# SCREENING OF LACTIC ACID BACTERIAL AND FUNGAL STRAINS FOR THEIR EFFICIENCY IN BIOCONTROL OF MYCOTOXIGENIC CONTAMINANTS OF FOOD

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***Abstract:*** *The goal of the present paper was to present the results of the research carried out for selecting the most efficient bacterial or fungal strains against certain mycotoxigenic contaminants of vegetal food products in order to use them as biological control agents. Generally, the presence of moulds in foods is associated with decay, spoilage and in certain cases with poisoning, due to dangerous fungal metabolites well-known as mycotoxins. In agriculture, the mycotoxin problem is results of a series of events started from the field, due to fungal infestation of a host plant during the vegetation cycle and the effect are found at the end of the chain, in food and feed. Inappropriate preservation of food during storage can lead to mycotoxin contamination with severe effects on human health. After discovering in 1960 the aflatoxins, carcinogenic metabolites of Aspergillus flavus, research showed that a large number of fungal species could form mycotoxins (e.g. a variety of Fusarium species can release mycotoxins of the class trichotecenes, zearalenone and fumosins). For the screening purpose, a series of lactic acid bacteria selected from fresh or fermented vegetables such as cucumbers, cabbages, from cereals, as well as fungal isolates from cereals, fruits and rhizosphere were tested for their antagonistic effect on the mycotoxigenic fungal species belonging to genera Fusarium, Aspergillus and Penicillium, contaminants of fresh and preserved fruits, vegetables or cereals. Adapted dual culture technique was employed for testing the antagonism between lactic acid bacteria and mycotoxigenic fungi on MRS agar medium and between non-mycotoxigenic and mycotoxigenic fungi on Czapek agar medium.Bacterial and fungal extracts from liquid culture media were used on paper disks method to assess the inhibitory effect on the growth of Fusarium mycotoxigenic species. The paper presents original photographs of the Petri dishes with visible inhibition zones around bacterial extracts from lactic acid bacteria, pseudomonads, as well as from fungi belonging to genus Trichoderma when interacted with mycotoxigenic species of Fusarium. Lactic acid bacteria, Pseudomonas fluorescens, Trichoderma spp. and Myrothecium verrucaria showed important antagonistic effect against mycotoxigenic Aspergillia and Fusaria. They can also be useful for including in biotechnological methods of food preservation.*

***Key words:*** *antagonistic effect, fungi, inhibition zone, lactic acid bacteria, mycotoxins.*

## INTRODUCTION

Mycotoxigenic fungi such as those belonging to genera *Penicillium, Aspergillus* and *Fusarium* can cause important yield loss or damage to agricultural products during the storage or processing due to the synthesis of secondary metabolites named mycotoxins (TSITSIGIANNIS ET AL., 2012).

Six major classes of mycotoxins appear with variable frequency as a function of geographic zone: aflatoxins, fumosins, ochratoxins, trichotecenes, patulin and zearalenone.

The effect of mycotoxins on animal and human health range from immediate toxic response to potential long term carcinogenic and teratogenic effects (WOO ET AL., 2012; LILLARD-ROBERTS, 2013).

Strategies proposed for reducing the effect of mycotoxin ingestion with contaminated food have as purpose to prevent contamination with mycotoxigenic fungi, to detoxify the mycotoxins from feed and food and to inhibit their absorption through digestive tract (KABAK et al., 2006). Physical, chemical microbiological and biotechnological methods are involved (MAGAN AND ALDRED, 2007) with special emphasis on biocontrol methods using selected bacterial and fungal species (CUCIUREAN, 2008; EL-MABROK ET AL., 2012).

Among natural biological antagonists, lactic acid bacteria known for their beneficial role on human health are able to synthesize antagonistic products for the control of pathogenic bacteria or mycotoxigenic fungi (DALIE ET AL., 2010; ADEBAYO ET AL., 2013) and various stains of genera *Pseudomonas* and *Bacillus* (TRIAS EL AL., 2008).

Literature cites antibiotic products by fungi known as important biocontrol agents, such as *Trichodermas* isolates (HASSAN ET AL., 2013). Available commercial biocontrol products using selected *Trichoderma* are: BioFungus, BinabT, RootPro, T-22G, Root Shield/Plant Shield and Trichodex. BioJect Spot Less, Bio-save, BlightBan and Cedomon are products that use bacteria from genus *Pseudomonas* as biocontrol organism.

