Biological Control of Mycotoxigenic Fungi and Ochratoxin A by The *In-Vitro* Application of a Qatari *Burkholderia cepacia* Strain (QBC03).

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Abstract

Mycotoxins are secondary metabolites synthesized by mycotoxigenic fungi which belong mainly to three major genera that are *Aspergillus*, *Fusarium* and *Penicillium*. They contaminate plants and different food commodities and cause health concerns. In this research, we have used the Qatari strain *Burkholderia cepacia* (QBC03) as a biological agent against mycotoxigenic fungi, and the strain has possessed a wide antifungal spectrum against 21 different species. Additionally, the antifungal activity of QBC03's supernatant was explored on the fungal biomass and OTA synthesis of *A. carbonarius* in liquid media and they were both reduced upon treatment. The effect of QBC03's supernatant on the spores' germination was examined, and as a result, the conidial germination was completely inhibited. The thermal stability of the antifungal compounds in QBC03's culture supernatant was investigated, and it was shown that QBC03's metabolites were distinctively thermostable and were still active even when heated at 100ºC. The findings of this research prove that strain QBC03 is an excellent candidate for the biological control of mycotoxigenic fungi, particularly in Qatar.

Introduction

One of the major causatives for food and feed spoilage is the contamination of mycotoxigenic fungi and their associated mycotoxins. The contamination with mycotoxins occurs during pre-(field fungi) or post-harvest (storage fungi) stages. The health effects of mycotoxins range from transitory illness to severe consequence (carcinogenesis, immunosuppression and death) in humans and animals as well. The physio-chemical nature of majority of the mycotoxins makes them resistant to extreme temperature and other physical procedure used in routine food and feed processing. The bio-control of mycotoxins/fungi with environment-friendly microbes is considered most safe and economically benefitable. The other strategies include physical (heat, UV, sorting of contaminated grains, etc.) and chemicals (acids, basis, ammonia treatment, etc.) treatments.

Materials & Methods

1. Exploration of the antifungal spectrum of QBC03

- QBC03 was inoculated on the surface of nutrient agar and were incubated at 30ºC/48 h. Afterwards, the fungal spores in soft PDA were poured around the colony and the plates were incubated at 26ºC for 4 days. The diameters of the inhibition zones were recorded.

2. Evaluation of QBC03's antifungal compounds on *A. carbonarius* biomass and mycotoxin production (OTA).

- Different volumes of QBC03 culture extract were incorporated in 20 mL of PDB. 10 µL of *A. carbonarius* spores were inoculated in all flasks and were incubated at 26ºC/140rpm/72h (Kilani-Feki & Jaoua, 2011). The culture was filtered to measure the biomass and mycotoxin in the filtrate by ELISA.

3. Investigation of the thermostability of QBC03's antifungal compounds against mycotoxigenic fungi

- The extract of QBC03 culture was collected and treated with different temperatures (-80 to 100) and was loaded into wells of PDA that had fungal spores suspended on its surface. The diameters of the inhibition zones around the wells were recorded on the 4th day of incubation at 26ºC.

4. Evaluation of the effect of QBC03's antifungal compounds on the spores' germination.

- Wells of 96-plate were loaded with 900 µL of PDB amended with 500 µg/L chloramphenicol, and 100 µL of 48 h QBC03's extract was added to the wells, and 2 µL of fungal spores were added too. In the control, the extract was replaced with NBY broth. The plate was incubated for 24 h and the spores were visualized using the inverted light microscope. Microscopic alterations in the treated mycelia were observed under the microspore.

