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ANTIFUNGAL ACTIVITY OF SOME LACTIC ACID BACTERIA ISOLATED FROM MATERIALS OF VEGETAL ORIGIN

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Abstract

Lactic acid bacteria have been considered to be promising natural biological antagonists for mycotoxigenic fungi that contaminate various food commodities. Apart from important economic losses, mycotoxin producing fungi have harmful effects on human and animal health. The public necessity for high quality food, without addition of chemical preservatives, with extended shelf life, determined the search of new strains of bacteria able to produce lactic acid and their use to control the fungal growth of plant pathogenic and mycotoxigenic species. The effect of 27 strains of lactic acid bacteria have been assayed on the growth of Alternaria solani plant pathogenic fungi isolated from heavily infected tomatoes as well as on potential mycotoxin producing fungi Aspergillus ochraceus isolated from tomato roots grown in greenhouse and Penicillium digitatum isolated from infected oranges. Interaction between lactic acid bacteria and selected mycotoxigenic fungi was tested by overlay assay method. Discrete spots of liquid cultures of tested lactic acid bacterial strains were placed on MRS agar and after incubation, overlaid with soft PDA containing propagules of tested fungi. Data were collected after 48 hours concerning the diameters of clear visible inhibition zones of fungal growth around the lactic acid bacterial strain spots. Preliminary tests have shown that selected lactic acid bacteria could inhibit the development of test fungi. A number of 11 lactic acid bacterial strains have shown antifungal activity on both Aspergillus ochraceus and Penicillium digitatum. Another 5 strains of lactic acid bacteria did not inhibit any of the two mycotoxigenic fungal species. The effect of lactic acid bacteria on plant pathogenic species Alternaria solani did not provide conclusive results and need further study. The results could be used in future experiments for obtaining performing biological agents with application in food safety.

Keywords: *antifungal activity, lactic acid bacteria, mycotoxigenic fungi.*

INTRODUCTION

Various food commodities can be contaminated by a wide spectrum of filamentous fungi, leading to important economic losses.

Fungal infection causes food spoilage with high impact on organoleptical properties. The contamination of various food commodities with mycotoxigenic fungi has harmful effects on human and animal health (Bryden, 2012).

The major species of mycotoxigenic fungi responsible for food spoilage belong to genera *Aspergillus, Fusarium* and *Penicillium* (Gerez et al., 2009). Literature cites various effects of mycotoxins that are highly toxic metabolites synthetsised by various fungal species on human health such as carcinogenic, teratogenic, immunotoxic, neurotoxic, hepatotoxic and nephrotoxic (Bryden, 2007; Wild and Gong, 2009).

Prevention of fungal growth on various food commodities is the best method of protection from the harmful effects on human and animal health. Several physical and chemical methods have been developed to control fungal growth, but some moulds acquired the ability to resist chemical treatment and some preservatives.

Due to general public demands for preservatives free food and feed, biopreservation, the control of one organism by another, has become an alternative that was subject to further investigation (Schnurer and Magnusson, 2005).

Lactic acid bacteria have been considered to be promising natural biological antagonists for mycotoxigenic fungi that contaminate various commodities (Magnusson et al., 2003; Trias et al., 2008). Dalie et al. (2010) reviewed the main mechanism involved in antimicrobial efficiency of lactic acid bacteria: the yield of organic acids, competition for nutrients and production of antagonistic compounds. Representatives belonging to genera *Lactococcus* and *Lactobacillus* and, to a lesser extent, to *Pediococcus* and *Leuconostoc* are recognized for their ability to prevent and limit the growth of mycotoxigenic fungi by the synthesis of antimicrobial proteins called bacteriocins (Savadogo et al., 2006).

The public necessity for high quality food, without addition of chemical preservatives, with extended shelf life, determined the search for new strains of lactic acid bacteria that are able to control the fungal growth of plant pathogenic and mycotoxigenic species.

