Broad spectrum biocontrol of plant pathogens with Bacillus amyloliquefaciens strains through selective enhancement of its antibiotic production using carbon sources and culture additives

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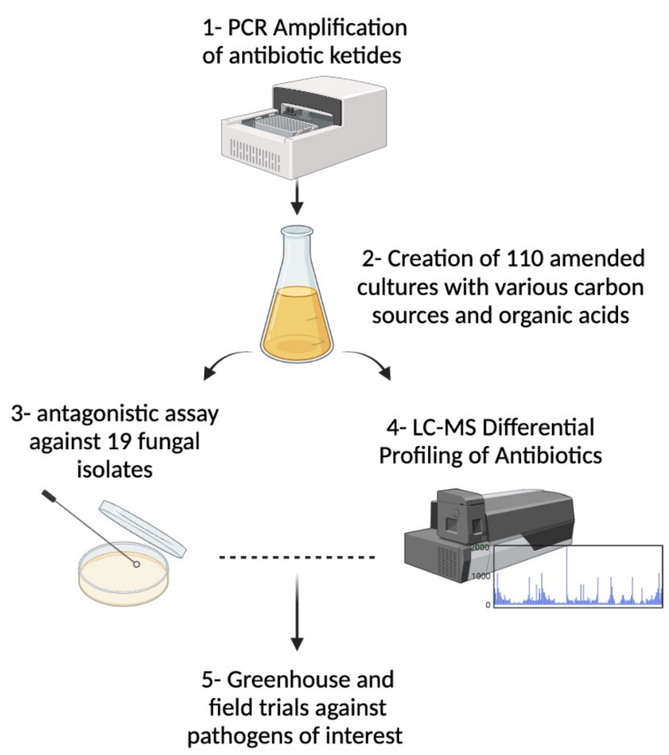
Introduction

- Fungal pathogens are the major cause of pathogen-related yield loss in agriculture, threatening global food security[1]
- Although fungicides are used to mitigate such plant diseases, chemical fungicide use has become less effective due to the emergence of fungicide resistance in pathogen populations[2]
- Thus, utilization of bacterial species as sustainable and natural alternatives continues to receive considerable attention
- *Bacillus amyloliquefaciens* are promising as potential biocontrol species, as nearly 8% of their genome is involved in antimicrobial metabolite synthesis[3]
- Interestingly, Bacilli show differential lipopeptide production depending on culture conditions[4]

Objectives –

- Increase the metabolite synthesis of three Bacillus strains (94, 113, 279) through the addition of various culture additives such as growth hormones and carbon sources
- Evaluate the biocontrol ability of the amended cultures against economically relevant plant pathogens

