VERSION: 1.1	**DATE:** 2022
PATHOGEN: Pseudomonas syringae pv. glycinea (syn: Pseudomonas amygdali pv. glycinea)	
HOST: Soybean (Glycine max)	
COMMON NAME: bacterial blight	
METHOD: Sb 4.2 Ground bulk seed–Serological and pathogenicity confirmation (Alvarez et al.,1995)(formerly Sb2.2)	
METHOD CLASS: STANDARD (A)	
SAMPLE: 5,000 seeds	

REVISION HISTORY: Version 1.1: 2.21.2022 Updated King's B Medium.

PROCEDURE:

1. Five subsamples of 1000 dry soybean seeds are grinded in a Stein Mill for 1 min, then added to 600 ml of sterile saline (0.85% NaCl) and the suspension placed on a rotary shaker for 2 hr at 25°C at 220 rpm.

2. Threefold serial dilutions are made from the suspension and 0.1 ml aliquots plated on King's B medium amended with cephalexin.

3. After incubation at 25°C for 2-3 days, presumptive colonies of P. s. glycinea, exhibiting a blue fluorescence under UV light (370 nm), are re-isolated onto KBC.

4. Presumptive colonies of each subsample are confirmed as P. s. glycinea by the following pathogenicity and slide agglutination tests.

Pathogenicity

1. Pathogenicity is determined by inoculating 15-day-old, greenhouse-grown soybean seedlings (cvs. Oakland, Beeson, Acme, and Flambeau) by rubbing leaves with a sterile cotton swab dipped in an aqueous suspension of the presumptive colony (approximately 10^5 cfu/ml).

2. The seedlings are incubated in light for 48 hr at 90% relative humidity in a mist chamber at 25°C, then transferred to the greenhouse and observed for necrotic lesions on leaves 4-7 days after inoculation.

Agglutination

1. Ten microliters of bacterial suspension of each colony (10^5 cfu/ml) was mixed in polystyrene Micro ELISA plates (Dynatech Corp.) with 10 µl of a 1:1,000 aqueous dilution of the antiserum obtained from A. Calzolari (Osservatorio Regionale per le Malattie delle Plante, Bologna, Italy).

2. The plates are agitated for 1 hr at 25°C on a rotary shaker at 220 rpm, and agglutination is determined under a stereoscopic microscope.

MEDIA:

King's B Medium

DI water	1 liter
Proteose peptone #3	20g
K_2HPO_4 (anhydrous)	1.5g
Glycerol	15ml
$MgSO_4$ * $7H_2O$	1.5g
Agar	20g

*4ml cephalexin from the stock solution per liter applied after autoclaving. (Stock = 1g per 100ml water)

REFERENCES:

Alvarez, E., Braun. E. J., and McGee, D.C. 1995. New assays for detection of Pseudomonas syringae pv. glycinea in soybean seed. Plant Dis. 79:12-14.