The aim of this work was to assess the effect of different lactic acid bacterial strains on growth of mycotoxigenic fungal species and to select performant strains as biopreservatives for fruit and vegetable commodities.

MATERIALS AND METHODS

The 27 strains of lactic acid bacteria were obtained from dr.Medana Zamfir, Institute for Biology Bucharest and have been assayed on the growth of three fungal isolates from fruits and vegetables: *Alternaria solani*, a plant pathogenic fungus isolated from heavily infected tomatoes, and two potential mycotoxin producing fungi – *Aspergillus ochraceus* isolated from tomato roots grown in greenhouse, and *Penicillium digitatum* isolated from infected oranges. Interaction between lactic acid bacteria and selected mycotoxigenic fungi was tested by overlay assay method (Magnusson et al., 2003). Discrete spots of liquid cultures of tested lactic acid bacterial strains were placed on MRS agar and after 24 h incubation at 30^{o}C, overlaid with soft PDA containing propagules of tested fungi. Data were collected after 48 hours incubation at 25^{o}C concerning the diameters of clear visible inhibition zones of fungal growth around the lactic acid bacterial strain spots. Values obtained represented the mean of three replicates per assay.

RESULTS AND DISCUSSIONS

Interaction between the 27 lactic acid bacterial strains and potential mycotoxigenic *Aspergillus ochraceus* isolated from greenhouse tomato roots (Figure 1) ranged between no visible inhibition and clear visible inhibition zone as shown in the aspect of petri plates (Figure 2). The assay results (Figure 3) showed that 4 lactic acid bacterial strains (15, 61, 122 and 113) had inhibition zone diameter over 25 mm and 18 strains below this value. Five strains had no inhibition effect on mycotoxigenic fungus *Aspergillus ochraceus*.

Figure 1. *Aspergillus ochraceus* - pure culture

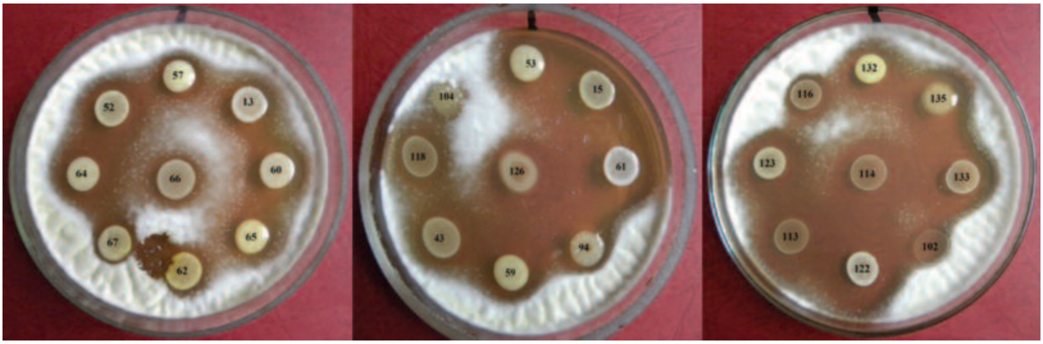

Figure 2. Petri plates with 27 lactic acid bacteria tested against *Aspergillus ochraceus*

