The potential of *Pseudomonas flourescens*, I336 and *Bacillus velezensis*, I113 to restore garlic seed stocks with latent *Fusarium* infections

A. HAWKINS[1,2], R. JOYCE[2,3,4], H. HORNICK-MARTYK[2,5], K. DUMONT[2], J. HOAGE[2], S. SALDIAS[2], S. KANDASAMY[2]

1. Department of Basic Medical Sciences, University of Western Ontario, 1151 Richmond St, London, ON N6A 3K7, Canada
2. A&L Biologicals, Agroecological Research Services Centre, 2135 Jetstream Rd, London, ON N5V 4H7, Canada
3. Department of Microbiology and Immunology, University of Western Ontario, 1151 Richmond St, London, ON N6A 3K7, Canada
4. Department of Pathology and Laboratory Medicine, University of Western Ontario, 1151 Richmond St, London, ON N6A 3K7, Canada
5. Department of Earth Sciences, University of Western Ontario, 1151 Richmond St, London, ON N6A 3K7, Canada

INTRODUCTION

The rising prevalence of Fusarium Dry rot (FDR) presents significant challenges to the global garlic market, resulting in yield losses of up to 30%[1,2].

Despite efforts to combat FDR through chemical interventions such as seed treatments and field spraying, these approaches have exhibited limited efficacy[2].

Consequently, a predominant emphasis has been placed on the management of post-harvest conditions as a means of disease control[2].

While biocontrol agents have shown promise in controlled *in-vitro* environments, a comprehensive investigation to ascertain their practical utility and suitability in *in-vivo* trials is imperative[2].

OBJECTIVES

- **To investigate the bio-control capabilities of *Bacillus velezensis*, I113 and *Pseudomonas flourescens*, I336 on both healthy and diseased garlic cloves. This evaluation will encompass a range of approaches including grow room trials, inhibition assays and metabolite analysis through high performance liquid chromatography (HPLC).**
- **To employ community fingerprinting methods (TRFLP) and Next-Generation Sequencing (NGS) for the comparative analysis of bacterial and fungal community structures between healthy and diseased garlic specimens.**

METHODS

Garlic seed stock was separated into diseased and healthy groups based on visual and textural criteria. A subsample of each were plated and the resulting microbes isolated and identify by sequencing. DNA of healthy and disease cloves was extracted and analyzed through NGS and TRFLP. Fungi isolated from the garlic seed stocks underwent an inhibition assay with I336 and I113. Both isolates were grown in liquid, and their metabolites harvested and analyzed using HPLC. I113, various dilutions of I336 or water were used to soak 18 cloves of garlic seed stock each. The cloves were subsequently planted, and the remaining culture decanted on top. Plants were monitored throughout the trial, receiving water and fertilizer as needed. Harvest occurred 42 days after planting (DAP) and biometric information was recorded.

GROW ROOM TRIALS

Diseased seeds treated with I113, I336 as-is, and I336 1/100 dilution exhibited increased emergence in grow room trials when compared to diseased control (Figure 4). This suggests that the applied treatment assisted in alleviating some disease symptoms.

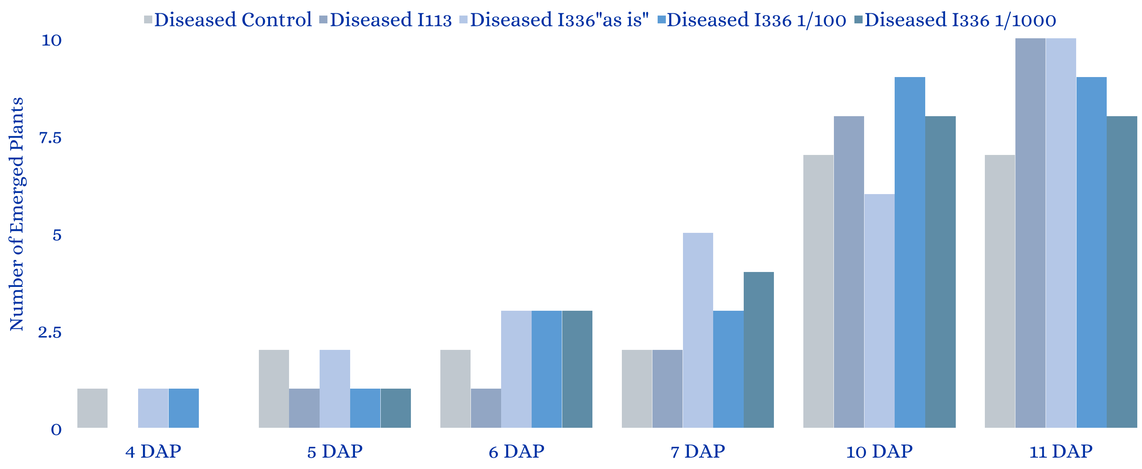

FIGURE 4: NUMBER OF EMERGED PLANTS FOR EACH TREATMENT 4 TO 11 DAYS AFTER PLANTING (DAP).

