92.25, 85.98 and83.45) for *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Bifidobacterium lactis* groups, respectively. There for the highest reduction % for both Mould& yeast counts and Aspergillus count caused by *Lactobacillus casei* followed by *Lactobacillus delbrueckii* then *Bifidobacterium lactis.*

Table 3 The effects of different of probiotics on *Aspergillus flavus* (cfu/g) in the examined oriental sausage samples

Groups	Control	*L. Casei*	*L. delbrueckii*	Bifidobacterium
Zero day	$3.83x10^{5}\pm2.85x10^{4a}$	$2.57x10^{5}\pm3.28x10^{4b}$	$3.13x10^{5}\pm2.85x10^{4ab}$	$3.37x10^{5}\pm2.67x10^{4ab}$
3rd day	$5.90x10^{5}\pm1.73x10^{4a}$	$1.37x10^{5}\pm2.19x10^{4c}$	$2.10x10^{5}\pm1.53x10^{4b}$	$2.43x10^{5}\pm8.82x10^{4b}$
6thday	$8.57x10^{5}\pm3.28x10^{4a}$	$8.17x10^{4}\pm1.86x10^{3c}$	$1.30x10^{5}\pm5.77x10^{3c}$	$1.90x10^{5}\pm5.77x10^{3b}$
9th day	$1.87x10^{6}\pm1.45x10^{5a}$	$5.03x10^{4}\pm4.26x10^{3b}$	$8.03x10^{4}\pm1.20x10^{3b}$	$9.13x10^{4}\pm1.20x10^{3b}$
12th day	$5.13x10^{6}\pm3.38x10^{5a}$	$2.97x10^{4}\pm1.86x10^{3b}$	$5.37x10^{4}\pm1.45x10^{3b}$	$6.34x10^{4}\pm1.45x10^{3b}$

The values represent Mean ± SE of three experiments. Means within a row followed by different letters are highly significantly different ($P < 0.05$). The data was statistically treated by one-way ANOVA using SPSS program.

Table 4 Reduction % of different probiotics on *Aspergillus flavus* (cfu/g) in the examined oriental sausage samples

Groups	*L. casei*	*L. delbrueckii*	Bifidobacterium
Zero day	32.9	10.44	12.01
3rd day	64.23	45.17	36.55
6thday	78.67	66.06	50.39
9th day	86.87	79.03	76.16
12th day	92.25	85.98	83.45

The data was statistically treated by one-way ANOVA using SPSS program.

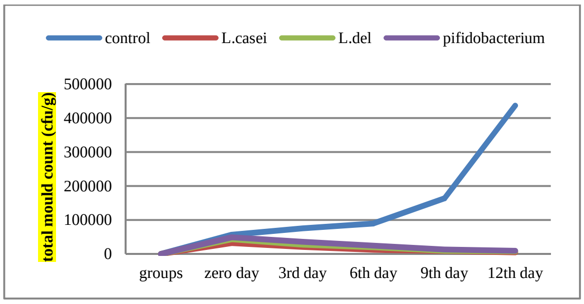

Fig. 1 The effects of different probiotics on total mould count (cfu/g) in the examined oriental sausage samples

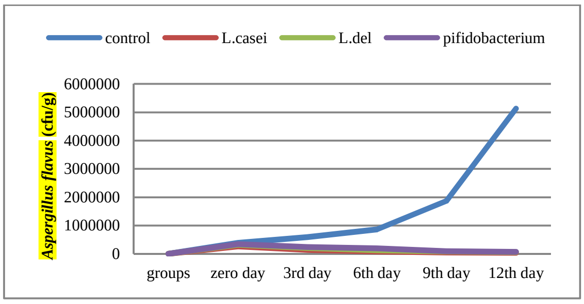

Fig. 3 The effects of different of probiotics on *Aspergillus flavus* (cfu/g) in the examined oriental sausage samples

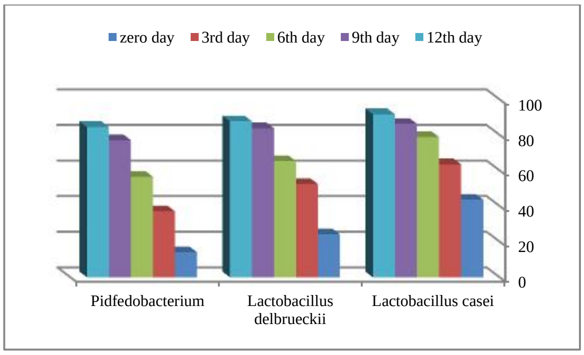

Fig. 2 Reduction % of different probiotics on total mould count (cfu/g) in the examined oriental sausage samples

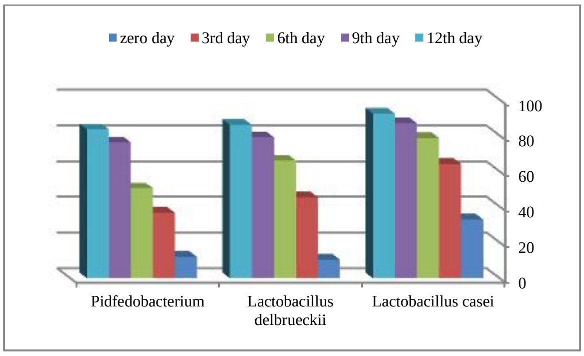

Fig. 4 Reduction % of different probiotics on *Aspergillus flavus* (cfu/g) in the examined oriental sausage samples

Actually, LABs were used as a starter culture, this using offering functionalities other than acidification has recently been explored. Also, LABs are capable of inhibiting different microorganisms in food, providing antimicrobial effects with a strong impact on preservation and safety (Yang et al., 2014 and Ahmad et al., 2017).

