Bacillus spp as a Biological Control Agent against Panama Disease in Banana

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INTRODUCTION

The banana originated in Southeast Asia and its cultivation is now widespread and dominant in tropical regions. In Africa, about 30 million tons of bananas are produced annually and most are consumed locally as either a food source or income generator for subsistence farmers. The continuous production of banana is however, threatened by infections, which are mainly caused by fungal pathogens. Panama disease is a notorious disease caused by *Fusarium oxysporum sp cubence*. Major losses of up to 50 percent in banana crops have been reported in some banana producing regions (**Figure 1**). The ability of the fungal pathogens (especially race 4) to infect a wide range of banana cultivars and to establish resistance to chemical pesticides is a threat to the continued cultivation of bananas. Alternative remedies to curb proliferation of Panama disease have therefore been initiated. CSIR Biosciences is extensively engaged in the development of biological control strategies and has identified an isolate of *Bacillus spp.*, which demonstrated inhibition of *Fusarium oxysporum* during *in vitro* experiments. Investigation to establish whether the isolate could demonstrate similar effects against the pathogen in banana crops was investigated. Preliminary green house trials, with a limited number of pot plants, were commissioned and were planned for two banana cultivars (cvs Williams and Grand Nain). The isolate was applied to the banana plants as a prophylactic and as a treatment.

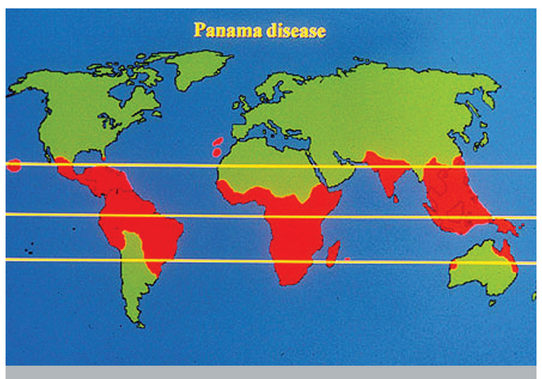

Figure 1: The global prevalence of Panama Disease

OBJECTIVES

The objectives of this study were:

- To demonstrate the ability of an isolate of *Bacillus spp.* to inhibit a wide spectrum of fungi; and
- To demonstrate inhibition of *Fusarium oxysporum* in banana plants in preliminary green house trials.

MATERIALS AND METHODS

- A candidate *Bacillus spp.* was tested *in vitro* against a spectrum of potential fungal pathogens. The fungi were either suspended in peptone and 0.1mL inoculated to a well (**Figure 4**) or they were aseptically cut into a block and placed on PDA plates (**Figure 5**). The antagonist was prepared in modified sporulation media (Feng *et al*, 2002) and incubation at 30°C, after 18 hours incubation and having stabilised the contents, 0.1mL of broth was aseptically transferred to the centre of the PDA plate that had been pre-inoculated with fungi. The tested fungal isolates were *Rhizoctonia spp, Fusarium oxysporum, Fusarium spp, Fusarium subglutinans, Verticillium dahliae* and plates were incubated at 25°C. Fungal development was monitored over seven days and a positive result was determined by uneven edge development of the fungi.

- The *Bacillus spp.* prepared as per above was tested *in vivo* for continued efficacy on two banana cultivars and treatments were designated as follows:
  - Positive control, with healthy plants and no treatment with antagonist
  - Negative control, with plants being artificially infected with test pathogen and no treatment
  - Treatment 1, where pre-infected plants were treated with the antagonist and
  - Treatment 2, where healthy plants were also treated with the antagonist.

  Treatments were done once every week with manual irrigation on all test plants. Visual inspection of test plant was done weekly over five weeks with the test pathogen being *Fusarium oxysporum* (race 4).

RESULTS AND DISCUSSION

The putative isolate demonstrated potential for the inhibition of all tested fungal pathogens (**Figure 4**). This was a valuable observation as some of the fungi inhibited by the isolate are known to be great challenges in the agricultural sector. The inhibition of the fungi by the putative isolate indicates strong potential as an agricultural biological agent against a wide spectrum of fungal pathogens.

The results of the inhibition tests against *Fusarium oxysporum f. sp. cubence* race 4 demonstrated the ability of the antagonist to inhibit the fungal pathogen *in vitro* (**Figure 5**). The tested fungal variant (race 4) is known for infecting even the less susceptible crop cultivars e.g. the Cavendish variant. Infections as caused by the tested variant, together with the other fungal variants, are generally widespread through out the world (**Figure 1**) with overwhelming damage to crops (**Figure 2**) and plantations (**Figure 3**).

The application of the isolate on the two tested crop variants showed positive antagonism against all infected test plants treated with the isolate (**Figure 6**). The difference between positive, negative controls and treatment 1 was visually observed with test plants of treatment 1 showing high foliar expression compared to the positive control. The negative control resulted in attenuated plant development due to fungal infection. Application of the isolate on non-infected plants (treatment 2) did not indicate any negative impact on plant development, indicating that the putative isolate demonstrated strong potential for use as a commercial biological treatment agent in agriculture (**Figure 7**). The ability of the product to revive infected plants was also observed in the test where infected plants were treated with the product.

Figure 2: Cross sectional view of an infected plant

Figure 3: Banana plantation infested by Panama in Malaysia (Image from *Plant health progress* article)

Figure 4: Inhibition by isolate of fungal isolates

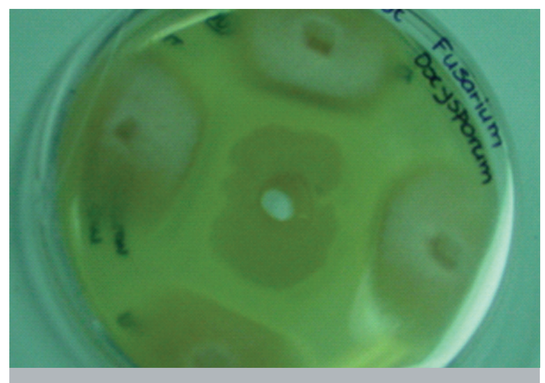

Figure 5: Inhibition by isolate of *Fusarium oxysporum*

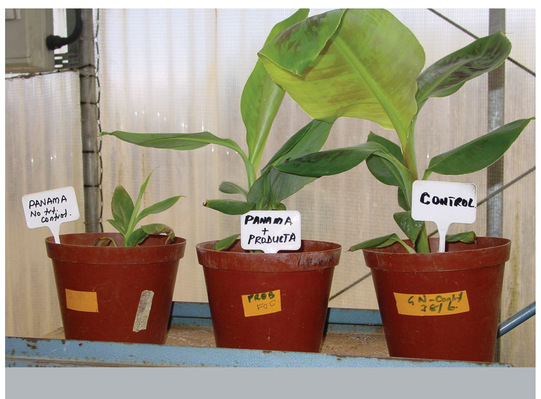

Figure 6: Comparison of control, treated and untreated crop

Figure 7: Comparison of control, uninfected treated with product and untreated crop

CONCLUSION

The data clearly demonstrated that the putative isolate has potential for development into a successful biological control agent. The isolate was mostly effective as a prophylactic but continued application to infected plants resulted in improvement in health, indicating potential application as a treatment. Larger scale field trials are planned to progress the putative isolate towards a commercial agricultural biological product.

ACKNOWLEDGEMENTS

Acknowledgments are due to the Product and Process Development Research Group for the hard work together with the Plant Biotechnology Research Group at CSIR Biosciences.