Treated diseased bulbs displayed healthier foliage compared to diseased control (Figure 5).

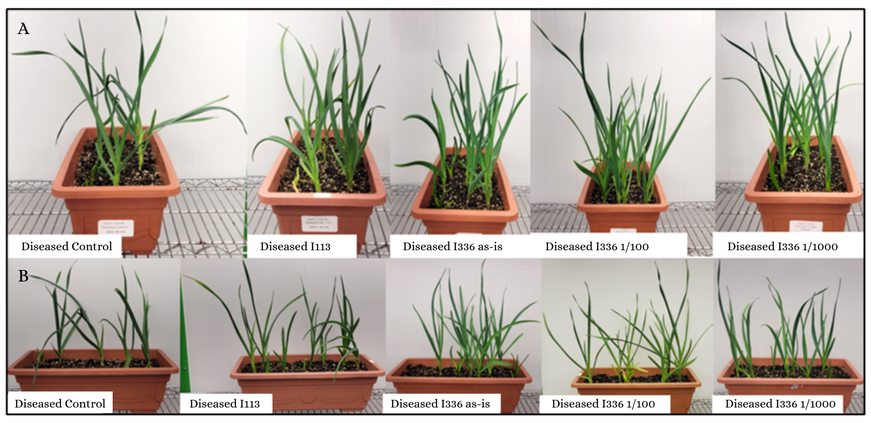

FIGURE 5: DISEASED TREATMENT PROGRESS PHOTOS FROM FRONT VIEW (A) AND SIDE VIEW (B), TAKEN 26 DAYS AFTER PLANTING.

At harvest, treated diseased garlic exhibited an increase in bulb mass varying from 21-211% when compared to diseased control, becoming comparable to the healthy control (Figure 6). Diseased bulbs treated with I336 exhibited the largest increase when compared to diseased control, followed by I113. This demonstrates the ability of I113 and I336 to restore garlic seed stocks with latent *Fusarium* infections.

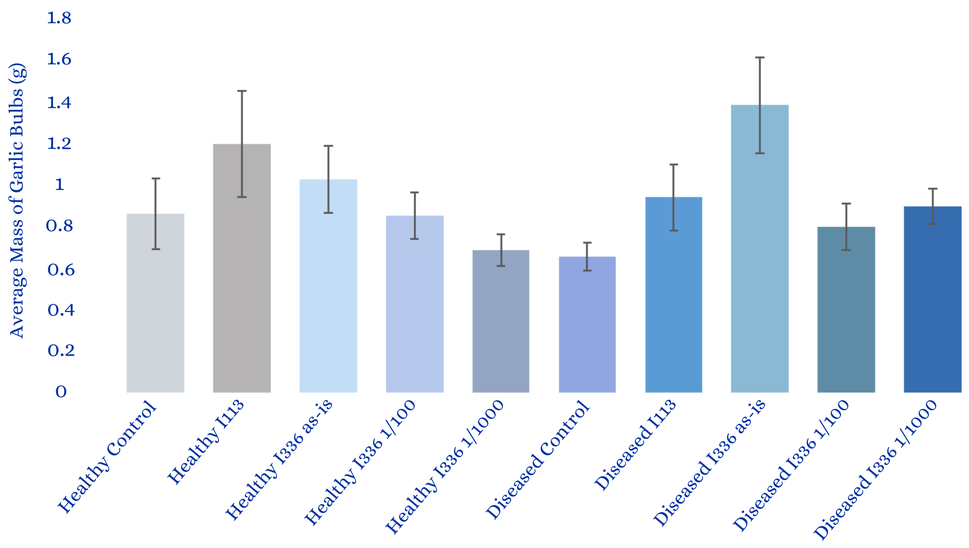

FIGURE 6: DRY MASS OF GARLIC BULBS (g).

MOLECULAR ANALYSIS

TRFLP revealed that there was significantly more microbes present in diseased cloves compared to healthy cloves, the majority of which were fungi (Figure 1). The microbial communities differed significantly between healthy and diseased cloves.

NGS revealed that the most common fungi in the diseased seed stock, both initially and after a one month storage period were those part of the *Fusarium fujikuroi* complex, the main cause of FDR.