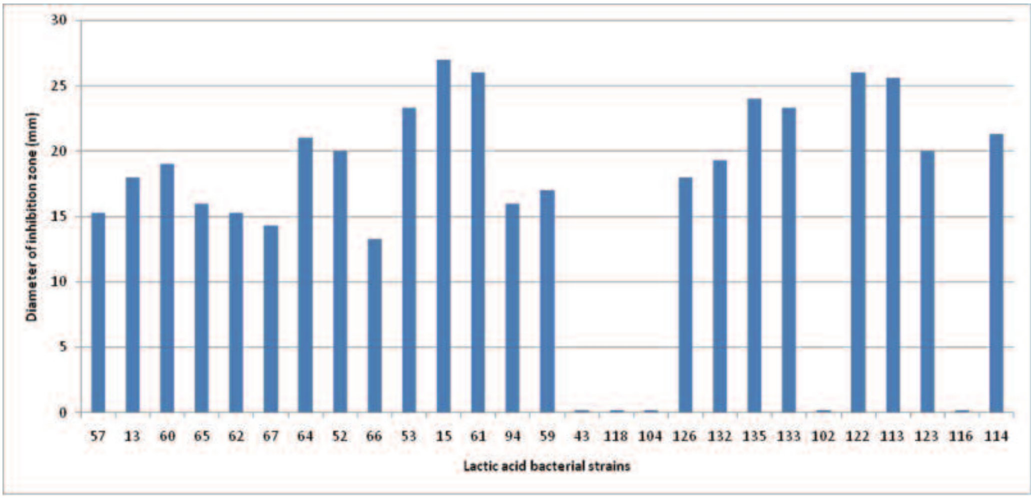

Figure 3. Comparative inhibition zone diameter of 27 lactic acid bacteria tested against *Aspergillus ochraceus*

Our research is in concordance with similar assay carried out by Munoz et al. (2010) when the growth of mycotoxin-producing *Aspergillus nomius* VSC 23 was inhibited by *Lactobacillus fermentum* and *Lactobacillus rhamnonus* in both consecutive and simultaneous inoculation. Djossou et al. (2011) showed that ten lactic acid bacterial strains isolated from silage coffee pulp demonstrated antifungal effect against ochratoxigenic mould *Aspergillus carbonarius*. Two of them belonging to *Lb.plantarum* showed an inhibition with clear zone area between 20-30 mm diameter for three assays against two isolates of test mould.

The inhibitory effect of lactic acid bacterial strains was stronger when they assayed against mycotoxigenic *Penicillium digitatum* isolated from orange (Figure 4). Petri plates showed large inhibition zones around 17 lactic acid bacterial strains (Figure 5). These strains presented inhibition zone diameters over 25 mm and 4 strains had lower values (Figure 6). No inhibitory effect was registered for 6 lactic acid bacterial strains. Research carried out by Trias et al. (2008) showed that 4 lactic acid bacterial strains isolated from fresh fruits and vegetables were able to inhibit the development of infection produced by *Penicillium expansum* on apples by 20%. Organic acids were the preferred mediators of inhibition. Inhibitory effect of lactic acid bacteria on development of another *Penicillium* isolate (*P. nordicum* BFE 487) was demonstrated by modified agar spot assay (Blagojev et al., 2012). Concentrated culture filtrate of *Lactobacillus plantarum* 21B isolated sourdough presented efficient antifungal activity against *P. corylophilum*, *P. roqueforty*, *P. expansum*, *Aspergillus niger*, *A.flavus* and *Fusarium graminearum* due to the capacity to produce phenyllactic and 4-hydoxy-phenyllactic (Lavermicocca et al., 2000).

Figure 4. Orange infected with *Penicillium digitatum*

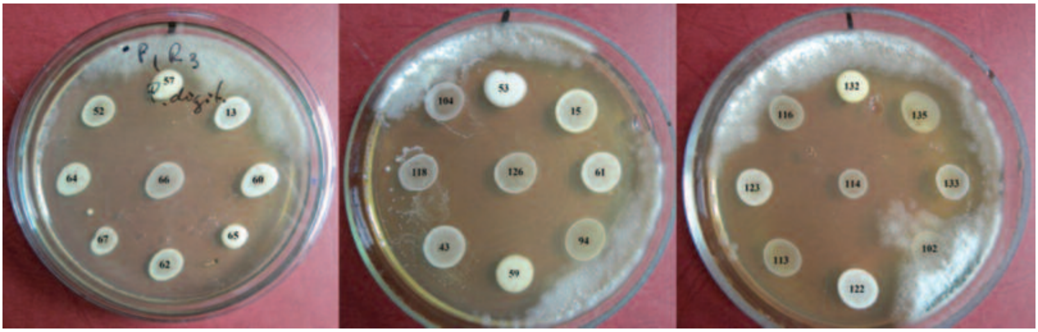

Figure 5. The inhibitory action of 27 lactic acid bacteria tested against *Penicillium digitatum*

