Glycoalkaloids from *Solanum spp* leaves modify virulence factors in *Dickeya solani* and *Pectobacterium brasiliense* sp. nov.

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The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute

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Pectobacterium brasiliense (Pcb)

Strain: Pcb3M16

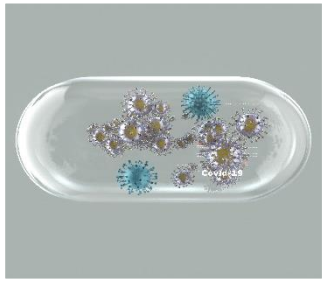
Reference: Lebecka & Michalak, 2020

Dickeya solani (Ds)

Strain: IFB0099

Reference: Golanowska et al., 2015

Introduction to bacterial pathogens and their impact



Classification:

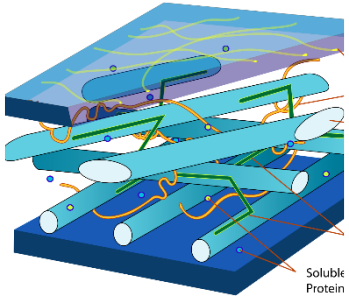
Gram-negative bacteria

family *Pectobacteriaceae.*

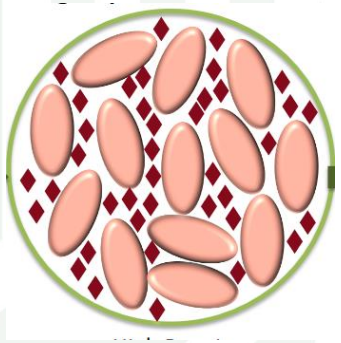
Major threats to potato crops, causing big yearly losses.

Ds and Pcb cause **soft rot** and **blackleg**, among top **10** destructive plant pathogens.

Chemical protection against bacterial diseases is **not** practiced.



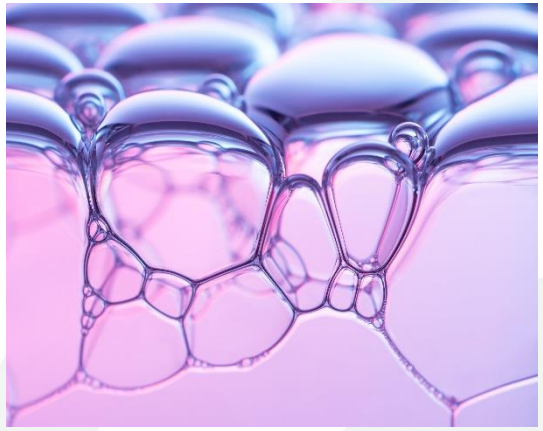
Ds and Pcb virulence is mainly due to plant cell wall degrading enzymes (**PCWDEs**).



The expression of these enzymes is controlled by **quorum sensing (QS)** systems.

Glycoalkaloids (GAs) in Potato Plants

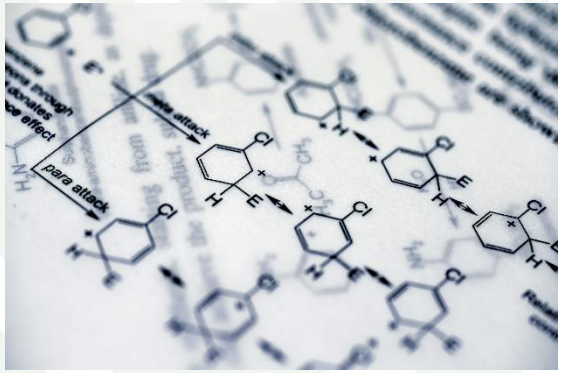
Potato plants contain **protective metabolites** that defend against threats like insects, herbivores, and pathogens.



Potato plants produce **glycoalkaloids (GAs),** toxins that defend against bacteria, fungi, viruses, and insects.

GAs Composition:

α-chaconine and α-solanine: 95% of total GAs.