The aim of the present paper was to present the results of the research carried out for selecting the most efficient fungal and bacterial strains against certain mycotoxigenic contaminants of vegetal food products in order to use them as biocontrol agents.

## MATERIAL AND METHODS

### Test organisms used in this study

Lactic acid bacteria were selected from fresh or fermented vegetables such as: cucumbers, cabbages, from cereals, as well as bacterial and fungal isolates from cereals and plants rhizosphere and preserved in test tubes as pure cultures.

Potential mycotoxigenic fungal species belonging to genera *Fusarium, Aspergillus* and *Penicillium*, contaminants of fresh and preserved fruits, vegetables or cereals were also isolated in pure cultures on PDA medium.

### Detection of antimicrobial activity by agar spot test

Antagonistic activity of 27 lactic acid bacterial strains was assayed against the mycotoxigenic fungus *Aspergillus ochraceus*. Discrete spots of each lactic acid bacterial strain suspension were inoculated at constant distances on Petri dish containing MRS agar medium and after 48 hours of incubation at 25$^{o}$C the fungus inoculum was poured mixed with soft PDA over the plate surface. The antimicrobial activity was determined by measuring the diameters of the inhibition zones around the spot after 48 hours of incubation.

### In vitro assay of antagonistic activity

The inhibition of mycelial growth of mycotoxigenic fungi from genus *Fusarium* was assessed by dual cultures with nonpathogenic fungi and bacteria, using 7 mm diameter pieces of pure cultures of the test species placed in opposite halves of the Petri dish with Czapek agar and incubation, or by using variants with spore suspensions belonging to variable number of microbial test species.

### Assay of antimicrobial activity of culture filtrates

Culture filtrates were obtained from MRS broth for lactic acid bacteria, Topping broth for *Pseudomonas spp.* and Czapek broth for fungi, by centrifuging followed by filtration of the supernatant through 0,2 mm pore size filter paper to remove viable structures. 0,1 ml of filtred supernatant were placed on 10 mm diameter filter paper disks onto the surface of the Petri dish

inoculated with mycotoxingenic *Fusarium* species and incubated. The inhibition zones around the filter paper disks were measured.

All assays were carried out in triplicate.

## RESULTS AND DISCUSSION

Results of in vitro assay of antagonistic activity of fungal and bacterial species are synthesized in the Table 1.

*Table 1*

Interaction between mycotoxigenic species of Fusarium and microbial strains with antagonistic properties

| Mycotoxigenic Fusarium species | Microbial strains | Interaction |
|---|---|---|
| *Fusarium avenaceum* | *Trichoderma viride L11* | +++ |
| *Fusarium culmorum* var. *roseum* | *Trichoderma viride L11* | ++/+++ |
| *Fusarium culmorum* var. *roseum* | *Trichoderma harzianum 8* | ++/+++ |
| *Fusarium culmorum* var. *roseum* | *Trichoderma harzianum 57* | +++ |
| *Fusarium culmorum* var. *roseum* | *Trichoderma harzianum 5* | ++ |
| *Fusarium culmorum* var. *roseum* | *Trichoderma viride L11* | +++ |
| *Fusarium culmorum* var. *roseum* | *Gliocladium catenulatum* | ++ |
| *Fusarium culmorum* var. *roseum* | *Fusarium oxysporum* | +/++ |
| *Fusarium culmorum* var. *roseum* | *Myrothecium verrucaria* | ++/+++ |
| *Fusarium culmorum* var. *roseum* | *Pseudomonas fluorescens* | +++ |
| *Fusarium culmorum* C14 | *Trichoderma viride L11* | ++/+++ |
| *Fusarium culmorum* C14 | *Trichoderma harzianum 57* | ++/+++ |
| *Fusarium culmorum* C14 | *Trichoderma harzianum 8* | ++ |
| *Fusarium culmorum* C14 | *Fusarium oxysporum* | +++ |
| *Fusarium culmorum* C14 | *Gliocladium catenulatum* | ++ |
| *Fusarium culmorum* C14 | *Trichoderma viride L5* | +/++ |
| *Fusarium culmorum* C14 | *Pseudomonas fluorescens* | ++ |
| *Fusarium graminearum* | *Trichoderma viride L11* | +++ |
| *Fusarium graminearum* | *Trichoderma harzianum 57* | +++ |
| *Fusarium graminearum* | *Trichoderma harzianum 8* | ++/+++ |
| *Fusarium graminearum* | *Trichoderma viride L5* | ++ |
| *Fusarium graminearum* | *Pseudomonas fluorescens* | +++ |
| *Fusarium verticillioides* | *Trichoderma viride L11* | +++ |
| *Fusarium verticillioides* | *Myrothecium verrucaria* | ++ |
| *Fusarium verticillioides* | *Gliocladium catenulatum* | + |
| *Fusarium verticillioides* | *Trichoderma harzianum 8* | ++ |
| *Fusarium verticillioides* | *Pseudomonas fluorescens* | ++ |

