PRELIMINARY RESULTS OF *In Vitro* ANTAGONIST BACTERIA ON DEVELOPMENT OF FUNGI ISOLATED FROM *Brachiaria brizantha* SEEDS.

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Introduction

Regeneration and multiplication of *Brachiaria*. grass germplasm are normally carried out under field conditions in Popayán (2°25'n;76°40'w, altitude 1750 m.a.s.l.), Colombia (Fig1). Under such conditions fungi, such as *Drechslera*., *Phoma*., *Curvularia*., *Sphacelia*., *Cerebella*., *Curvularia* and *Fusarium*. can either infest or infect seeds and significantly reduce the quality of the seed produced and stored. Trials with different fungicides proved to be inadequate for disease control. We used an antagonistic bacteria as a preliminary approach of biological control of these fungi.

Figure1. Regeneration and multiplication of *Brachiaria,* grass germplasm.

Material and Methods

Fifteen bacterial isolates were obtained from *Brachiaria brizantha* seeds produced at the Santa Rosa Experimental Station in Popayán, Colombia. Isolates that showed a direct antagonistic activity (growth inhibition zones) against fungi growing on either seed or media were selected. (Fig 2). Isolates were grown on Nutrient Agar and were purified.

Bacterial isolates were separated into three groups based upon colony morphology and Gram staining. Group 1 (G1) was comprised of 4 isolates with white colonies, dry appearance, rough edges, and were bacillus Gram positive. Group 2 (G2) was comprised of 6 isolates with cream colonies, moist appearance, smooth edges, and were bacillus Gram positive, and Group 3 (G3) was comprised of 5 isolates with yellow colonies, moist appearance, smooth edges, and were bacillus Gram negative.

The antagonistic effect of isolates from these bacterial groups was evaluated on five different fungi: *Drechslera., Phoma. , Curvularia., Fusarium.,* and *Epicoccum*.

Direct antagonistic studies

Sections of fungal isolates (3 mm in diameter) previously grown on PDA were placed on an edge of new PDA plates.

Bacterial isolates that were grown on nutrient agar were streaked on the same PDA plate at the opposite edge of the fungal section and keeping the bacteria and fungal isolates separated by about 7 cm.

Figure 2. fungi identification growing on either seed or media.

Filtrate studies.

Bacterial isolates from Group 3 were inoculated separately in nutrient broth. Cultures grew for 24 hours at 26ºC. They were centrifuged and filtered using a 0.8µ Millipore membrane. The filtrate (10ml/100ml of media) was added to PDA media after autoclaving and before solidification. Sections of fungal isolates (3 mm in diameter) were then placed on these PDA plates as was done previously in our direct antagonistic studies.

Fungal colony growth was measured at 3, 5 and 8 days after plating both organisms onto the same plates or control plates**.** Percentage of inhibition was estimated for each bacterial isolate by relating the maximum growth (MG) possible in control plates and the growth (g) reached under presence of the bacteria isolate in the test plates using the following equation:

% inhibition= (MG-g/MG) x 100

Results and Discussion

Bacterial isolates from groups G1 and G2 had a low inhibitory effect on fungal growth based upon the time required by the fungi to reach the maximum growth in presence of the bacterial isolate. Isolates from group G3 produced more significant results.

These results indicated a possible antibiosis bacterial mechanism better than an antagonistic mechanism. Large inhibition zones suggested a presence of a metabolic compound in the media that restricted mycelia growth in that area (Fig 3).

Filtrate studies were similar to the first test and confirmed the inhibition effect of these isolates against the same fungi. Fungal growth inhibition was 98.5% in *Drechslera.*, 98.4% in *Fusarium* ., 97.4 % in *Epicoccum*., 96.5% in *Curvularia.,* and 90.0% in *Phoma*., respectively.

Lab and field tests to validate and verify these results are underway. Preliminary results from a field test using G3 inoculum and propiconazole showed some level of control in *Drechslera.,* and *Phoma.,* but not in *Curvularia., Epicoccum* ., and *Fusarium* .

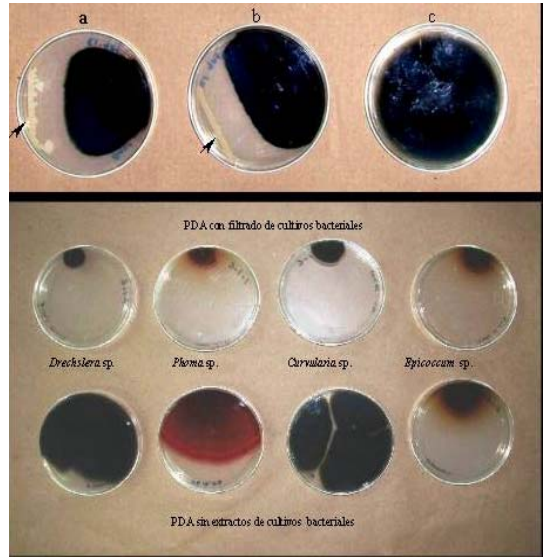

Figure 3. Antagonistic effect of bacterial isolates from group G3 against *Drechslera*., a fungi associated with *Brachiaria brizantha* seeds onto PDA plates: Top pictures (**a)** isolate 1 G3, **(b)** isolate 2 G3 and **(c)** control (fungi alone). Bottom picture. Inhibitory effect of bacteria filtrates on four fungal growth on PDA.

References

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Poster presented at the 2nd International Symposium on Biological Control of Bacterial Plant Diseases, Orlando, USA, 4-7 November 2008.