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Special Research Report #141: *Optimizing the efficacy of beneficial bacteria against botrytis blight in greenhouse crops, Part 1*

Category: Disease Management

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BACKGROUND

Botrytis cinerea is the causal agent of botrytis blight or gray mold, which is the most common and economically devasting disease for greenhouse crops. Fungicides are routinely used to control Botrytis; however, some Botrytis are developing resistance to those chemicals. Beneficial bacteria have been used successfully as biological control agents for disease control. Our laboratory has screened a collection of bacteria for their ability to control botrytis blight in petunia. We have identified some of those bacteria as effective microbial biocontrol agents (MBCAs). Further investigations with these bacterial isolates are required to characterize the bacteria and evaluate the efficacy of Botrytis disease control.

There are three objectives for this project, which have been divided into two reports. This report covers the first objective.

- Objective 1 - Identify potential modes of action for Botrytis control by selected MBCAs.

MATERIALS AND METHODS

Bacteria modes of action and in-lab assays

Exp 1.1 – To determine if the bacteria can directly inhibit the growth of Botrytis, we grew the bacteria strains and Botrytis on single culture plates in the lab. Bacterial growth was measured on the plate with a ruler.

Exp 1.2 – To evaluate if bacteria strains produce volatile compounds that could indirectly inhibit the growth of Botrytis, we grew bacteria strains on one culture plate and Botrytis on a second culture plate. The two plates were sandwiched together such that they were sharing the same air space but not in direct contact. We determined if the Botrytis growth was affected by the presence of bacteria by comparing growth to that on the no-bacteria control plates.

Exp 1.3 - To determine if combining bacteria strains together would improve the control of Botrytis blight, we needed to learn more about these bacteria. Some bacteria have the ability to form a biofilm, which is a matrix of bacteria cells. Biofilms can be involved in protecting plants from pathogens that cause disease, like Botrytis. We therefore conducted an assay to determine which bacteria or combinations of bacteria can produce a biofilm.

Exp 1.4 - Some bacteria are capable of directly inhibiting the growth of other bacteria. If any of our strains have that ability, this experiment will help us determine which bacteria

cannot be combined as MBCAs. The combinations of bacteria were cultured together on nutrient-rich agar media. Growth inhibition or growth promotion of a bacteria by another was quantified.

Exp 1.5 - Genomic analyses can be used to identify the bacteria (genus and species), and to identify potential genes that may be involved in biocontrol. For example, bacterial genes of interest may include those involved in the production of antibiotics or volatile compounds or those involved in secretion systems or pathogen resistance. Antibiotics produced by bacteria can directly inhibit fungal pathogen growth. Some bacteria produce volatiles that could inhibit the growth of Botrytis in surrounding plant tissue. Secondary secretion systems are sophisticated systems in bacteria that allow them to move molecules, like toxins and antibiotics, across their cell membranes so that they can interact with and inhibit pathogens. They influence the relationship between beneficial bacteria and host plants and are therefore an important feature of MBCAs.

RESULTS

Exp 1.1 Three bacteria strains directly inhibited Botrytis when grown together on a plate.

Three bacteria strains inhibited the growth of Botrytis in a lab assay. These included strains 14B11, 15H3, and 94G2. This Botrytis growth inhibition can be seen in the blue circle in Figure 1A. These bacteria may produce antibiotics or other chemicals that diffuse into the agar media and directly inhibit the growth of Botrytis.

Exp 1.2 Six bacteria strains inhibited Botrytis growth in shared air experiments.

Six of the seven tested bacteria (14B11, 15H3, MBSA-3BB1, 89F1, 94G2, and AP54) inhibited the growth of Botrytis when grown with the same air space (but not on the same plates) (Figure 1B). This suggests that these bacteria produce and release volatile compounds into the air that inhibit Botrytis growth.