*- the antagonist doesn't inhibit pathogen and the pathogen overgrows it*
*+ the antagonist inhibits the pathogen growth but the pathogen overgrows it*
*++ the antagonist inhibits the pathogen growth but stops to inhibition limit*
*+++ the antagonist inhibits the pathogen and overgrows it*

Variable antagonistic activity was registered for one species against various mycotoxigenic *Fusarium* species. Interaction ranged from no inhibition to strong inhibition, when the antagonist inhibits mycotoxigenic *Fusarium* species and overgrows it.

Best results with high anagonistic activity was recorded for *Trichoderma viride* L11, *Trichoderma harzianum* 57 and *Trichoderma viride* L5 followed by *Pseudomonas fluorescens.*

For example, *Pseudomonas fluorescens* co-cultivated with mycotoxigenic *Fusarium* species on Topping agar showed stronger growth inhibition on *Fusarium graminearum* than on *Fusarium verticillioides* (Figure 1).

Figure 2 illustrates the large antagonistic zones around *Myrothecium verrucaria* and its stronger inhibitory effect on mycelial groth of *Fusarium culmorum* var. *roseum* than on *Fusarium verticillioides* after 7 days of incubation.

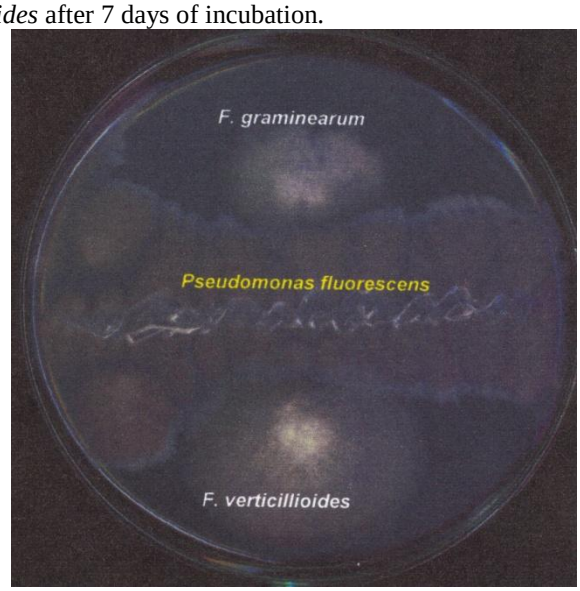


Figure 1. Antagonistic zone between *Pseudomonas fluorescens* and *Fusarium graminearum* and *Fusarium verticillioides* (4 days)

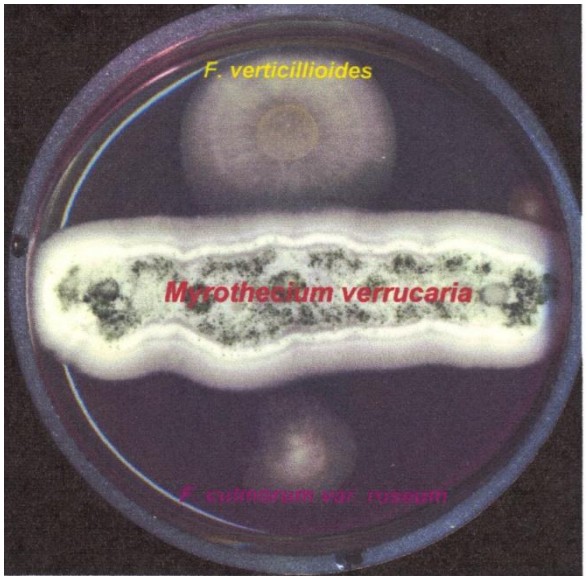


Figure 2. Antagonistic zone between *Myrothecium verrucaria* and *Fusarium verticillioides* and *Fusarium culmorum* var. *roseum* (7 days)

It was also evidenced that strong antagonistic isolates *Trichoderma viride* L11, *Trichoderma harzianum* 57 and *Trichoderma viride* L5 are compatible each other and may act synergistically against 3 mycotoxigenic *Fusarium* species. The aspects of petri dishes after 12 days of incubation reflects the stronger inhibitory influence of *Trichoderma* metabolites on the development of mycotoxigenes isolates of *Fusarium culmorum* C14 and *Fusarium culmorum* var. *roseum* and weaker of *Fusarium graminearum* (Figure 3).