Other GAs:

solasonine, solamargine, leptinine I, & leptine II.

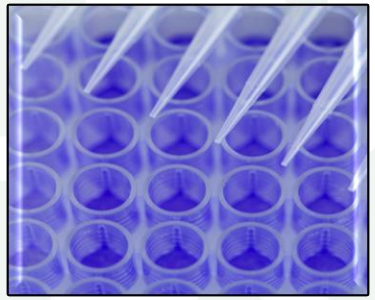
Materials & Methods

GAs sources:

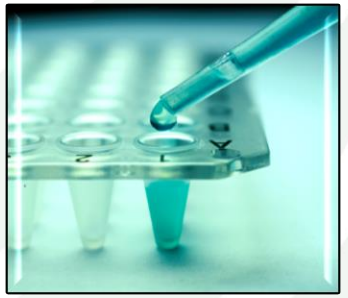
- ✓3 potato cultivars (Mieszko, Owacja, Tajfun)
- ✓3 wild species (*S. chacoense, S.maglia, S. garsiae*)
- ✓2 interspecific *Solanum* spp. hybrids (DG 00-683; DG 08-305)

Analytical technique:
High-Performance Liquid Chromatography-Mass Spectrometry (**HPLC-MS)**

1. GAs & Pectinolytic Activity - **Crystal Violet Pectate (CVP) medium**

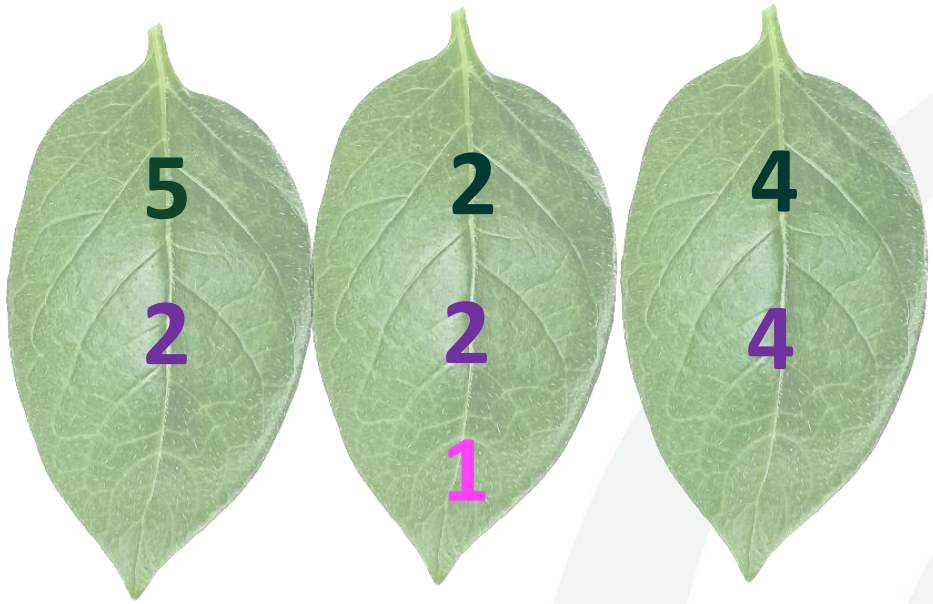


2. GAs & Biofilm Formation- **Microtiter plate assay stained with Crystal Violet**



3. GAs & QS **Gene Expression- quantitative PCR**

Selected GAs and their composition



S. chacoense DG 00-683 Tajfun

α-chaconine

α-solanine

leptinine I

peak area on HPLC-MS histograms:

C = 0;

1 = 0 < C < 25,000;

2 = 25,000 < C < 50,000;

3 = 50,000 < C < 75,000;

4 = 75,000 < C < 100,000;

5 = 100,000 < C < 125,000

Objective: To explore the potential of GAs, particularly from *Solanum* spp. leaves, as inhibitors against *Pectobacterium* and *Dickeya*.

Hypothesis: GAs can inhibit the growth, QS, enzymatic activity, and biofilm formation of these bacteria.

