

Pathogenicity of *Xanthomonas axonopodis* pv. *glycines*, the Causative Agent of Bacterial Pustule in Soybeans

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Introduction

- *Xanthomonas axonopodis* pv. *glycines* (Xag)
 - Gram (-), aerobic, rod-shaped bacterium
 - Member of family Pseudomonadaceae
 - Produces extracellular polysaccharides and xanthum gum
 - Causes bacterial pustule in soybeans

- Bacterial Pustule - Disease cycle
 - Spread by splashing water, wind blown rain, and cultivation when foliage is wet
 - Enters plant through stomatas and wounds
 - Multiplies intracellularly (25-30°C)
 - Disease symptoms appear after 7-10 days

- Bacterial Pustule - Disease Symptoms
 - Small, yellow-green spots initially appear
 - Later reddish brown spots
 - Pustules rupture and dry out



- Incidence
 - Pustule is most prevalent bacterial disease of soybeans
 - Brazil, China, India, Korea, USA and Taiwan
 - Reduces crop yield by 40%
 - Found to reduce yield by 4-11% in USA

- Control
 - Crop rotation
 - Burial of crop residues
 - Planting resistant varieties
 - Resistance developed by *rxp* gene
 - Maintained by traditional breeding

Reasons to study Xag

- Little is known about Xag
- World-wide pathogen causes significant reduction in soybean yield
- Occasional outbreaks observed in USA
- Symptoms similar to that of soybean rust

Objectives

1. Isolation and comparison of Xag isolates by biochemical and molecular techniques
2. Development of greenhouse assay for evaluation of factors that impact pathogenicity of Xag
3. Screen wide variety of commercial soybean cultivars for resistance to Xag

Methods

- Isolation of Xag
 - Area of the leaf with pustule was cut and homogenized in sterile dH₂O
 - Streaked for isolation on potato dextrose agar (PDA) and incubated at 25-30°C for 48 h
 - Isolates verified by gram stain and ability to cause pustule on susceptible soybean cultivars
 - Comparison of isolates: Growth profiles, metabolic fingerprinting (Biolog Plates), SDS-PAGE profiles, and pathogenicity testing using a greenhouse assay

- Greenhouse assay for pathogenicity testing:
 - Isolates were grown in Potato Dextrose Broth
 - Incubated at 25°C for 8 h (mid log) on shaker
 - Cells harvested and washed with sterile dH₂O
 - O.D. adjusted to 0.3 (10⁸ cells/ml) before spraying on two-week old plants (PI 520.733)
 - 16 plants used per experiment; plants were damaged with perforation needle prior to inoculation (20 ml of inoculum per 16 plants)
 - Pustule severity recorded after 7-10 days