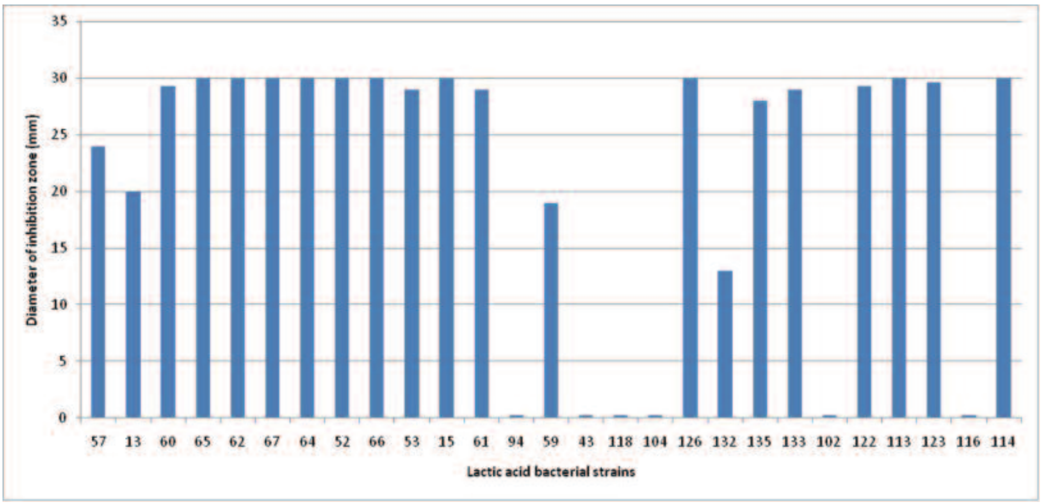

Figure 6. Comparative inhibition zone diameter of 27 lactic acid bacteria tested against *Penicillium digitatum*

The results of the assay against *Alternaria solani* isolated from tomatoes (Figure 7) was not conclusive enough because the fungus sporulation needs a longer incubation period than the 48 hours until the reading of inhibition diameters was done in the present assay.

Figure 7. Tomato infected with *Alternaria solani*

As a general aspect in the assay carried out, a number of 11 lactic acid bacterial strains have shown antifungal activity on both *Aspergillus ochraceus* and *Penicillium digitatum* (strains 15, 52, 53, 60, 64, 113, 114, 122, 123, 133, 135). Four of these strains (15, 61, 113 and 122) presented inhibitory zone diameters over 25 mm when tested against both potential mycotoxigenic fungal species. Another 5 strains of lactic acid bacteria (43, 102, 104, 116 and 118) did not inhibit any of the two mycotoxigenic fungal species. Literature also cites data from agar well diffusion assay where one isolate *Lactobacillus lactis* subsp. *lactis* CHD 28.3 showed a broad spectrum of antifungal activity against *Aspergillus flavus* IARI, *Aspergillus flavus* NCIM 555, *Aspergillus parasiticus* NCM 898 and *Fusarium* spp., six strains showed inhibitory effect against one fungal species and other lactic acid bacterial strains didn't present the capacity to inhibit the growth of any mycotoxigenic fungal species (Dalie et al., 2010).

CONCLUSIONS

A number of 11 lactic acid bacterial strains have shown antifungal activity on both *Aspergillus ochraceus* and *Penicillium digitatum.*

The lactic acid bacterial strains 15, 61, 113 and 122 presented the highest antifungal activity against both potential mycotoxigenic fungi.

Another 5 strains of lactic acid bacteria did not inhibit any of the two mycotoxigenic fungal species.

The effect of lactic acid bacteria on plant pathogenic species *Alternaria solani* did not provide conclusive results and need further study.

The results could be used in future experiments for obtaining performing biological agents with application in food safety.

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