GAs impact on pectinolytic activity of bacterial isolates

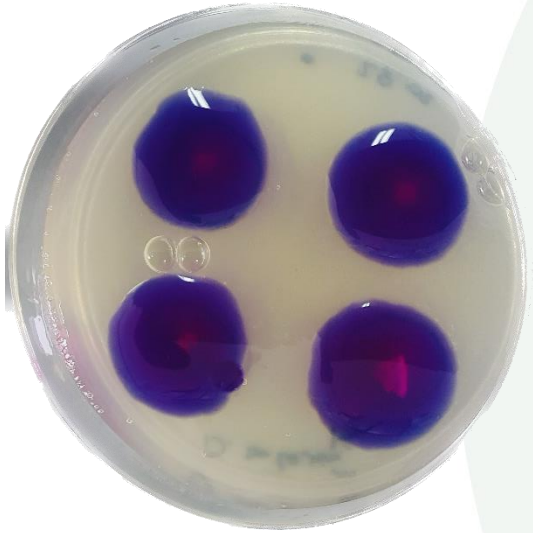
Assay Medium: Crystal Violet **Pectate** (CVP) medium

GAs Supplementation: 0.8 mg/ml of CVP

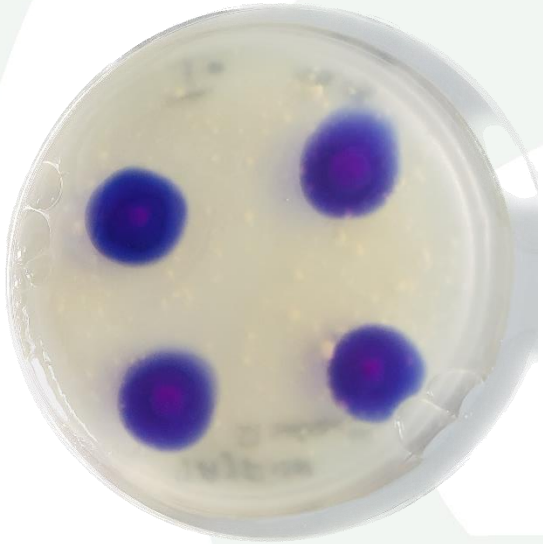
Assessment: incubated at T 31°C for 48 h

