Specificity and Sensitivity comparative study between phage PVP-S1 and monoclonal antibody as receptor in polydiacetylene vesicles for *Salmonella* colorimetric detection

T. V. de Oliveira[1,2*], N. de F. F. Soares[1], C. M. Carvalho[2,3], J. S. dos R. Coimbra[1], N. J. de Andrade[1], J. Azeredo[2,3], E. A. A. Medeiros[1], P. P. Freitas[2]

[1] University Federal of Viçosa, Department of food technology, Packaging laboratory, Viçosa, MG, Brazil.

[2] International Iberian Nanotechnology Laboratory, Braga, Portugal.

[3] Centre of Biological Engineering, University of Minho, Braga, Portugal.

Abstract: Polydiacetylene polymer (PDA) has been intensively studied because of its properties as colour change from blue to red and change from non-fluorescent to fluorescent form due to an external stimulus that lead to a reorientation of the PDA within the organized structure. External stimulus could be temperature, pH, solvent influence, bacteria presence, mechanical stresses and others (Oliveira *et al.*, 2012). Pires *et al.* (2010) support the hypothesis that such phenomena occurred due to conformational changes associated with the functional group rotation around the simple carbon-carbon bond present in PDA chains. When the backbones of PDA conjugated polymer chains are perturbed, the delocalized π-network induces changes in electronic absorption and emission properties (Huo *et al.*, 1999). For a particular colour change, it is possible to incorporate a compound in the polydiacetylene carboxyl groups that will work as a specific receptor for the bacteria detection. This technology can be used for the detection of pathogens and thus is important to avoid food contamination once the standard technology demands long time and people trained.

The selection of the receptor used in the PDA is the first critical step to develop a biosensor with improved selectivity, selectivity and stability. For this reason, the aim of this study was to make a comparative study between two recognition molecules: phage PVP-S1 (Santos et al., 2011) and a monoclonal antibody in the PDA sensor for the detection *of Salmonella*. Antibodies lack specificity, poor separation efficiency and sensitivity. Phages are extremely specific, withstand harsh environments, are economically and easily produced, show high stability during storage and thus present potential for bacterial detection. Overall the selection of the recognition molecule that show the best features is important to develop a simple and rapid sensor for the industry and consumer's life. The specificity of the sensor was proven by using *Staphylococus aures* and *Escherichia coli* as gram-positive and gram-negative controls, respectively.

Other controls as LB medium and with PDA were performed to ensure that the colour change did not occurred due to another external stimulus. Controls maintained the colour around blue (peaks in 640 nm) and upon *Salmonella* Enteretidis presence the colour changed to red (peaks in 540 nm) as verified by spectroscopic analysis. Colorimetric response was calculated as Charych *et al.* (1993) to quantify colour transition (Figure 1).

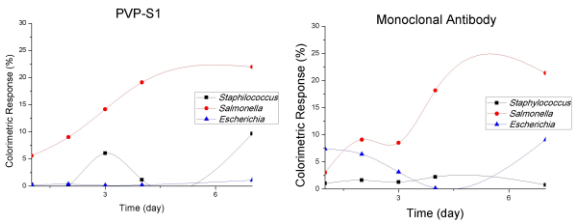

Figure 1: Colorimetric response obtained after the interaction of the different bacteria with with phage PVP-S1 (a) and antibody (b) as specific receptors in the PDA sensor

Monoclonal Antibody showed the highest colorimetric response and the PVP-S1 was more stable. Therefore both receptors improved the PDA sensor sensitivity and specificity and thus can be used as biorecognition molecules to the development of the PDA sensor.

Keywords: polydiacetylene vesicles, monoclonal antibody, *Salmonella* detection, phages, specificity, sensitivity.

References:

Oliveira, C. P. de; Soares, N. de F. F.; Fontes, E. A. F.; Oliveira, T. V. de; Maradini Filho, A. M. (2012). Behaviour of polydiacetylene vesicles under different conditions of temperature, pH and chemical components of Milk. Food Chem. 135, 1052-1056.

Huo, Q.; Wang, S.; Pissloup, A.; Verma, D.; Leblanc, R. M. (1999) Unusual chromatic properties observed from polymerized dipeptide diacetylenes. Chem. Com. 16, 1601-1602.

Pires, A. C. S.; Soares, N. de F. F.; Silva, L. H. M. da; Silva, M. C. H. da; Mageste, A. B.; Soares, R. F; Teixeira, A. V. N. C.; Andrade, N. J. de. (2010). Thermodynamic Study of Colorimetric Transitions in Polydiacetylene Vesicles Induced by the Solvent Effect. J. of Phys. Chem. 114, 13365-13371.

Charych, D. H.; Nagy, J. O.; Spevak, W. (1993) Direct colorimetric detection of a receptor-ligand interaction by a polymerized bilayer assembly. Scien. 261, 585-588.

Santos, S.; Kropinski, A.M.; Ceyssens, P.-J.; Ackermann, H.W.; Villegas, A.; Lavigne, R.; Carvalho, C.M.; Krylov,V.N.; Ferreira, E.C.; Azeredo, J. (2011). Genomic and proteomic characterization of the broad host range Salmonella phage PVP-SE1 - The creation of a new phage genus. Journal of Virology 85:21, 11265-11273