Project number: 6272
Funding source: Teagasc
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Valorisation of fruit and vegetable waste for use as inhibitors of the plant pathogen *Ralstonia solanacearum*

Key external stakeholders: Vegetable growers, vegetable processors, pharmaceuticals, pesticide manufacturers, government authorities/legislators, food research scientists

Practical implications for stakeholders: As much as 20% of the fruits and vegetable processed are discarded as waste. This project highlighted that this waste could in itself serve as a potential source of natural inhibitors of plant pathogen *Ralstonia solanacearum* Lectin. Modulations of the pathogen protein may have potential pharmaceutical applications.

Main results:

- Extensive NMR-spectroscopy characterisation of food-plant pathogen protein *Ralstonia solanacearum* lectin (RSL) upon interactions with eight different commercially available sugar monomers. Chemical shift perturbations, binding maps and dissociation constants were determined to help in understanding binding efficacy of these sugars towards RSL. RSL was found to interact with both the α- and the β-anomer of L-fucose and the "fucose like" sugars D-arabinose and L-galactose.
- Weak interactions of crude extracts from 5 food-plant species with RSL were observed, which is more likely due to residual sugars after the extraction with organic solvents. Instead simple water extracts from vegetables (tomatoes and carrots) showed strong interactions with RSL confirming that they are rich source of compounds (sugars) that can strongly bind to RSL.
- Using NMR spectroscopy, significant differences in the interactions of sugar-free and bound forms of RSL were demonstrated.
- Novel way of PEGylation of protein lectin with glycopolymers was achieved that may have potential biomedical applications.

Opportunity / Benefit:

Outcomes of the project will especially be of use to the vegetable growers/processors as the discovery of compounds that bind-efficiently with the *Ralstonia solanacearum* lectins and thereby prevent the invasion of this common pathogen. Development of methodologies for the recovery of potential compounds from fruits and vegetable waste stream will allow to exploit the untapped cheap resource.

The project's outcomes can especially be applied in pharmaceuticals. A well characterized interactions with sugars helps in designing inhibitors that can potentially bind to the protein tighter than fucose. PEGylation is an abundantly used method to improve the pharmacokinetics of therapeutic proteins.

Contact Dilip Rai Email: dilip.rai@teagasc.ie.

Collaborating Institutions: National University of Ireland, Galway; Durham University, UK; European Synchrotron Radiation Facility Grenoble, France.

Teagasc project team: Dr. Dilip Rai
Pawel Antonik, M.Sc. Eng.

External collaborators: Dr. Peter Crowley, NUIG
Dr. Alex Volkov, VUB
Prof. Nico van Nuland, VUB
Dr. Adam Round, ESRF
Prof. Neil Cameron, Durham

1. Project background:

Ralstonia solanacearum is a bacterium which causes fatal damage to hundreds of agricultural plants such as potato, tomato, banana, tobacco, etc. It produces two proteins (lectins) that are involved in attachment of the bacterium to the host. Lectin-mediated anchoring to target cell-walls enables maximal cell destruction by bacterial virulence factors. Inhibitors, which block the active site of lectins will reduce or eliminate the interaction between Ralstonia and plant hosts. The project aims to examine the potential of vegetable waste as a source of lectin-inhibiting compounds and/or discovery of the inhibitors towards the development of effective pesticides for Ralstonia. In addition, an in-depth investigation of the lectin-inhibitor(s) binding activity using advanced NMR spectroscopy, which is crucial initial step towards pesticidal development, will be undertaken.

2. Questions addressed by the project:

The project addresses the following specific questions:

- What are the binding efficacies of different monosaccharides with *Ralstonia solanacearum* Lectin (RSL)?
- What is the interaction of extracts from fruits and vegetable waste with RSL in the inhibition studies?
- Could the RSL protein be modified for potential pharmaceutical/biomedical applications?