Inoculation: Suspension: 10^9 CFU mL^{-1} Method: toothpick

Replicates: 3 biological 4 technical



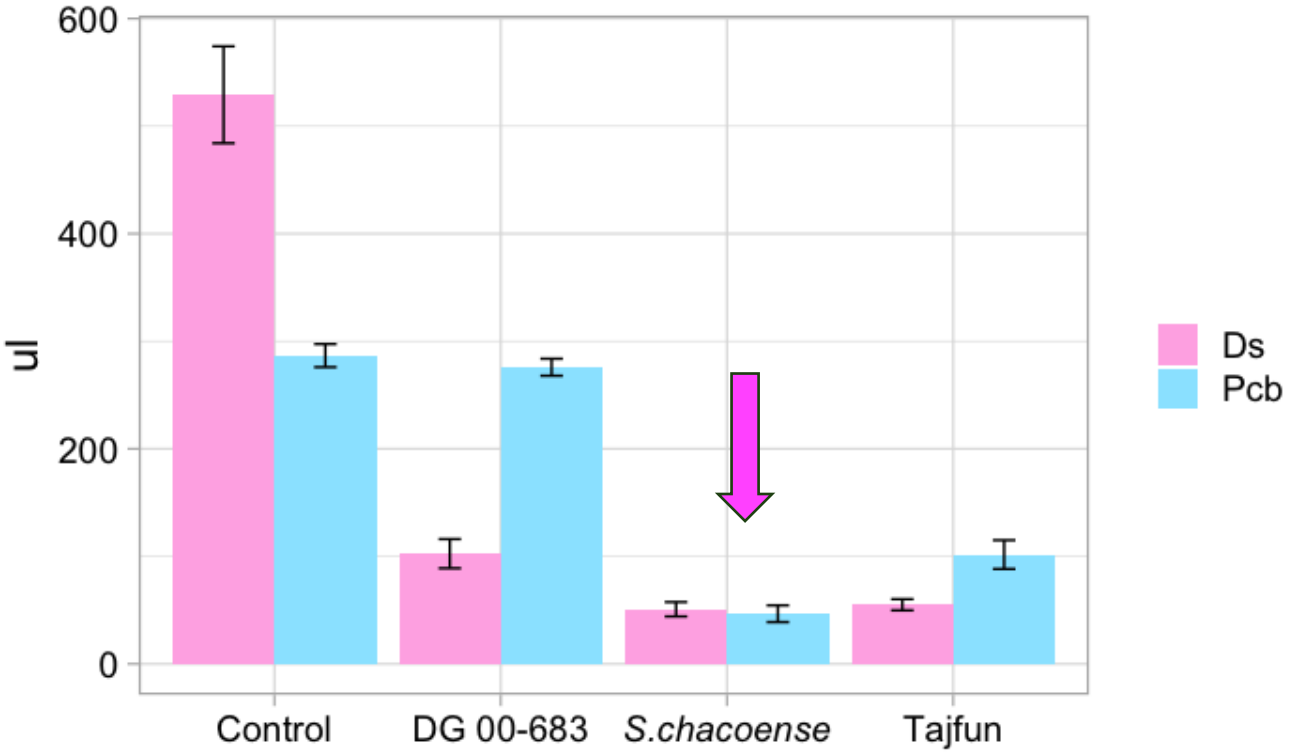
Control without GAs



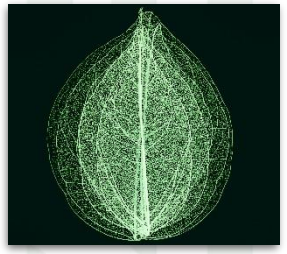
GAs from the cultivar Tajfun

Measurement of cavity volumes formed by Ds in CVP medium with and without GAs.

Effect of GAs on pectinolytic activity of bacterial isolates



GAs, from ***S. chacoense***, significantly inhibited the pectinolytic activity of both bacterial strains.



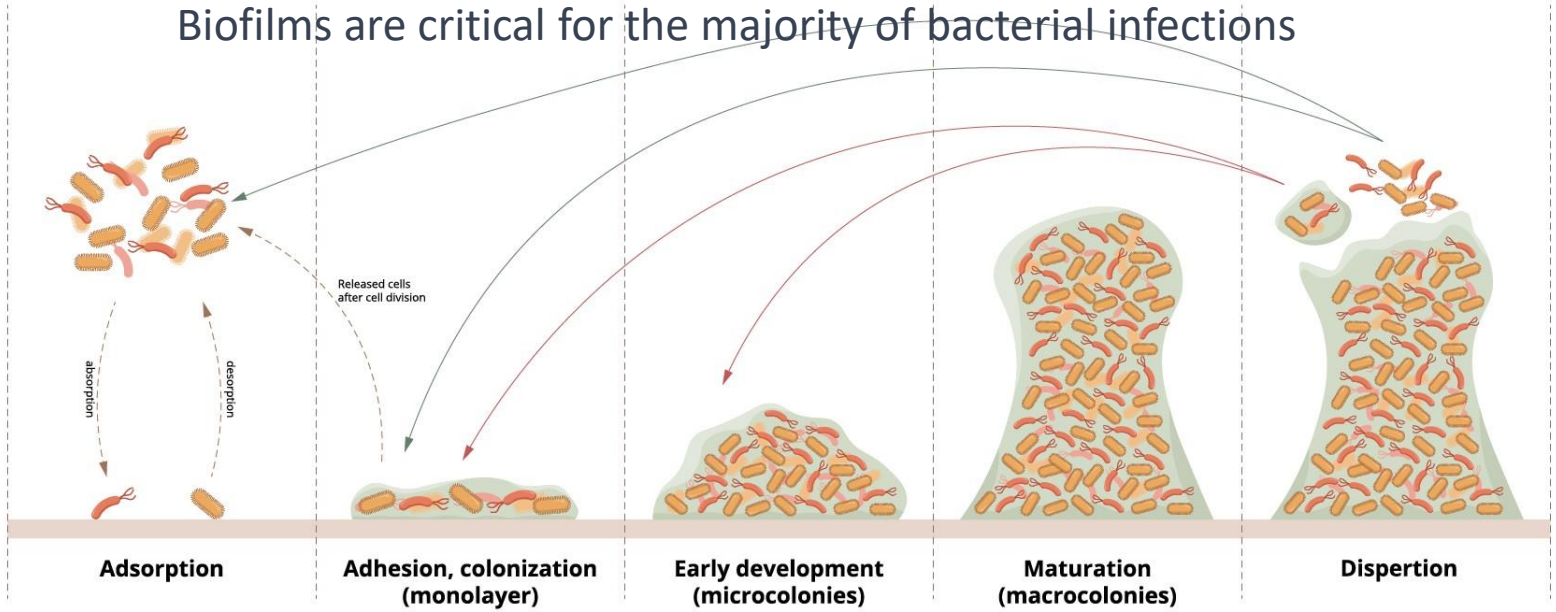
Ds showed similar responses to GAs from **DG 00-683** and **Tajfun**, but both were weaker than the response to GAs from ***S. chacoense***.

