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 4.1 Soaked bulk seed – Biochemical confirmation (Chauveau, 1988) (formerly Sb 2.1)	
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 soybean seeds are soaked for 24 hr at 4-5°C in 600 ml of sterile tap water adjusted to pH 6.5 with a phosphate buffer solution.

2. Threefold serial dilutions are made from the soaking solution and 0.1ml aliquots plated on King's B medium amended with cephalexin (KBC).

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. Five presumptive colonies of each subsample are subculture onto King's B medium.

5. These subcultures are then confirmed as P. s. glycinea by a positive reaction for levan production and negative reactions in oxidase and esculin hydrolysis tests.

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)

Esculin Hydrolysis Agar

DI water	1 liter
$NH_4H_2PO_4$	0.5g
K_2HPO_4	0.5g
$MgSO_4 * 7H_2O$	0.2g
NaCl	5g
Yeast Extract	5g
Ferric ammonium citrate	0.5g
Esculin	1g
Agar	12g

*pH should be about 6.8

Levan Agar

DI water	1 liter

Sucrose	50g
Nutrient agar	23g

REFERENCES:

Chauveau, J. F. 1988, personal communication