Supernatant of three probiotic bacteria were effective either to inhibit the growth and the aflatoxin production of two Aspergillus spp. But also, Probiotic culture supernatant (PCS) of three of probiotic bacterial strains; *Lactobacillus plantarum, Lactobacillus acidophilus and Bifidobacterium bifidum* was tested as antifungal against two mycotoxin producing fungi; *Aspergillus flavus* and *Aspergillus parasiticus*. Using the probiotic supernatant as food additives was considered (Hamad et al., 2018).

Probiotics mechanisms of action by which they exert their effects are largely unknown, but different species of LAB produce different antimicrobial substances. Here are some examples of those substances: *Lactobacillus reuterii* produce a low molecular weight antimicrobial substance called *reuterin*; subspecies of *Lactococcus lactis* produce a class I bacteriocin, known as nisin A; *Lactobacillus plantarum* produces a class II bacteriocin plantaricin S; and *Lactobacillus acidophilus* produces a class III bacteriocin acidophilucin A. Production of bacteriocins is highly affected by the factors of the species of microorganisms, ingredients and pH of medium, incubation temperature and time Elbagory et al., 2015).

Results demonstrated that all three used (LAB) strains had the strong antimicrobial activities, these activities led to observable decrease in total yeast and mould count and total *Aspergillus flavus* count as *Lactobacillus casei* cause the highest significant inhibition of total yeast and mould count and total *Aspergillus flavus* count, followed by *Lactobacillus delbrueckii* then *Bifidobacterium lactis*.

Antimycotic and antifungal effect of probiotics was previously detected byAly-Soher et al. (2009),Prathivadi-Bayankaram and Sellamuthu(2016),Ghazvini et al. (2016), Ahmed -Amira (2018) and Ibrahim- Hemmat et al (2018).

Reaching complete inhibition of fungal growth and aflatoxin production by using cell free supernatants (CFS) from several Lactobacillus species. Results reported that *L. acidophilus* recorded the highest inhibitory effect on the germination of aflatoxigenic *A. parasiticus* and actually prevented aflatoxin production which could be considered a safe process for controlling aflatoxin contamination in different food products (Aly-Soher et al., 2009).

Aflatoxin inhibiting ability and antifungal activity of four different probiotic strains against Aspergillus flavus and *Aspergillus parasiticus* were studied. Results illustrated that *Lactobacillus delbrueckii subsp. lactis* showed maximal antifungal (67.43% reduction) and anti-aflatoxigenic (94.33% reduction) activity against A. flavus whereas *A. parasiticus* was inhibited by *Lactobacillus brevis* with the antifungal reduction of 69.38% and anti-aflatoxigenic reduction of 96.12% (Prathivadi-Bayankaram and Sellamuthu, 2016).

Lactobacillus fermentum and *Bifidobacterium bifidum were used to reduce aflatoxin production and growth rate of A. parasiticus in comparison with the controls ($p \leq 0.05$).*Results illustrated that *LAB reduced total aflatoxins and B1, B2, G1 and G2 fractions by more than 99%. Also, LAB metabolites reduced the level of standard AFB1, B2, G1 and G2 from 88.8% to 99.8% ($p \leq 0.05$). Authors concluded that, B. bifidum and L. fermentum were recommended as suitable bio control agents against the growth and aflatoxin production by aflatoxigenic* Aspergillus *species* (Ghazvini et al., 2016).

The effect of two different probiotics on the reduction of total aflatoxin residues in minced beef sample containing previously known amount of TAF during cold storage (4ºC) was studied. Results showed that two probiotic strains (*L. acidophilus and B. lactis*) could be able to cause gradual reduction in total aflatoxin residues which reach up to 88% and 98.2%, respectively of total aflatoxin residues within 6 days of refrigerated storage (Ahmed -Amira, 2018).

The effect of different probiotics on the reduction of aflatoxin residues in naturally contaminated minced meat sample was studied. Results illustrated that the two probiotic strains (*Lactobacillus acidophilus and Bifidobacterium lactis*) could be able to cause gradual reduction in total aflatoxin residues up to 88% and 98.3%, respectively of total aflatoxin residues within 8 days of experiment. *Bifidobacterium lactis* was concluded to be more effective than *Lactobacillus acidophilus* (Ibrahim et al., 2018).

5. CONCULSION

Different Lactic acid bacteria (LAB) strains had shown an antimicrobial effect on total yeast and mould count and total *Aspergillus flavus* count as *Lactobacillus casei* cause the highest significant inhibition of total yeast and mould count and total *Aspergillus flavus* count, followed by *Lactobacillus delbrueckii* then *Bifidobacterium lactis*. Because of their antimycotic effect Probiotics can be used as food preservatives in oriental sausage. To improve the hygienic status of meat products Probiotics should be used in food industry. Probiotics (*Lactobacillus casei, Lactobacillus delbrueckii and Bifidobacterium lactis*) can be used to antagonize the growth of pathogenic microorganisms. Probiotics can be applied in food and feed processing industries as natural preservatives especially they are considered GRAS.

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