Pcb exhibited no change in activity with GAs from DG 00-683 compared to the control.

Biofilm Formation

Structured community of microbial cells enclosed in a self-produced polymeric matrix adherent to a surface.

Components:
Microbial Cells: "Bacteria or microorganisms forming layers."
Extracellular Polymeric Substances (EPS): "Mixture of polysaccharides, proteins, nucleic acids, and lipids."



Biofilm lifecycle

Ma et al., 2022

Our research focused on observing the **early stages of biofilm formation**.
We specifically analyzed the biofilm after **6** hours of bacterial growth.

GAs role in bacterial biofilm formation

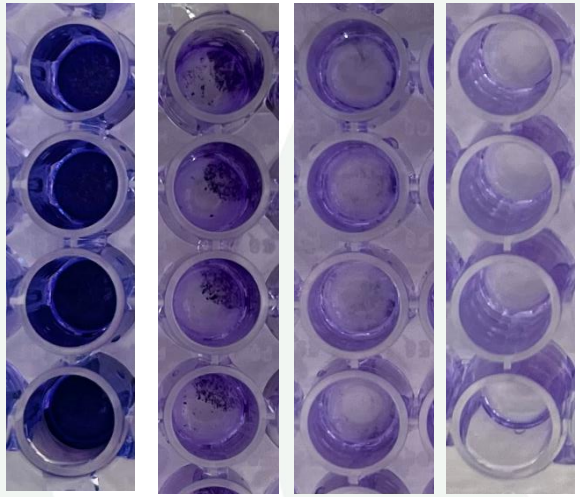
Biofilm Assessment
Microtiter plate assay
Stained with Crystal Violet