When plated with *Fusarium* isolated from garlic tissue, I113 displayed strong inhibitory characteristics and I336, while weaker, displayed sufficient inhibition (Figure 2).

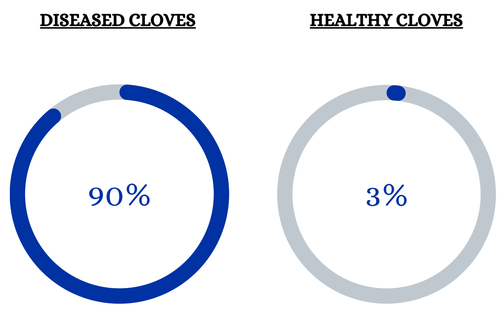

FIGURE 1: AMOUNT OF FUNGI IN RELATION TO BACTERIA PRESENT IN DISEASED AND HEALTHY GARLIC SEED STOCK.

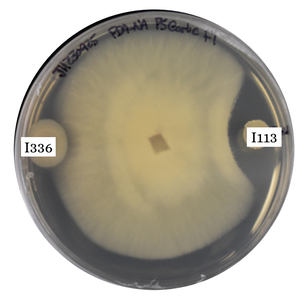

FIGURE 2: INHIBITION ASSAY PLATE OF I336 AND I113 AGAINST FUSARIUM ISOLATED FROM DISEASED GARLIC TISSUE.

HPLC revealed that I113 produces several antifungal metabolites such as, fengycins, surfactins, and iturins while I336 produces phenazines, 2,4-Diacetylphloroglucinol (2-4-DAPG) and surfactins, making them good biocontrol agents (Figure 3, Table 1).

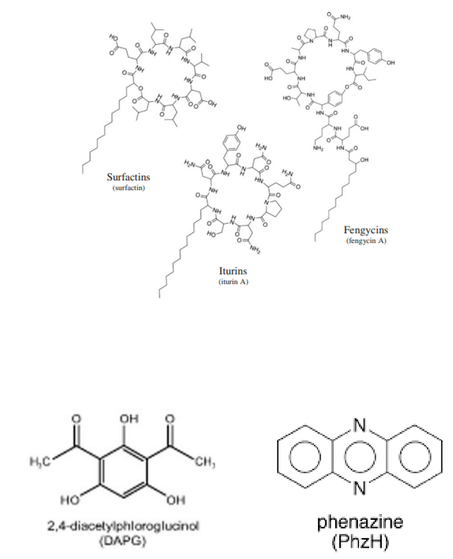

FIGURE 3: STRUCTURES OF FENGYCINS, SURFACTINS, ITURINS, PHENAZINES AND DAPG[3].

Metabolite	Mode(s) of Action	Isolate
Fengycins	Induced systematic resistance Inhibits phospholipase A2, resulting in membrane pore formation	I1113
Surfactins	Induced systematic resistance Membrane pore formation	I113 and I336
Iturins	Membrane pore formation	I113
Phenazines	Accumulation of ROS	I336
DAPG	Disrupt membrane integrity	I336

TABLE 1: METABOLITES PRODUCED BY I113 AND I336 AND THEIR MODES OF ACTION[3].

CONCLUSION

Diseased garlic seed stock displayed an increased ratio of fungi to bacteria with the most common fungi present being *Fusarium*.

Both I113 and I336 demonstrated biocontrol mechanisms both *in-vitro* and *in-vivo* due to their broad range of antimicrobial metabolites.

The introduction of I113 or I336 to diseased seed stock can lead to an improved garlic bulb yield that is comparable with the healthy seed stock, suggesting its potential to improve seed health.

Further studies will examine the effect of these isolates in combination as well as their potential biostimulant activity, both in laboratory and field settings.

RELATED LITERATURE

1. Mondani, L., Chiusa, G., & Battilani, P. (2021b). Fungi associated with garlic during the cropping season, with focus on fusarium proliferatum and F. oxysporum. Plant Health Progress, 22(1), 37–46. https://doi.org/10.1094/php-06-20-0054-rs
2. Gálvez, L., & Palmero, D. (2022). Fusarium dry rot of garlic bulbs caused by fusarium proliferatum: A Review. Horticulturae, 8(7), 628. https://doi.org/10.3390/horticulturae8070628
3. Falardeau, J., Wise, C., Novitsky, L., & Avis, T. J. (2013). Ecological and mechanistic insights into the direct and indirect antimicrobial properties of bacillus subtilis lipopeptides on Plant Pathogens. Journal of Chemical Ecology, 39(7), 869–878. https://doi.org/10.1007/s10886-013-0319-7