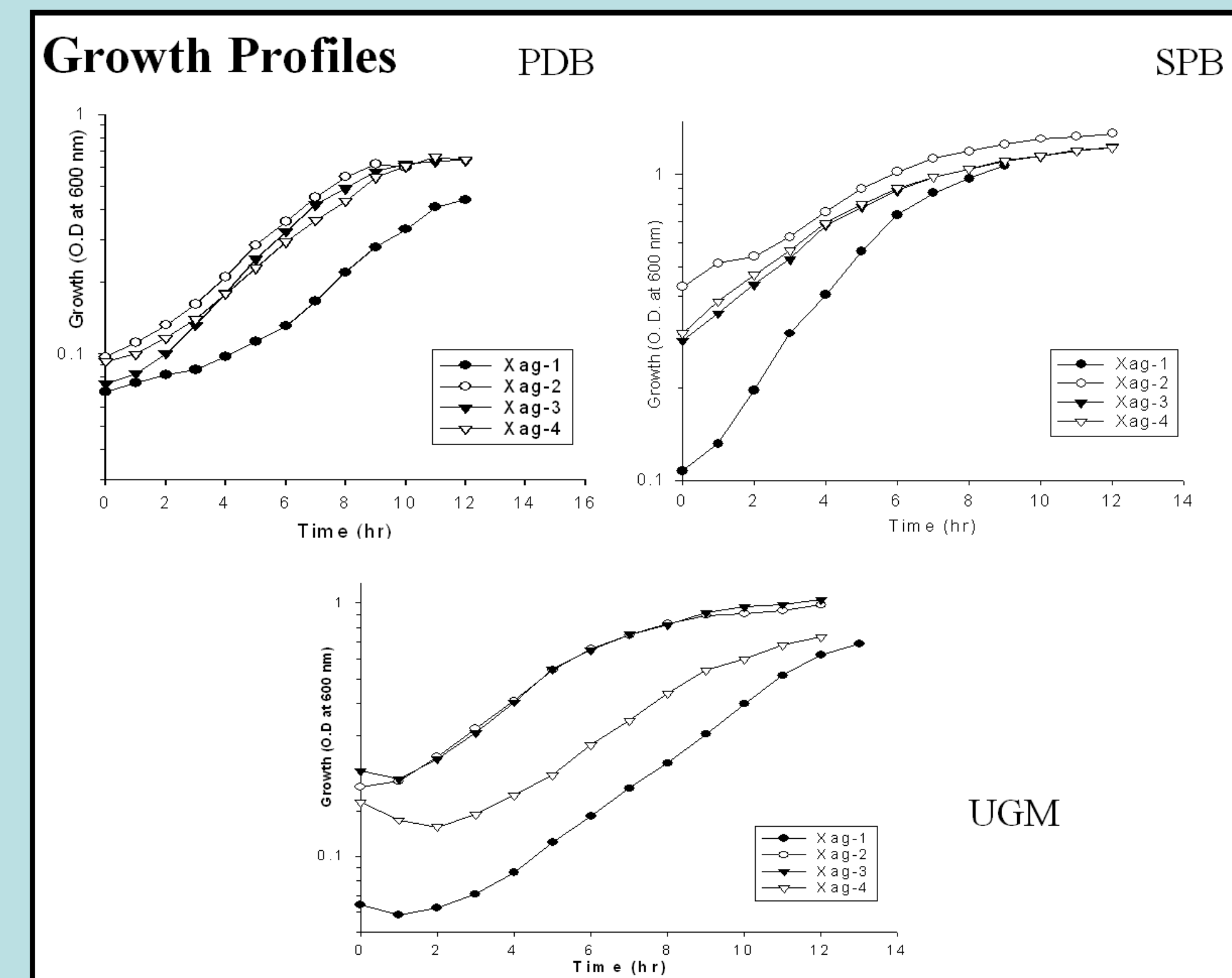
Results

- Xag-1 and Xag-3 isolated from susceptible soybeans PI 520.733 and Spencer, respectively
 - Tests confirmed Xag-1 and Xag-3 produced pustule on PI 520.733
- Gram staining showed they were gram (-) rods
- Produced yellow, mucoid colonies on PDA

Bacteria used in this study

Bacterium	Source ^a
<i>X. axonopodis</i> pv. <i>glycines</i> (Xag-1)	This study
<i>X. axonopodis</i> pv. <i>glycines</i> (Xag-2)	ATCC
<i>X. axonopodis</i> pv. <i>glycines</i> (Xag-3)	This study
<i>X. axonopodis</i> pv. <i>glycines</i> (Xag-4)	UF

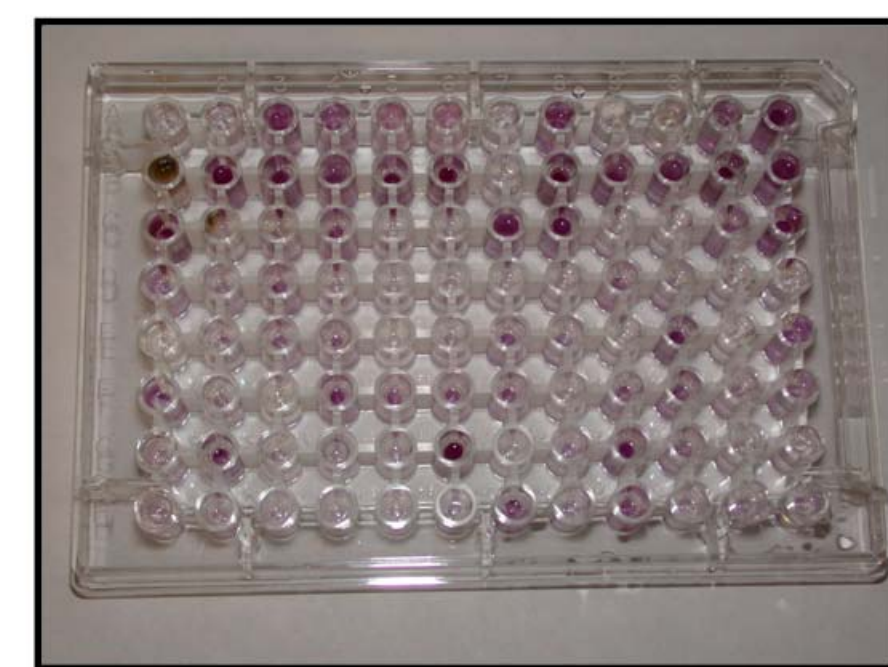
^a ATCC, American Type Culture Collection; UF, University of Florida