Bacterial suspension
10^6 CFU

Incubation
6 h at 30°C

Biofilm Quantification
560 nm OD

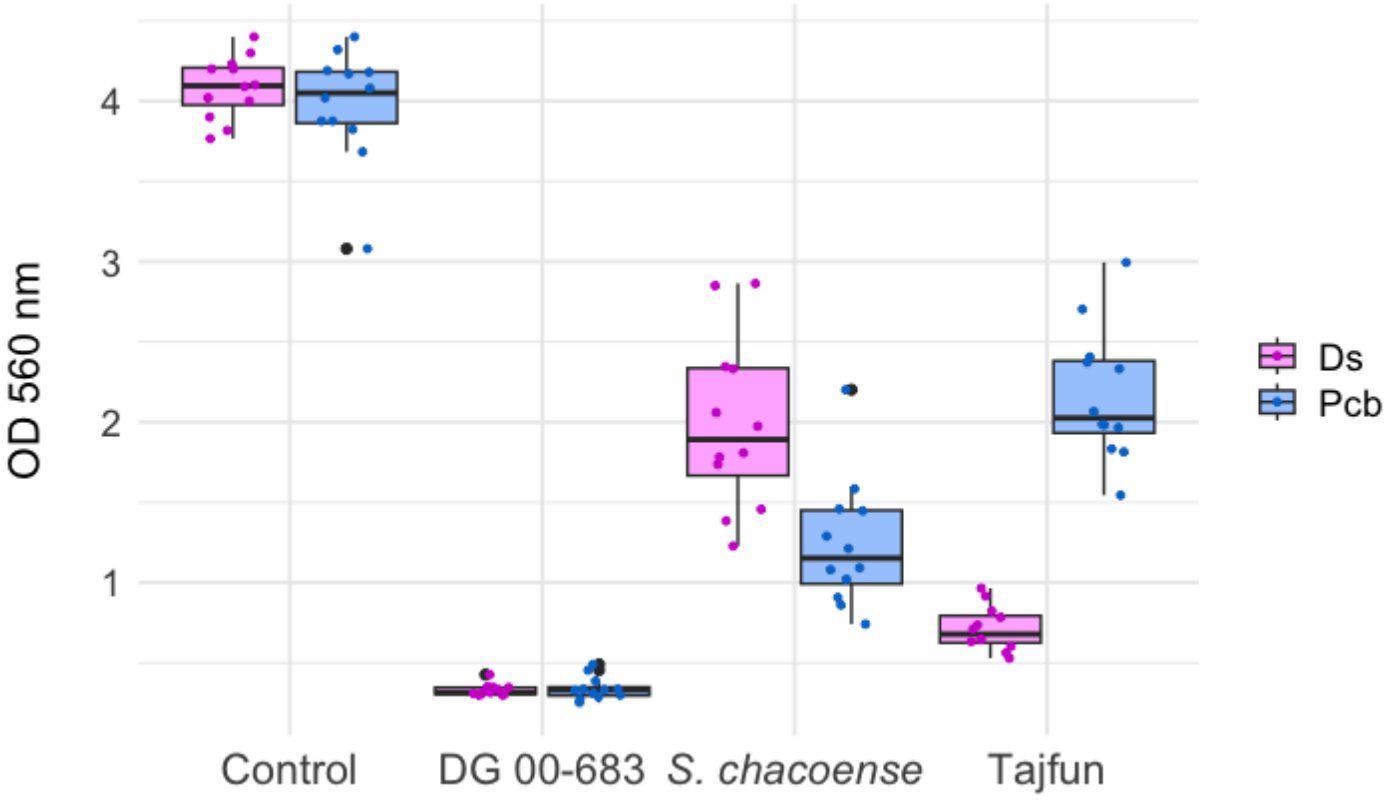
Replicates
3 biological
4 technical



1 2 3 4

1. Control
2. Tajfun
3. ***S. chacoense***
4. **DG 00-683**

Biofilm formation inhibition by GAs in *Ds* and *Pcb*



DG 00-683 GAs: Most effective for both Ds and Pcb

***S. chacoense* GAs:** Noticeable reduction, but less than DG 00-683

Tajfun GAs: Highly effective against Ds

Quorum Sensing in *Dickeya* & *Pectobacterium*: A Key player in plant pathogenicity

QS plays an important role in bacterial growth, virulence, motility and biofilm formation.
It operates through auto-inducers (AIs), which give an idea of bacterial density.
These auto-inducers are chemical signals, such as acyl-homoserine lactones (AHL).