Results & Discussion

1. Broad spectrum range of antifungal activities of QBC03's diffusible compounds

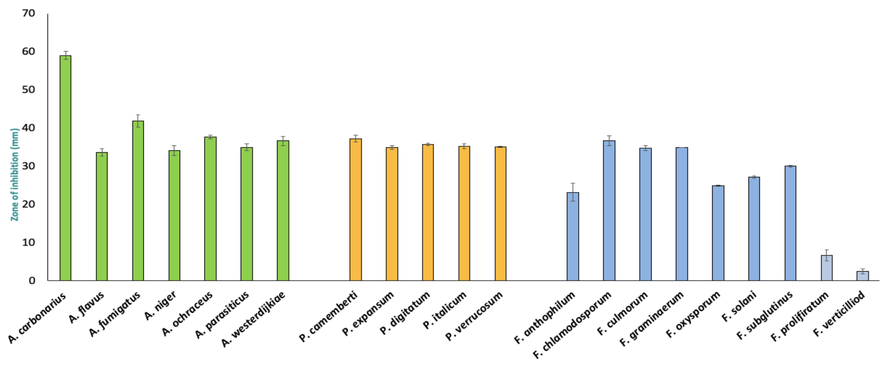
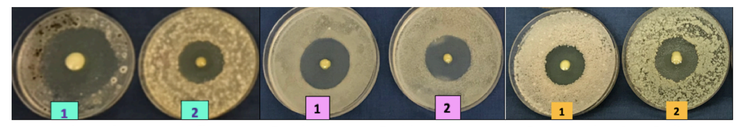


Fig.1: Determination of the antifungal activity of QBC03 strain against *Aspergillus, Fusarium and Penicillium* species in overlaying assay method.

- QBC03's diffusible compounds have an excellent antifungal activities against different mycotoxigenic fungal genera.
- The antifungal activity of QBC03 is specie specific, where some species were too sensitive to the bacterial compounds and others where not affected



2. Effect of different concentrations of QBC03's antifungal compounds on OTA synthesis by *A. carbonarius* in PDB.

- Increasing the concentration of QBC03's antifungal compounds in the fungal culture has significantly reduced the mycelia biomass and OTA concentration in *A. carbonarius* culture.
- At 3% onward, the biomass had reduced significantly to reach almost 1.82 mg.
- OTA's concentrations followed the biomass trend. 3% of the bacterial extract reduced the biomass to more than half compared to the control.
- QBC03's supernatant had induced aggregates formation and thickening in the cell wall (chlamydospores formation) (Zeidan *et al.*, 2019).

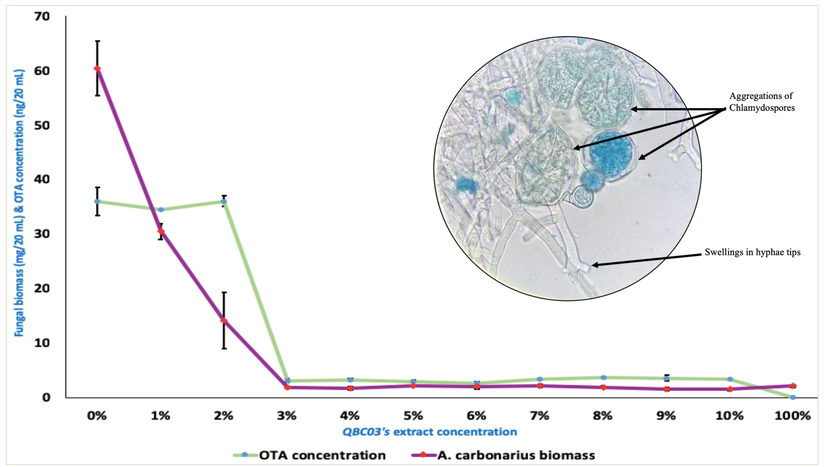


Fig.2: Effect of QBC03's supernatant on the mycelia biomass and OTA concentration by *A. carbonarius* upon different treatments of QBC03's antifungal extract in PDB.

