The effects of α-solanine and α-chaconine on the activity of bacterial efflux pumps and biofilm formation in pectinolytic bacteria

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Introduction

Bacterial pathogens such as *Dickeya solani* and *Pectobacterium brasiliense* pose a significant threat to global food security by affecting major crops, including potato (*Solanum tuberosum* L.). These pathogens rely on plant cell wall-degrading enzymes, quorum sensing, biofilm formation, and efflux pumps to facilitate infection. As a defence mechanism, potato synthesises glycoalkaloids (GAs) such as α-solanine and α-chaconine, which exhibit antimicrobial activity and may disrupt bacterial virulence pathways. To evaluate their potential as natural inhibitors of virulence, we assessed the effects of synthetic GAs on bacterial growth, viability, biofilm formation, and efflux pump activity in *D. solani* and *P. brasiliense*.

Material & Methods

Bacterial strains: *Dickeya solani*, *Pectobacterium brasiliense*
Treatments: Synthetic glycoalkaloids — α-solanine (2 mM), α-chaconine (2 mM); applied individually and as a 1:1 mixture
Viability assay: Flow cytometry using the BacCount™ Viable Kit after 24 h incubation
Biofilm assay: Crystal violet staining after 24 h incubation
Efflux pump activity: Ethidium bromide (EtBr) accumulation assay (fluorescence-based) using LightCycler® 480, 24 h incubation
Growth assay: Growth inhibition measured over 24 h with GAs (Tecan Infinite F50)

Results

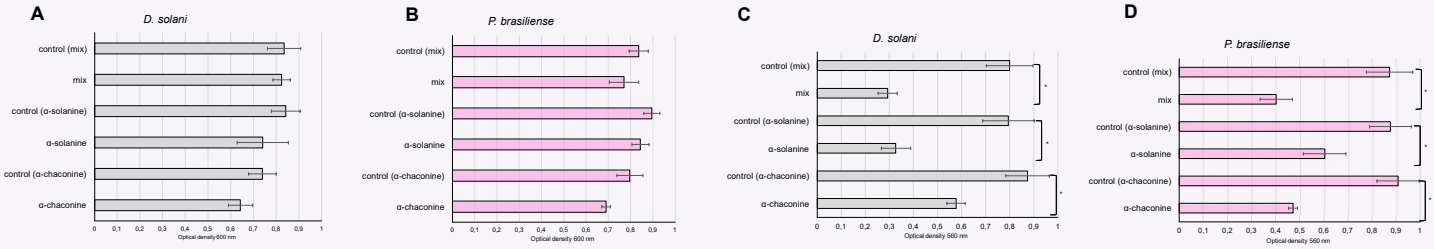

Figure 1: Minimum inhibitory concentration (MIC) and biofilm formation of *D. solani* (A, C) and *P. brasiliense* (B, D) after 24 h incubation with synthetic glycoalkaloids (α-solanine, α-chaconine, or their 1:1 mixture). Asterisks indicate significant differences compared to the corresponding control (Duncan's test, $p < 0.05$).

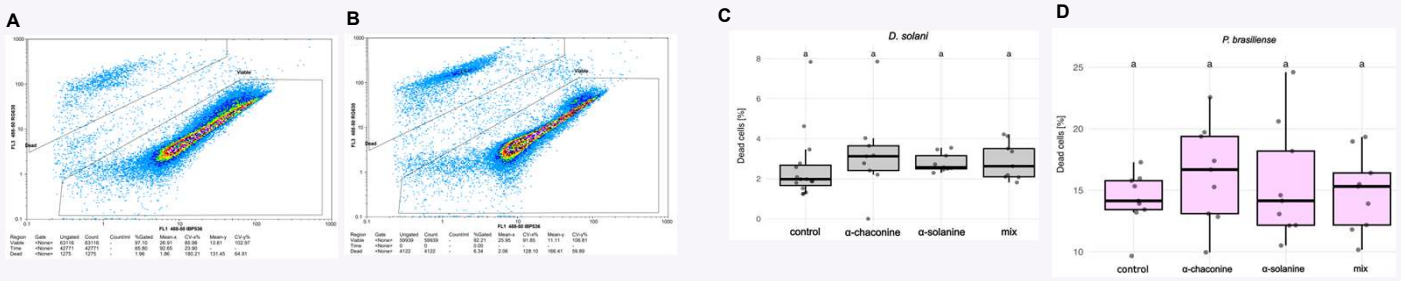

Figure 2: Effects of synthetic glycoalkaloids on the viability of *D. solani* and *P. brasiliense* cells.
(A) Representative flow cytometry dot plot for *D. solani* control (untreated).
(B) Flow cytometry dot plot for *D. solani* treated with α-chaconine (2 mM).
(C) Quantification of dead *D. solani* cells after 24 h incubation with synthetic glycoalkaloids (α-solanine, α-chaconine, or their 1:1 mixture).
(D) Quantification of dead *P. brasiliense* cells under the same conditions.
Significant differences were determined using Duncan's test ($p < 0.05$).

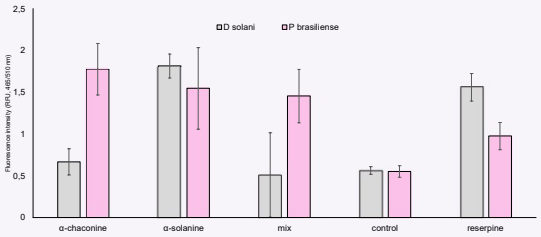

Figure 3. Efflux pump activity in *D. solani* and *P. brasiliense* assessed by ethidium bromide (EtBr, 2 µg/mL) accumulation. Treatments included glycoalkaloids and reserpine (positive control). Bars show mean RFU ± SD.

Conclusions

Synthetic GAs, α-solanine and α-chaconine, both individually and in combination, significantly reduced biofilm formation in *D. solani* and *P. brasiliense*. Moreover, increased EtBr accumulation in treated cells suggests inhibition of efflux pump activity by GAs. These findings suggest that GAs disrupt key virulence mechanisms, highlighting their potential as a natural, sustainable control method for soft rot pathogens.

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