Ds

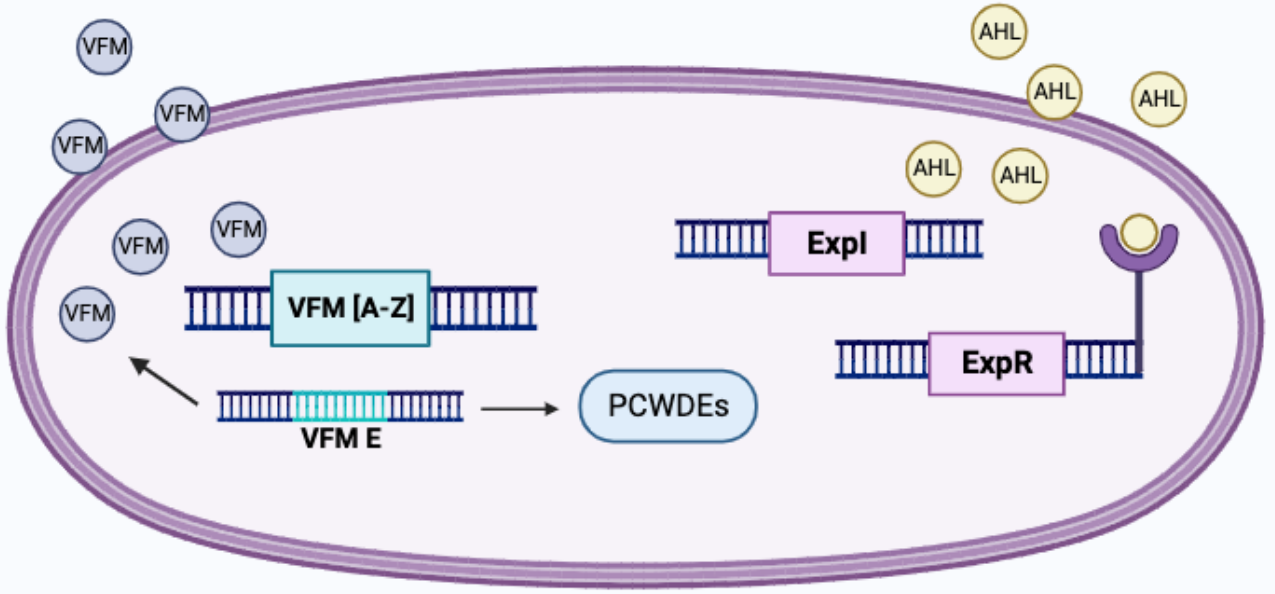
Uses two QS systems:

- AHL-based (synthase ExpI and sensory protein ExpR)
- Vfm system which has 26 genes (VFM A-Z)

Notably, **VFM E plays a crucial role in PCWDEs production**.

Pcb

QS is focused on AHL production, detection, and response.



As bacteria grow, they produce AHLs via the enzyme ExpI. When AHL levels are high, they bind to ExpR and activate QS-related genes.