3. Effect of temperature on the antifungal compounds of QBC03 against mycotoxigenic fungi

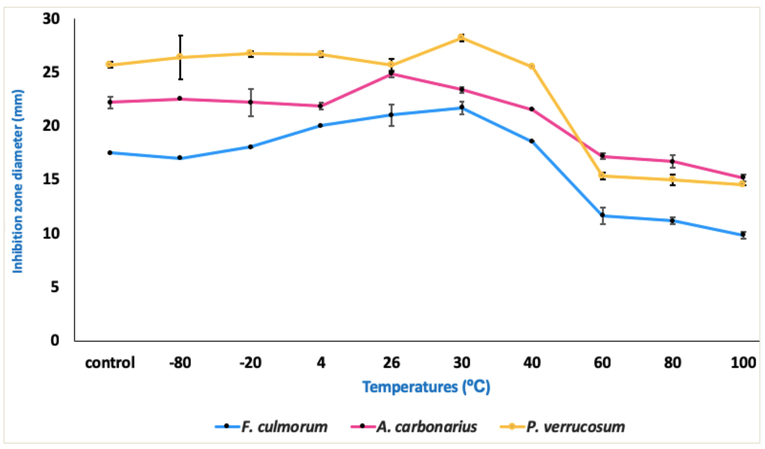
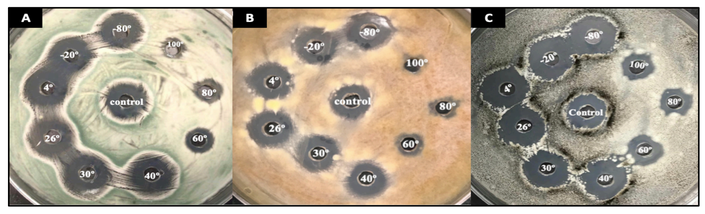
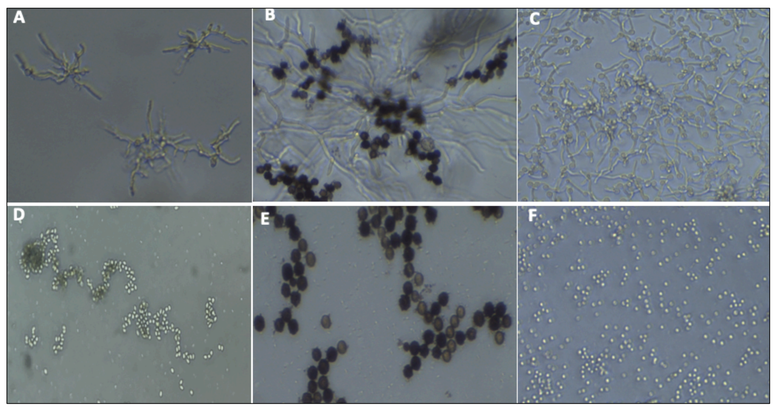


Fig.3: Wells-diffusion method used here to test the effect of temperature on the activity of QBC03's antifungal compounds against the mycotoxigenic fungi *P. verrucosum* (A), *F. culmorum* (B) & *A. carbonarius* (C). The inhibition zones around the wells were measured and recorded.

- The activity of QBC03's antifungal compounds was shown to be stable upon the treatment with wide range of temperatures starting from -80º to 100º.
- Increasing the temperature has reduced the antifungal activity.
- The thermal stability of the antifungal compounds at very high temperatures (100ºC) makes them good candidate to be used locally since the temperatures can elevate very much in the gulf region.



4. Effect of QBC03's supernatant on the fungal spores' germination



- The extract of the 48 h QBC03 culture has completely inhibited the conidial germination and no germination tubes were seen after 24 h incubation.
- The tray was kept incubated for 3 more days, but the spores didn't germinate at all.

Fig.4: Effect of QBC03's antifungal compounds on the fungal spores' germination. (A-C) are spores that are not treated with the bacterial extract and (D-F) are spores that are treated with the bacterial extract for *P. verrucosum, A. carbonarius* & *A. westerdijikae,* respectively.

Conclusion

- The Qatari *Burkholderia cepacia* (QBC03) strain has antifungal activities against 21 different fungal species from different genera.
- QBC03's antifungal compounds had significantly reduced the growth of *A. carbonarius* in liquid media, in addition to a significant reduction in OTA production.
- The antifungal extract of QBC03 has efficiently inhibited the spores' germination of the mycotoxigenic fungi and the conidia couldn't germinate at all after the treatments with the extract.
- The antifungal compounds of QBC03 were shown to be thermostable and therefore have a future use in *in-vivo* applications.

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