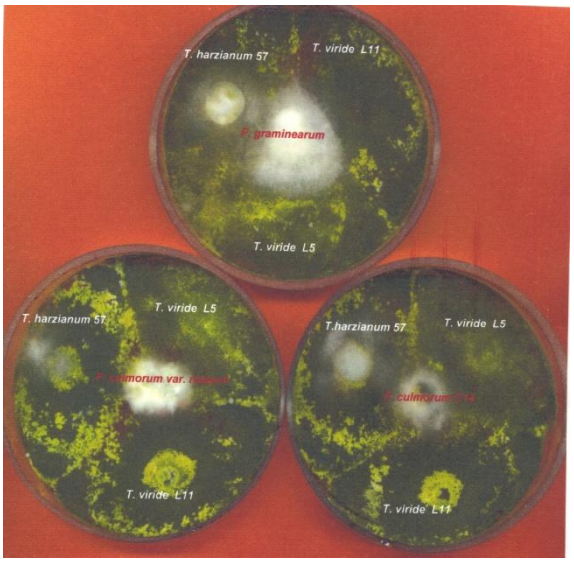


Figure 3. Inhibitory potential of 3 *Trichoderma* isolates on mycelial growth of three mycotoxigenic *Fusarium* species (12 days).

*Trichoderma viride* L11 prezented good growth rate and inhibition capacity against mycotoxigenic *Fusarium culmorum* C14, *Fusarium verticillioides*, *Fusarium graminearum*, and *Fusarium culmorum* var. *roseum* when co-cultivated on the same Petri dish (Figure 4).

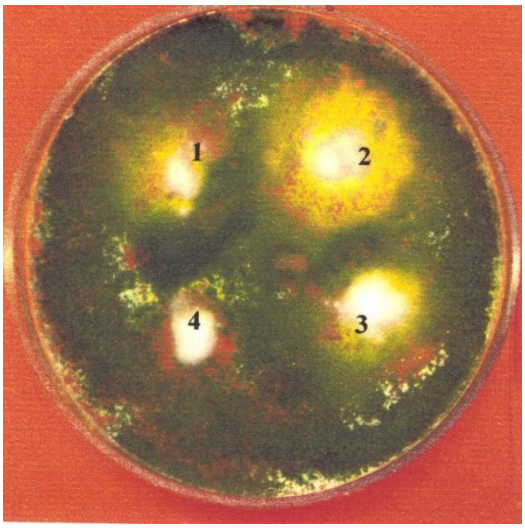


Figure 4. Inhibitory potential of *Trichoderma viride* L11 on mycelial growth of 4 mycotoxigenic *Fusarium* species: 1. *F. culmorum* C14, 2. *F. verticillioides*, 3. *F. graminearum*, 4. *F. culmorum* var. *roseum* (7 days).

Lactic acid bacterial strains presented various antagonistic activity levels against mycotoxigenic *Aspergillus ochraceus*, with large, small or no clear zones around the spots with inoculum. As illustrated in Figure 5, the largest antagonistic zone, even after 4 days incubation and fungal sporulation, was recorded for strain No 15.

Figure 5. Antagonistic zones between lactic acid bacterial strains and mycotoxigenic *Aspergillus ochraceus* (4 days).

Culture filtrates of bacterial and fungal antagonists were placed on filter paper disks, as showed in Figure 6 and after 72 hours of incubation presented specific inhibitory effect on growth of *Fusarium culmorum*. The effect determined as a function of the diameter of clear zone around the filter paper disk was for *Trichoderma viride* L11 > *Trichoderma harzianum* 57 > *Pseudomonas fluorescens* > *Trichoderma viride* L5 > Lactic acid bacterial strain No 15.