Methods



Results

In vitro Antagonistic Assay

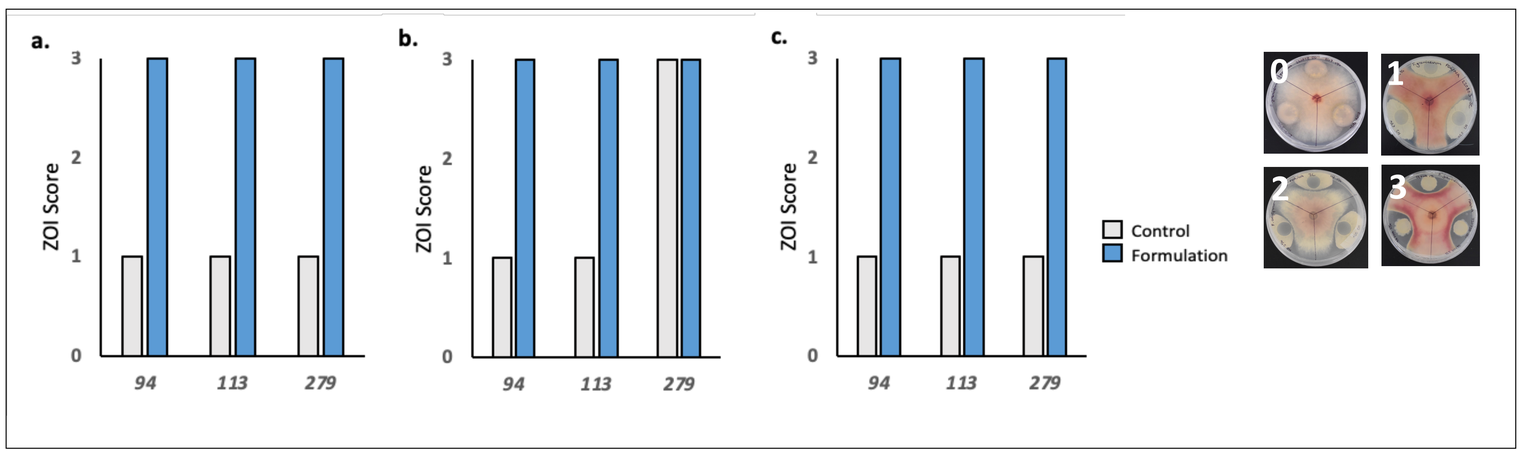


Fig. 1. *in vitro* antagonistic abilities of control and amended isolates against *Fusarium oxysporum* (a), *Fusarium graminearum* (b), and *Slerotinia sclerotiorum* (c). The isolates were assigned a score between 0 to 3 based on their zone of inhibition (ZOI), as seen in the example (d).

LC-MS Differential Screening

Table 1.

Isolate	Peak # increase
94	43%
113	47%
279	75%

Fig. 2. LC-MS results for amended cultures and respective controls. The total peak area for 94 (**a**), 113 (**b**), and 279 (**c**) are on the left, and the total peak number increase for the three isolates is exhibited in **table 1**.

Greenhouse & Field Trial

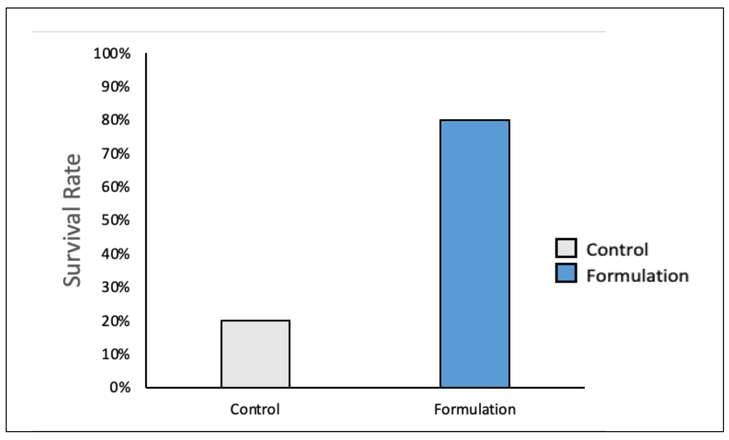


Fig. 4. Growth room trial data for tomatoes artificially infected with *Fusarium oxysporum*. The plants were either treated with amended 94, or left untreated as control; plant survival rate was quantified after 14 days.

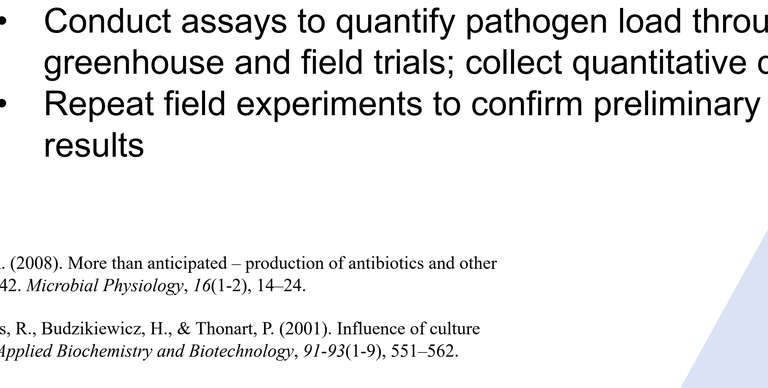


Fig. 5. Average yield of field squash infected with powdery mildew. Plants were treated with either amended cultures or a common chemical fungicide. An equal number were left untreated as control.

Conclusion

- All three *Bacillus amyloliquefaciens* strains showed improved lipoprotein production upon manipulation of growth media[4]; confirmed with LC-MS data (**Fig. 2.**)
- Amended cultures with highest lipoprotein production demonstrated increased antagonism against pathogens, notably *F. oxysporum, F. graminearum,* and *S. sclerotiorum* (**Fig. 1**)
- Modified cultures improved plant survival (**Fig. 4)** and delayed symptom expression (**Fig. 5**)
- Thus, the strains could potentially be used as natural biocontrol alternatives, mitigating issues associated with fungicide resistance[2]

Future Directions –

- Organize further growth room trials against additional pathogens of interest
- Conduct assays to quantify pathogen load throughout greenhouse and field trials; collect quantitative data
- Repeat field experiments to confirm preliminary results

References

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