

Supplementary Information

## Development of an Immunochromatographic Strip for Rapid Detection of *Pantoea stewartii* subsp. *stewartii*. *Sensors* 2015, 15, 4291-4301

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### 1. Experimental Section

#### 1.1. Buffers and Solutions

0.05 M sodium carbonate-bicarbonate buffer (CBS, pH 9.6); 0.05 M sodium carbonate-bicarbonate buffer containing 0.2% (w/v) gelatin as blocking buffer; 0.01 M phosphate buffered saline (PBS, pH 7.4); 0.01 M phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST, pH 7.4); 0.01 M phosphate-buffered saline containing 0.1% (w/v) gelatin as antibody dilution; 0.1 M citrate phosphate buffer (pH 5.0) containing 180  $\mu$ L of 30% H<sub>2</sub>O<sub>2</sub> (A solution) and ethylene glycol substrate solution containing 0.06% (w/v) 3,3',5,5'-tetramethylbenzidine (B solution), mixed at a ratio of 5:1 as substrate solution; 2 M sulfuric acid as stop reagent.

#### 1.2. Development of Monoclonal Sandwich ELISA Method

The procedure of sandwich ELISA method was as follows: microtiter plates were coated with capture mAb at 37 °C for 2 h with 100  $\mu$ L/well in CBS (pH 9.6). Plates were washed three times with PBST after incubation and then incubated with blocking buffer at 37 °C for 2 h (200  $\mu$ L/well). After washing three times, plates were incubated with heat-killed *Pantoea stewartii* subsp. *stewartii* in 0.01 M PBS or blank (0.01 M PBS) at 37 °C for 1 h (100  $\mu$ L/well). Then the plates were washed three times and incubated with HRP-mAb in antibody dilution at 37 °C for 1 h (100  $\mu$ L/well). After washing

four times, 100  $\mu\text{L}$ /well substrate solution was added and plates were incubated at 37  $^{\circ}\text{C}$  for 15 min in dark and then stopped by 50  $\mu\text{L}$ /well stop reagent. The absorbance at 450 nm was determined by a microtiter plate reader (BioTek, Winooski, VT, USA).

### 1.3. Pair-Wise Interaction Analysis

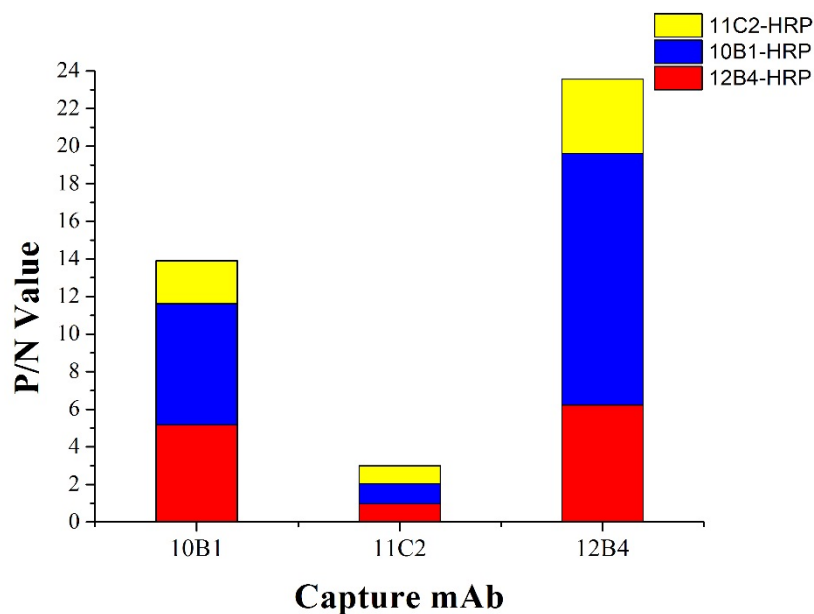
To establish the sandwich ELISA method, all the mAbs and HRP labeled mAbs were used as the capture and detection antibodies respectively in the pair-wise interaction analysis. Heat-killed *Pantoea stewartii subsp. stewartii* in 0.01 M PBS at the concentration of  $1 \times 10^8$  cfu/mL and blank (0.01 M PBS) were tested respectively by the sandwich ELISA method. The combination which provided the highest positive/negative value (P/N value, the ratio of the optical density values of the positive test sample to negative sample) was selected as the pair for sandwich ELISA method.

