P – 388

APS Meeting
Portland, OR 2009

Label-free Detection of Soybean Rust Spores Using Photonic Crystal Biosensor

Ramya Vittal[1], Wei Zhang[3], Leo L. Chan[3], Brian T. Cunningham[3], and Glen L. Hartman[1,2]

[1]Department of Crop Sciences, University of Illinois at Urbana-Champaign; [2]USDA-ARS, Urbana, IL; [3]Nano Sensors Group, Department of Electrical & Computer Engineering, University of Illinois at Urbana-Champaign

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Summary

The study represents the first demonstration of photonic crystal biosensor technology for the detection of *Phakopsora pachyrhizi* urediniospores. A detection limit of 1.5 x 10^5 uredinospores/ml was achieved. The platform has a potential to detect and forecast the disease

1.Goal

- To develop a photonic crystal based immunoassay for the quantification of soybean rust spores.

2. Background

Soybean rust

- Caused by the fungus *P.pachyrhizi*
- Early detection prior to visible leaf symptoms may provide data for forecasting the disease

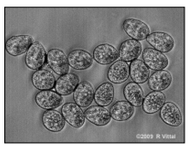

Fig. 1. *P.pachyrhizi* urediniospores

BIND system and photonic crystal biosensor

- The BIND System is comprised of the BIND Reader and 96-, 384- or 1536-well microplate photonic crystal biosensors
- Sensor is composed of a one-dimensional grating incorporated with low and high refractive index materials and is designed to function as a narrow band reflectance filter[1,2].
- Attachment of biomolecules onto the sensor surface changes the local refractive index and results in a shift of the resonant peak wavelength (PWV) to a longer value

Fig. 2. Photonic crystal biosensor embedded into the bottom of a 96-well microplate[3]

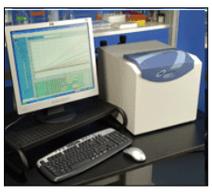

Fig. 3. BIND reader connected to a computer[3]

Subtractive inhibition assay

- It is an indirect method of detection based on the interaction of the antibody and urediniospores in solution
- Spore-bound antibody complex from the solution was removed by a stepwise centrifugation
- Free unbound antibody is detected on the sensor surface where a decrease in free antibody was observed with increasing spore concentration (Fig. 4)

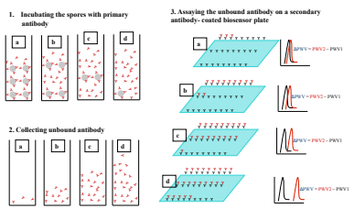

Fig. 4. Subtractive inhibition assay on a biosensor platform

3. Materials and Methods

- *P.pachyrhizi* urediniospores were used for immunization and all other applications in this study
- Corn rust (*Puccinia polysora*) spores were used to test for antibody cross-reactivity
- Various concentrations of spores were incubated with a 0.2 µg/ml of antibody for 1 h (Table 1) and free unbound antibody was collected by sequential centrifugation[4,6]
- An aldehyde-rich biosensor was coated with secondary antibodies to capture the supernatant
- The assay was performed in triplicates; Positive (only antibody) and negative (only PBS) controls were used
- Degree of antibody inhibition (R/R_0) for each spore concentration was calculated by dividing their mean PWV shifts (R) by the mean blank response (R_0)
- Detection limit was calculated as the lowest point exhibiting >10% inhibition [5]

4. Results

- The experiments showed that the PWV shifts were inversely proportional to the concentrations of spores, thus verifying the assay principle

Table 1. Dilution series of spores

Label	Number of spores per ml
0	0
1	7.5 x 10^4
2	1.5 x 10^5
3	3 x 10^5

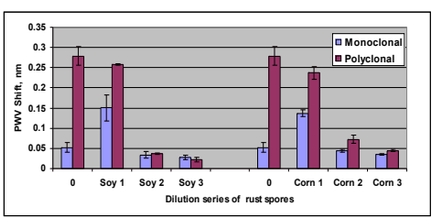

Fig. 5. Subtractive inhibition assay of *P.pachyrhizi* spores using polyclonal and monoclonal antibodies. *P. polysora* spores seem to cross-react with both the antibodies. The PWV shift decreased with an increase in spore concentration.

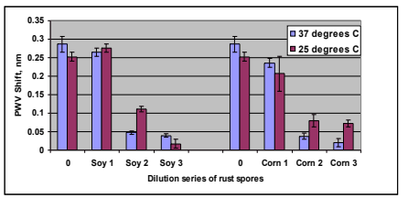

Figure 6. Graph showing the effect of incubation temperature on the binding between spores and antibody.

- Both monoclonal and polyclonal antibodies cross-reacted with corn rust spores
- Overall, polyclonal antibodies had higher mean response compared to the monoclonal antibodies (Fig. 5)
- Incubating the spores and antibodies at 25°C and 37°C yielded similar results (Fig. 6.)
- A detection limit of 1.5 x 10^5 has been achieved

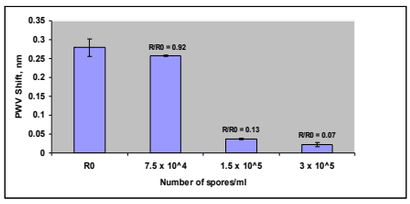

Figure 6. Calibration graph showing the degree of inhibition of antibody for each soybean rust spore concentration

5. Discussion

- The achieved detection limit is lower than the detection limit of 3.1 x 10^5 spores/ml reported for *Puccinia striiformis* on an biacore chip[6]
- The assay needs to be optimized in terms of analysis time and sensitivity
- Specificity will be tested using other rust fungi and on other spore types like basidio-, telio- and aeciospores

6. Acknowledgements

We gratefully acknowledge the Soybean Disease Biotechnology Center and Illinois Council on Food and Agricultural Research for providing financial support for this work. We thank Dr. James Haudenshield for his valuable guidance.

7. References

[1] B. T. Cunningham *et al.* 2004. Journal of Biomolecular Screening 9(6): 481 - 490
[2] B. T. Cunningham *et al.* 2002. Sensors and Actuators B, Chemical 1: 316 - 328
[3] www.srubiosystems.com
[4] Leonard *et al.* 2004. Biosensors and Bioelectronics 19: 1331-1335
[5] M.C. Hennion, D. Barcelo. 1998. Anal. Chim. Acta 362: 3 – 34
[6] P. Skottrup *et al.* 2007. Biosensors and Bioelectronics 22: 2724-2729