3. The experimental studies:

A number of analytical techniques including NMR and UV-Vis spectroscopy, mass spectrometry, small-angle X-ray scattering, isothermal titration calorimetry, and electrophoretic methods were employed to characterize RSL-carbohydrate interactions. Crude and semi-purified (flash chromatography fractions) extracts from 5 different vegetables' waste streams were prepared in Teagasc Ashtown and assessed for the RSL-interaction studies. The NMR-relaxation experiments were carried out in collaboration with Prof. van Nuland's lab in Vrije Universiteit Brussel and small-angle X-ray scattering was measured in European Synchrotron Radiation Facility Grenoble, France. Synthesis of glycolpolymers was performed in Durham University (Prof. Cameron's lab). peels (after removal of toxic glycoalkaloids) serve as source of bio-active peptides?

4. Main results:

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5. Opportunity/Benefit:

Outcomes of the project will especially be of use to the vegetable growers/processors as the discovery of compounds that bind-efficiently with the *Ralstonia solanacearum* lectins and thereby prevent the invasion of this common pathogen. Development of methodologies for the recovery of potential compounds from fruits and vegetable waste stream will allow to exploit the untapped cheap resource.

The project's outcomes can especially be applied in pharmaceuticals. A well characterized interactions with sugars helps in designing inhibitors that can potentially bind to the protein tighter than fucose. PEGylation is an abundantly used method to improve the pharmacokinetics of therapeutic proteins.

6. Dissemination: The technology has been transferred in a number of ways, primarily through scientific A1 publications and conferences as outlined below:

Main publications:

- P. Antonik, A.N. Volkov, U. Broder, D. Lo Re, M. Wimmerová, N.A.J. van Nuland, P.B. Crowley. (2016). Spectroscopic Discrimination of Anomer-specific recognition and dynamics in a fucose-specific Lectin. *Biochemistry*, 55 (8): 1195–1203.
- P. Antonik, A.M. Eissa, A.R. Round, N.R. Cameron, P.B. Crowley. (2016). Noncovalent PEGylation via Lectin–Glycopolymer Interactions. *Biomacromolecules*, 17 (8): 2719–2725.

Conference Abstracts

- P. Antonik, A.N. Volkov, M. Wimmerová, U. Broder, N.A.J. van Nuland, D. Rai, P.B. Crowley (2012). NMR study of the 30 kDa fucose-binding lectin from *Ralstonia solanacearum*. ÈUROMAR 2012, Dublin, Ireland. Jul. 1-5, 2012
- P. Antonik, A.N. Volkov, M. Wimmerová, U. Broder, N.A.J. van Nuland, D. Rai, P.B. Crowley (2013). NMR study of the 30 kDa fucose-binding lectin from *Ralstonia solanacearum*. 2nd Irish NMR Meeting, Dublin, Ireland. Apr. 16, 2013
- P. Antonik, A.N. Volkov, M. Wimmerová, U. Broder, N.A.J. van Nuland, D. Rai, P.B. Crowley (2013). NMR study of the 30 kDa fucose-binding lectin from *Ralstonia solanacearum*. EMBO Workshop, Glycoproteins: From structure to disease. Palma de Mallorca, Balearic Islands, Spain. Apr. 24-26. 2013
- P. Antonik, A.N. Volkov, M. Wimmerová, U. Broder, N.A.J. van Nuland, D. Rai, P.B. Crowley (2014). NMR study of the 30 kDa fucose-binding lectin from *Ralstonia solanacearum*. "Soft-Inter 2014" Soft interactions in Biological and Biomimetic self-assemblies. Saint Malo, France. Sep. 7-13, 2014
- P. Antonik, A.N. Volkov, M. Wimmerová, U. Broder, N.A.J. van Nuland, D. Rai, P.B. Crowley (2014). NMR study of the 30 kDa fucose-binding lectin from *Ralstonia solanacearum*. Biophysical Society Conference "Disordered Motifs and Domains in Cell Control", Dublin, Ireland. Oct. 11-15, 2014

Popular publication:

- P. Antonik (2016). Protein-Carbohydrate Interactions and Structural Characterization of Ralstonia solanacearum Lectin. *PhD Thesis.*

7. Compiled by: Dr. Dilip Rai

Contact Dilip Rai Email: dilip.rai@teagasc.ie.