### 1.4. Characterization of the Sandwich ELISA Method

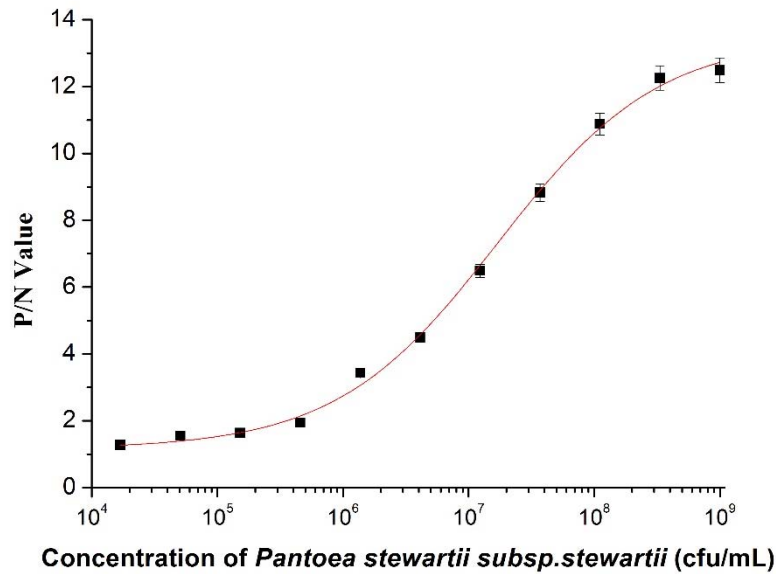
A series of bacterial standards ( $1 \times 10^9$ ,  $3.3 \times 10^8$ ,  $1 \times 10^8$ ,  $3.3 \times 10^7$ ,  $1 \times 10^7$ ,  $3.3 \times 10^6$ ,  $1 \times 10^6$ ,  $3.3 \times 10^5$ ,  $1 \times 10^5$ ,  $3.3 \times 10^4$  and  $1 \times 10^4$  cfu/mL in 0.01 M PBS) were tested by the sandwich ELISA method. The standard curve was generated with P/N value as the ordinate and concentrations of microorganism standards as the abscissa.

### 1.5. Cross-Reactivity of the Sandwich ELISA Method

*Pantoea stewartii subsp. stewartii* NCPPB 449 and other four plant pathogens: *B. glumae* NCPPB 3591, *X. oryzae pv. oryzicola* NCPPB 1150, *P. syringae pv. syringae* NCPPB 2844, and *X. oryzae pv. oryzae* NCPPB 3002 were tested by the sandwich ELISA method at a concentration of  $10^8$  cfu/mL.



**Figure S1.** The pair-wise interaction analysis by sandwich ELISA (P/N value); Note: P/N value, the positive/negative value, which was the ratio of optical density value of the testing sample to the negative control sample.



**Figure S2.** The standard curve of *Pantoea stewartii subsp. stewartii* in monoclonal sandwich ELISA; Note: P/N value, the positive/negative value, which was the ratio of optical density value of the testing sample to the negative control sample.

**Table S1.** The cross-reactivity of the sandwich ELISA method (n = 8).

Microorganism	Sandwich ELISA	
	OD <sub>450</sub> Value	P/N Value
<i>Pantoea stewartii subsp. stewartii</i> NCPPB 449	1.901 ± 0.11	13.45 (+)
<i>B. glumae</i> NCPPB 3591	0.184 ± 0.017	1.54 (+)
<i>X. oryzae pv. oryzae</i> NCPPB 1150	0.212 ± 0.011	1.72 (+)
<i>P. syringae pv. syringae</i> NCPPB 2844	0.240 ± 0.013	1.99 (-)
<i>X. oryzae pv. oryzae</i> NCPPB 3002	0.193 ± 0.012	1.30 (-)

Note: P/N value, the positive/negative value, which was the ratio of optical density value of the testing sample to the negative control sample. Values were calculated according to the formula  $P/N > 2.1$ . (+) means positive, (-) means negative. NCPPB: National Collection of Plant Pathogenic Bacteria, Harpenden, UK