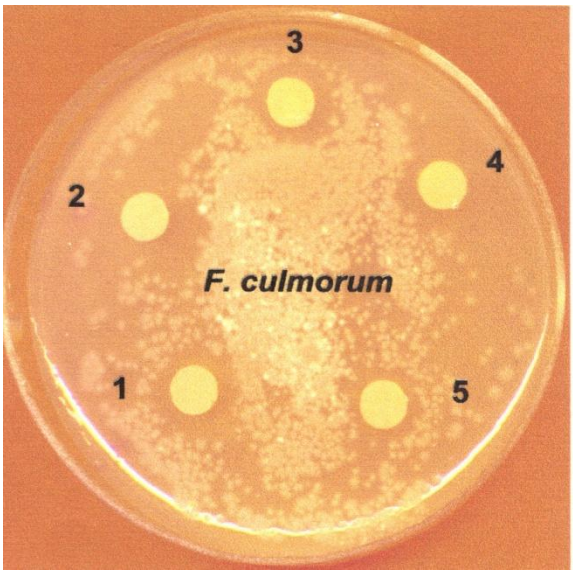


Figure 6. Inhibition zones of mycotoxigenic *F. culmorum* growth around filter paper disks with culture filtrates of: 1. *Pseudomonas fluorescens*, 2. *T. viride* L11, 3. *T. viride* L5, 4. *T. harzianum* 57, 5. Lactic acid bacteria strain 15 (72h).

Inhibitory effects of the same culture filtrates were obtained on the growth of toxigenic *Fusarium culmorum* var. *roseum*, with largest clear zones around the supernatants of *Trichoderma viride* L11, *Trichoderma harzianum* 57 and lactic acid bacteria strain No 15 (Figure 7).

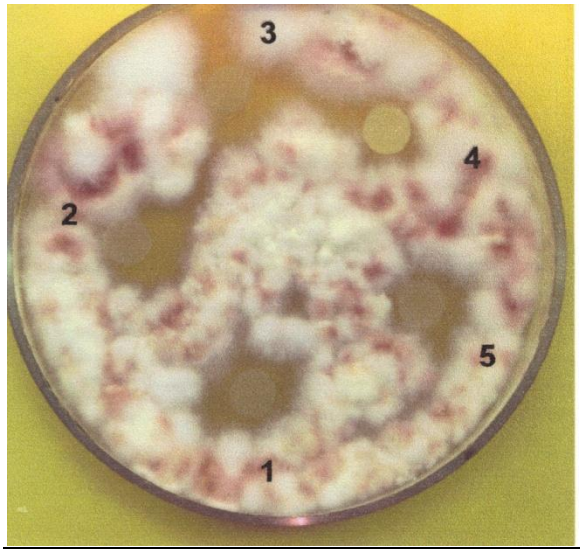

Figure 7. Inhibition zones of mycotoxigenic *F. culmorum* var. *roseum* growth around filter paper disks with culture filtrates of: 1. *T. viride* L11, 2. *T. harzianum* 57, 3. *T. viride* L5, 4. *Pseudomonas fluorescens*, 5. Lactic acid bacteria strain 15 (72h)

Our results are in concordance with literature data that showed the capacity of certain bacterial and fungal special to produce antagonistic effects on the mycotoxigenic species from genera *Fusarium, Aspergillus, Penicillium, Stachybotrys* (Agarry at al., 2005). Various rhizosphere bacteria from vegetables and cereals were reported to produce metabolites such as phenyl lactic acid, 2,4-diacetyl phloroglucinol with antagonistic effect against plant pathogenic and mycotoxigenic fungi (Picard et al., 2000; McSpadden et al., 2001).

In our research, a strongly antagonistic lactic acid bacteria (strain No 15) was selected from screening assay against ochratoxin-producing *Aspergillus ochraceus* and it was recommended for biocontrol purposes.

Similarly, lactic acid bacteria isolated from fresh vegetables were selected for biocontrol of tomato pathogens (Emerenini et al., 2014).

## CONCLUSIONS

Antagonistic activity of screened isolates varied as a function of the phytopatogenic species against it was assessed.

Three *Trichoderma* isolates, *Pseudomonas fluorescens*, lactic acid bacteria No 15 and *Myrothecium verrucaria* showed important antagonistic effect against mycotoxigenic *Aspergillus* and *Fusarium* species.

Screened fungal and bacterial strains may be useful for biocontrol of mycotoxigenic contaminants of fresh and fermented vegetable food.

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