Methodology for Quorum Sensing genes expression analysis

GAs Supplementation
0.8 mg/ml

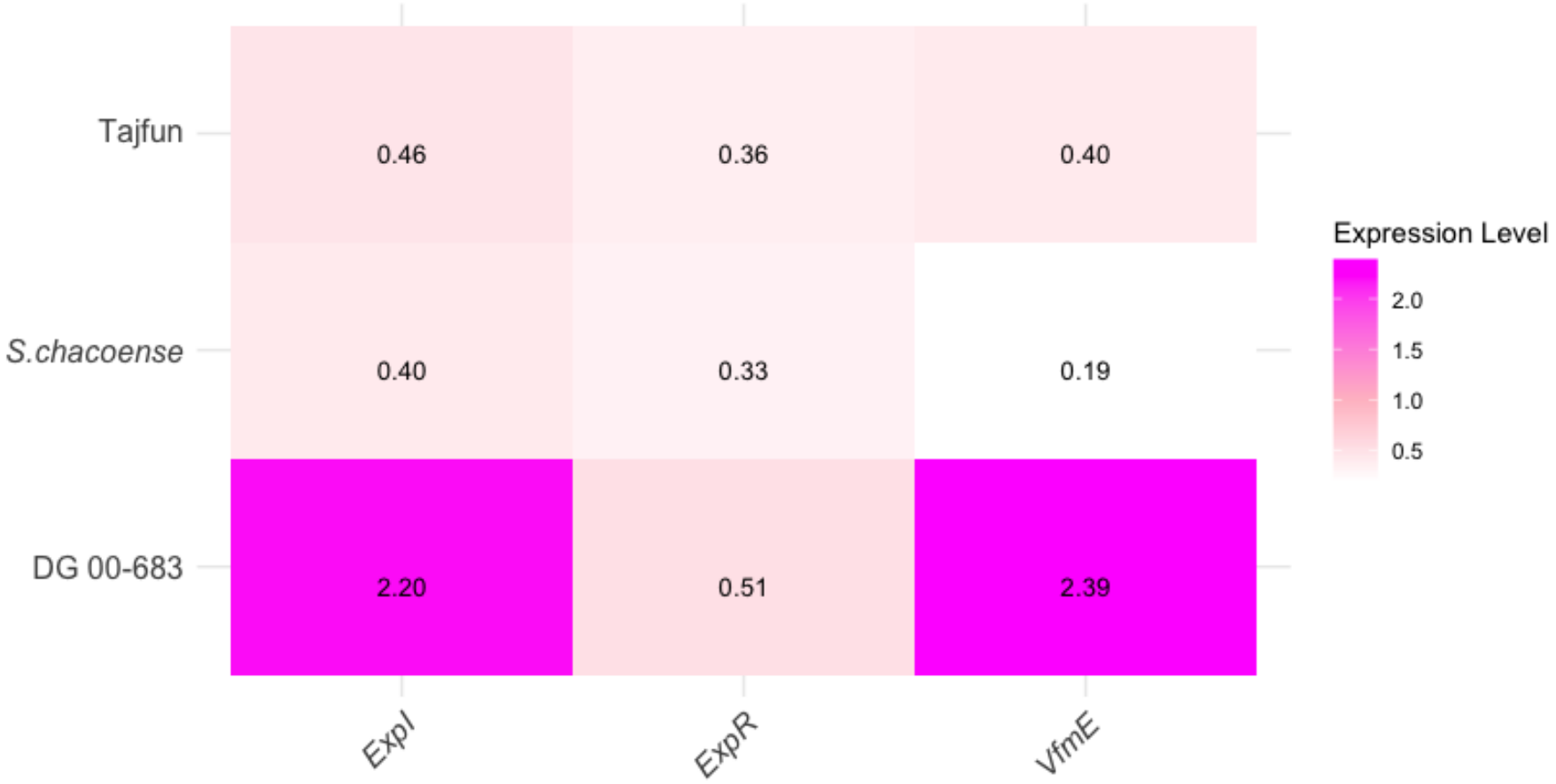
Incubation:
t 30°C for 8 h
180 rpm

RNA Isolation

qPCR to analyze the expression of **ExpI, ExpR**, & **VfmE**

Relative gene expression of ExpI, ExpR, and VfmE was calculated using $2^{-\Delta\Delta Ct}$ method.

Impact of GAs on QS gene expression in *Dickeya solani*



DG 00-683: highest expression of ExpI & VfmE.

S. chacoense: strongly suppresses all genes. White cell (0.19) for **VfmE** indicates minimal expression.

Tajfun: moderate expression levels.

Key Findings:

- GAs, particularly from *S. chacoense*, significantly **inhibited pectinolytic activity** of Ds and Pcb.
- GAs from DG 00-683 most effectively **inhibited biofilm formation** in both Ds and Pcb.
- Varying impacts on QS gene expression: DG 00-683 highest for ExpI & VfmE; *S. chacoense* suppressed all tested genes; Tajfun – at moderate levels.

Conclusion:

Glycoalkaloids show potential as natural inhibitors against key virulence factors of Ds and Pcb, suggesting a possible eco-friendly alternative for controlling potato bacterial diseases. Further studies are needed.

Thank you for your attention.

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