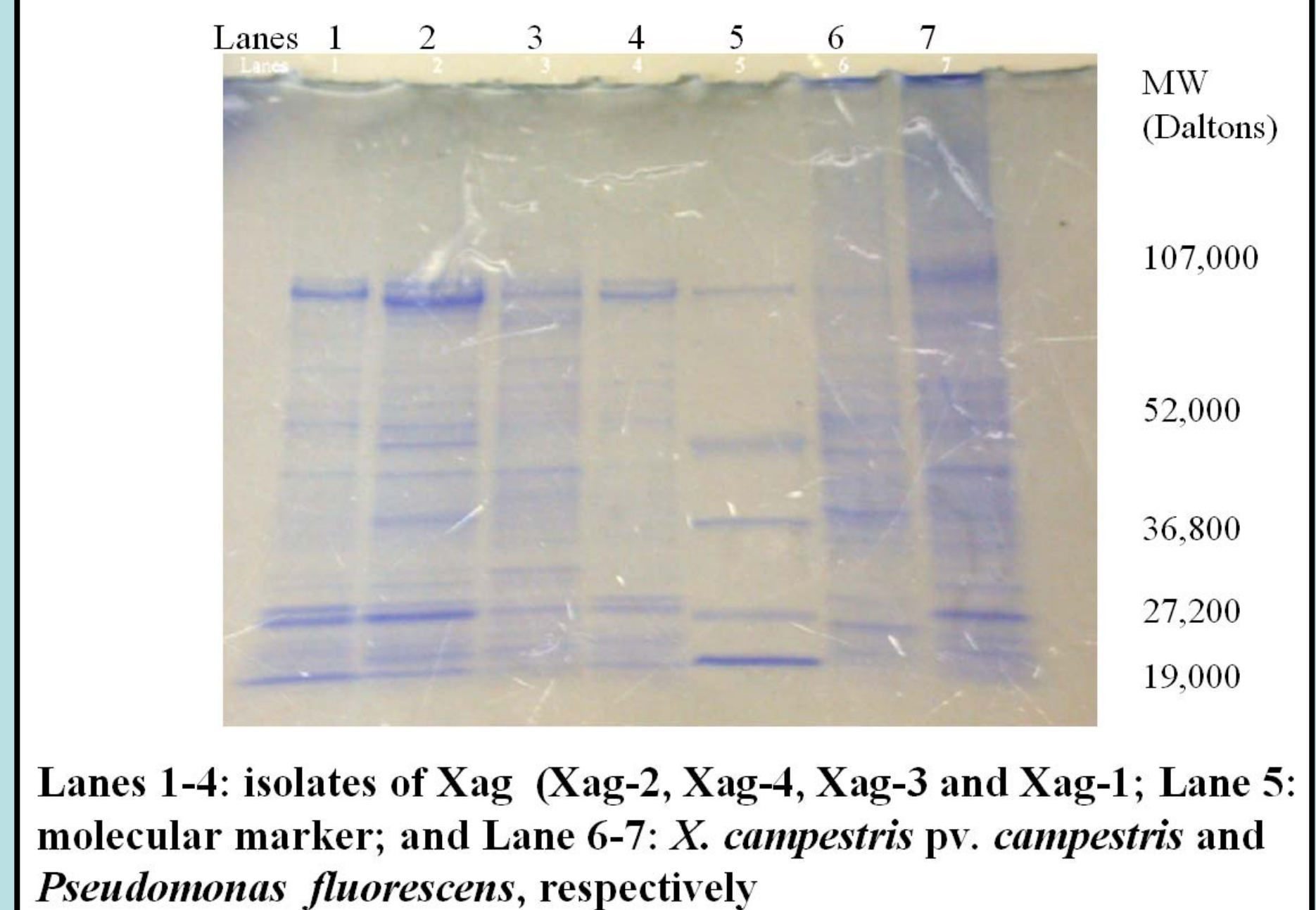
Metabolic fingerprinting

- 54 of the 95 substrates supported the growth of all 4 Xag isolates

Isolates	Growth on total # of substrates
Xag-1	59 (5)
Xag-2	74 (20)
Xag-3	91 (37)
Xag-4	77 (23)



SDS-PAGE Profiles of Isolates



Lanes 1-4: isolates of Xag (Xag-2, Xag-4, Xag-3 and Xag-1; Lane 5: molecular marker; and Lane 6-7: *X. campestris* pv. *campestris* and *Pseudomonas fluorescens*, respectively

Pathogenicity Testing

Average number of pustules on PI 520.733 inoculated with cells of different isolates of Xag

Isolates	Pustules per plant (X ± SE) ^a
Xag-1	72 ± 9
Xag-2	37 ± 5
Xag-3	29 ± 3
Xag-4	34 ± 5

^a Values represent the mean ± the standard error of 16 plants (p < 0.001)

Screening for Resistance to Bacterial Pustule: Soybean Variety Testing

- Initial screening showed 146 out of 525 Roundup-Ready cultivars were susceptible to Xag-1
- Of the 146, 66 cultivars were found to be more susceptible than PI 520.733

Summary

- Xag-1 and Xag-3 produced pustule on susceptible soybean cultivar
- Xag-1 was more aggressive and slightly different than other Xag isolates
- Factors that impact pathogenicity are age of cells and growth conditions
- Some Roundup ready soybean cultivars were susceptible to Xag-1
 - Resistance is not maintained
 - Potential to cause loss in yield
- Future studies
 - Isolation of Xag
 - Techniques for comparison
 - Variety testing with different Xag isolates