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Qualitative Evaluation of the Capacity of *Lactobacillus* Strains to Degrade Mycotoxins Developed and Accumulated by Strains of the Genus *Alternaria*

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ABSTRACT

Mycotoxins are secondary metabolites produced and accumulated by mould. This study assessed the ability of 89 lactic acid bacteria, isolated from different sources, to degrade two mycotoxins by *Alternaria*: alternariol and altenuene. The mehodology employed was the thin layer chromatography. The results obtained allow us to demonstrate that a 16.85% of the strains (15/89 strains) are capable of decreasing the intensity of the band related at the alternariol and 14.60% (13/89 strains) is capable of decrease the intensity of the band that manifests the altenuane in Alufolium. All tests have been observed under wavelengths of 360 nm and 255 nm. The results obtained allow carrying out a selection of lactic acid strains capable of degrading or decreasing the concentration of mycotoxins selected for this.

Keywords: Lactic Acid Bacteria; Mycotoxins; Alternaria; Thin Layer Chromatography

1. Introduction

Mycotoxins are compounds resulting from the condensation reactions which take place under certain physical, chemical and biological conditions when the reduction of ketone groups in the biosynthesis of fatty acids made by the moulds is interrupted. These acids are primary metabolites by the fungus used as energy source. Mycotoxins are often formed at the end of the exponential phase or early stationary growth phase of the microorganism.

These are pollutants of various food and feed, correlating with various diseases of animals and humans [1,2]. Exposure to mycotoxins can cause acute or chronic toxicity, and adverse effects on the central nervous system, on the cardiovascular, respiratory and digestive system and can also cause death. Agents may also be carcinogenic, mutagenic, teratogenic and immunosuppressive. At present it is mentioned as one of the most important effects, the ability of some mycotoxin compromise the immune response thus reduce the resistance to infectious diseases.

Moreover mycotoxins are of global concern due to major economic losses because of its effects at the level of human health, animal productivity and national and international trade. Miller [3] in a statement staff estimate that in the United States of America and Canada, the annual losses due to the effects of mycotoxins in feed and livestock industries are of the order of 5000 million. For prevention, decontamination, inactivation and detoxification have studied different methods: physical, chemical, fungistatic utilization, use of additives and enzymes mycotoxin absorbents.

The latter method is applicable to the degradation and/or inactivation of mycotoxins, which can include: natural extracts and/or essential oils from plants and pathogenic microorganisms.

Various tests have been conducted to study pathogenic microorganisms capable of degrading specific conditions certain mycotoxins. Such microorganisms are: *Saccharomyces cerevisiae*, *Flavobacterium aurantiacum*, *Rhizopus* spp. *Neurospora sitophila* and rumen microorganisms.

Some of these studies have provided effective results in the degradation of mycotoxins such as Aflatoxins, Patulin, Ochratoxin A, Zearalenone, T-2, Rubratoxin or Diacetoxyscirpenol A. These studies have been performed in the laboratory, but the practical application of these systems is under study and development [4].

Lactic acid bacteria (LAB) may be a possible alternative. This group of microorganisms is considered GRAS

(Generally Recognized As Safe) and are therefore ideal for use as biocontrol or as biopreservants. Bacteria of the genus *Lactobacillus* are widely distributed in nature and grow in a variety of conditions [5,6], during their development released into the metabolic products such as lactic acid, acetic acid, hydrogen peroxide, bacteriocins, among others [7] which can cooperate effectively to control undesirable microorganisms.

2. Materials and Methods

From 89 axenic strains of *Lactobacillus*, of various origins, as detailed in **Table 1**, and developed overnight in MRS broth was obtained. An aliquot was inoculated into 50 mL of MRS broth that is calculated according the proportion of 5% (v:v) corresponding to the final volume.

After inoculation of each of these cultures were aliquoted kinetics according to exponential phase growth of these strains, determined between 0 - 7 hrs of incubation [8].

During 30 minutes maintaining the interaction between each of the respective cultures mycotoxins and filtered under study. The minimum concentration of mycotoxins is 0.020 ppm [9].

Interaction elapsed 10 µl are obtained, which are deposited in silica gel chromatoplates (Ref: 0554.001, Merck). Previously these chromatoplates were activated at 100˚C for 15 minutes [9].

Positive controls:

- 10 µl alternariol (M1) + 10 µl MRS Broth;
- 10 µl altenuene (M2) + 10 µl MRS Broth.

Negative controls:

- Filtering 10 µl of strain + 10 µl strain MRS Broth;
- 10 µl cultivation of strain + 10 µl unfiltered MRS Broth.

Mobile phase was used as the following mixture: toluene/methanol/acetic acid (86:12:2) [9].

After the chromatography, the chromatoplates were observed under uv at 365 nm and 255 nm. The fluorescence bands of samples and controls problems allow us to compare the values of Rf and set in the semiquantitative results.

3. Results and Discussion

Several authors have shown in their work that secondary metabolites produced and accumulated by strains of the genus *Alternaria* are toxic [10-12]. In the course of our work we have selected two of the genus *Alternaria* mycotoxins: alternariol and altenuene, to conduct studies on the potential degradation of mycotoxins by the 89 strains of lactic acid bacteria that have submitted ability to inhibit *Fusarium moniliforme* and/or *Alternaria alternata*.

The results obtained allow us to demonstrate that a 16.85% (15/89) are capable of reducing the intensity of the band at alternariol observed at wavelengths 360 nn and 255 nn and 14.60% (13/89) is capable of decreasing the intensity of the band expressed by altenuene observed at wavelengths 360 nm and 255 nm.

The **Figure 1** shows chromatoplate, showing decreased intensity of the band representing the mycotoxin. Decrease is attributed to the interaction of the mycotoxin with lactic strains

This technique is relatively fast but only qualitative results can be achieved, thus allowing not deliver numerical results representing the decrease in concentration of the mycotoxin. However, facilitates carrying out a selection of strains BAL, with manifest ability to degrade or reduce the concentration of the two mycotoxins selected for this study: alternariol and altenuene.

4. Conclusion

The two mycotoxins used as substrates for degradation capacity tests, were modified or degraded by 24 strains

Table 1. Number of strains tested and origin.

Origin of *Lactobacillus* strains	Number of strains tested
Small intestine and caecum of birds	8
Dog faeces	29
Pig faeces	41
Snails faeces	11

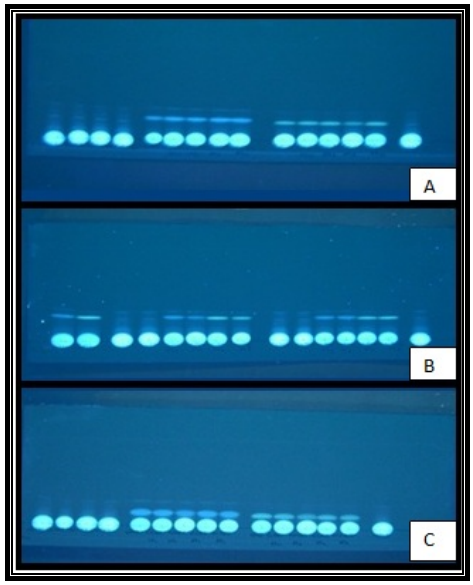

Figure 1. Testing thin layer chromatography mycotoxin reduction bands: A: interaction of M1 with strain C-P11; B: M2 interaction with strain C-C50; C: interaction with the M1 and M2 strain N-C61; C: Filtered N: Strain unfiltered; M1: M2 alternariol: altenuene.

of lactic acid bacteria, of which, fifteen strains showed activity on alternariol and thirteen on altenuene.

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