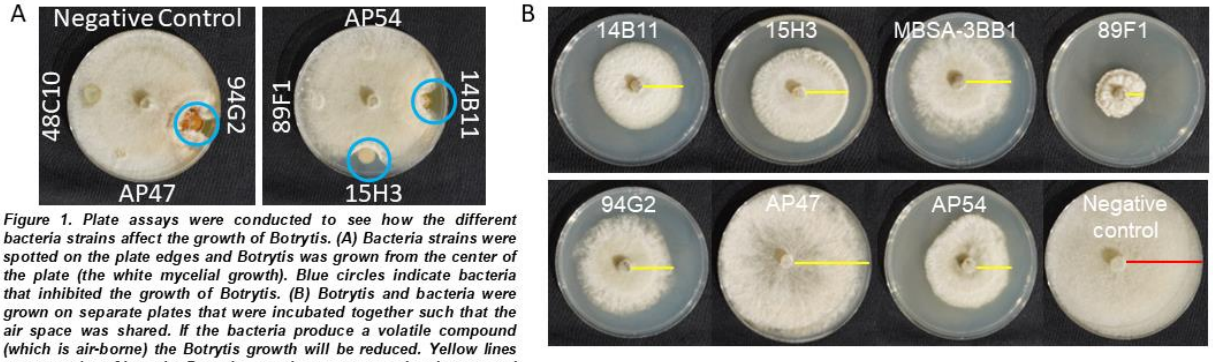

Figure 1. Plate assays were conducted to see how the different bacteria strains affect the growth of Botrytis. (A) Bacteria strains were spotted on the plate edges and Botrytis was grown from the center of the plate (the white mycelial growth). Blue circles indicate bacteria that inhibited the growth of Botrytis. (B) Botrytis and bacteria were grown on separate plates that were incubated together such that the air space was shared. If the bacteria produce a volatile compound (which is air-borne) the Botrytis growth will be reduced. Yellow lines are examples of how the Botrytis growth was measured and compared to the negative control (red line). Note the Botrytis in the negative control treatment (no bacteria) and AP47 has grown to the edge of the plate. Compare that to the growth of Botrytis when it shared the air space with the other bacteria strains.

Exp 1.3 Some tested bacteria strains created biofilms, and some strains reduced the ability of other bacteria to form biofilms. Biofilms are important for MBCA because they may act as a barrier to microbial plant pathogens. Strain 14B11 had the

highest biofilm formation. When 14B11 was grown in combination with other strains, biofilm formation was very high. However, when 14B11 was grown with 15H3, biofilm formation was significantly reduced. This is the only bacteria strain combination with a negative effect on biofilm formation (Figure 2).

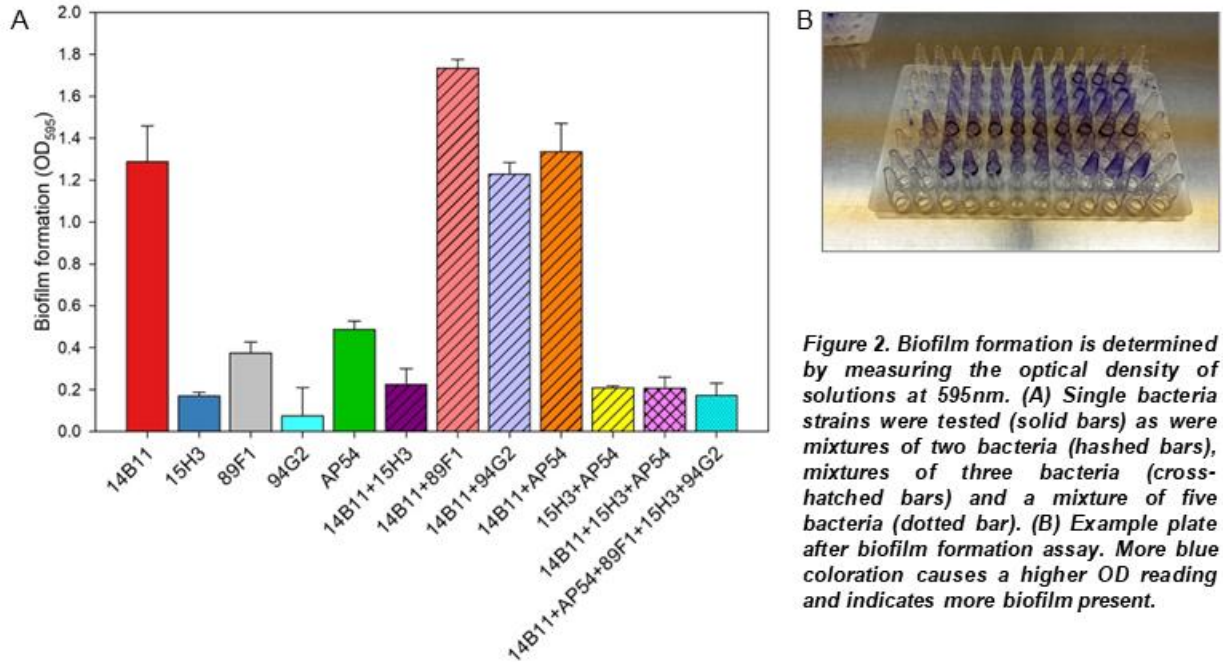

Figure 2. Biofilm formation is determined by measuring the optical density of solutions at 595nm. (A) Single bacteria strains were tested (solid bars) as were mixtures of two bacteria (hashed bars), mixtures of three bacteria (cross-hatched bars) and a mixture of five bacteria (dotted bar). (B) Example plate after biofilm formation assay. More blue coloration causes a higher OD reading and indicates more biofilm present.

Exp 1.4 Some strains directly inhibit the growth of other strains. If a bacteria strain negatively impacts the growth of other bacteria strains, that strain can not be utilized in a consortium (for product formulation). Co-culturing bacteria and evaluating the growth of each bacteria informed us that certain bacteria strains inhibit the growth of other bacteria. In general, the presence of 15H3 with any other bacteria strain resulted in an inhibition of bacteria growth (Figure 8 and Table 1).

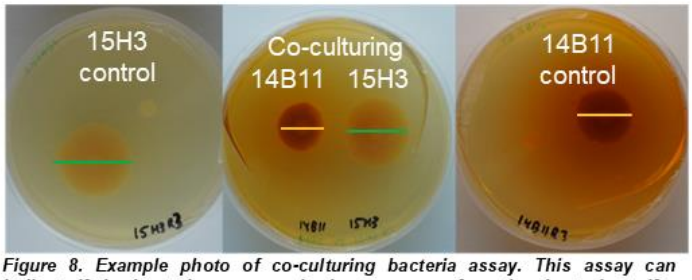

Figure 8. Example photo of co-culturing bacteria assay. This assay can indicate if the bacteria can grow in the presence of another bacteria or if a mixture of bacteria inhibits growth of other bacteria. In this figure, you can see that the growth of 15H3 alone (left plate) or 14B11 alone (right plate) is larger than the growth of those strains when they are grown together (middle plate). The diameter of the bacteria growth in the control plates is larger than the diameter of the bacteria in the co-culturing plate.

Table 1. Summary of co-culturing of bacteria strains together and if that co-culturing causes bacteria growth to be inhibited

Bacteria co-culturing	Growth inhibition
14B11+15H3	Yes
14B11+89F1	No
14B11+94G2	No
14B11+AP54	No
15H3+AP54	Yes
14B11+AP54+89F1+15H3+94G2	Yes

Exp 1.5 Bacteria identification and highlights of genetic analyses. To increase our knowledge about the identity and potential modes of biocontrol, the genomic DNA of the bacteria was sequenced. The sequencing information was used to identify the bacteria genus and species (Table 2). We also used this genomic information to investigate the genes of interest that may play a role in the biological control of fungal diseases like

Botrytis. We found that our strains have genes for antibiotic production, volatile production, and secretion systems. All of these features make the bacteria good candidates for MBCAs.

Table 2. The bacteria strains were sequenced, and phylogenetic analysis resulted in the following current taxonomic classifications

Isolate	Taxonomic identification
14B11	*Pseudomonas chlororaphis*
15H3	*Pseudomonas protegens*
89F1	*Pseudomonas moraviensis*
94G2	*Pseudomonas lini*
AP54	*Pseudomonas protegens*

CONCLUSIONS

Objective 1 - Identify potential modes of action of these bacteria for Botrytis control.

We have identified bacteria in our collection that control Botrytis with multiple modes of action. Additional experiments will focus on characterizing their potential for use in the development of an MBCA.

IMPACT OF RESEARCH TO THE INDUSTRY

Combining bacteria strains together can be a powerful tool in creating an MBCA with efficacy in a diverse variety of floriculture crops. We have identified key lab assays that should be conducted to evaluate bacteria so informed decisions can be made on what bacteria to include in a consortium for future product formulation. Through these lab assays and plant trials, we identified bacteria consortia that act as an MBCA for Botrytis control in petunia.

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