SELECTION OF MOLECULAR APTAMERS FOR IDENTIFICATION OF LIVE CELLS OF RALSTONIA SOLANACEARUM: A NEW POTENTIAL METHOD IN PLANT PATHOLOGY

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Outlines

- Introduction
- Objective
- Method & protocols
- Results
- Conclusion & perspectives
- Acknowledgements
Introduction

- *R. solanacearum* causes bacterial wilts on a wide range of planted crops, ornamentals and weeds worldwide.

- “Species complex”.
  - 5 races
  - 5 biovars
  - 4 phylotypes

- Race 3 biovar 2 (R3bv2).
  - World wide distribution except US and Canada
  - Major disease of potato
  - Introduction in the US in latently infected geranium cuttings
  - Strictest biosecurity regulations

- Critical need for unambiguous identification of R3bv2. Development of fast, sensitive, and specific detection methods.
Objective

Evaluate the effectiveness of cell-SELEX* to produce molecular aptamers for differentiation of strains of *R. solanacearum* (detection of live cells).

*System Evolution of Ligands by EXponential enrichment*
Method & protocols

- **Molecular aptamers.**
  - Single-stranded oligonucleotides.
  - Bind with high affinity to their target.
  - Detection of whole cells.
  - Small molecules, chemically stable.
  - Molecular probes for cancer studies.
  - Blocking molecules: reduced virulence in *M. tuberculosis*.

- **Cell-SELEX.**

  Repeated competitive binding from an initial DNA or RNA library (random sequences) to the target molecules.
Method & protocols

- Schematic representation of cell-SELEX.

From Shangguan D. et al. PNAS, 2006
Method & protocols

Principles of flow-cytometry

- **FSC** = Indication of particle’s size.
- **SSC** = Information about the outer composition of a cell.
- **FL** = Fluorescence detector (1 to 4).
Method & protocols

- Flow-cytometry data for cell-SELEX

A “shift” of the fluorescence intensity pick is an indication of enrichment of single DNA sequences with high affinity to the target cells (potential aptamers).
Method & protocols

Sequence analysis

From around $10^{15}$ unique random sequences

<table>
<thead>
<tr>
<th>Random sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous family 1</td>
</tr>
<tr>
<td>Homologous family 2</td>
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</tbody>
</table>
**Method & protocols**

- **Bacterial strains (cells)**
  - **UW485**: Race 3 biovar 2T
  - **Rs5**: Race 1 biovar 1 (Florida)

- **Cell growth conditions**
  - **Liquid medium**: MMP
  - **Phosphate-based buffer**
  - **36 hours growth**

- **ssDNA Library (78 nucleotides)**

  ![Diagram of ssDNA Library](Diagram)

  - **Library**
    - 5' - ACG ACA CAC AGC ACA CTC A - (NNNNNNNN...) - TC TTC TCC CTC GTC CTA CCT - 3'
    - 19  39  20
  - **Forward Primer**
    - 5' - ACG ACA CAC AGC ACA CTC A - 3'
    - **Carboxyfluorescein**
  - **Reverse Primer**
    - 5' - AGG TAG GAC GAG GGA GAA GA - 3'
    - **Biotin**

  **Fluorescence dsDNA (PCR product) → ssDNA**
Results

- Flow-cytometry histograms (10 rounds)
  - 20,000 cells

Target cells (UW485)

Negative cells (Rs5)
Results

- Flow-cytometry histograms (14 rounds)
  - 20,000 cells
  - Shift

Target cells (UW485)  Negative cells (Rs5)

- Library
- 11th
- 12th
- 14th
Results

- Flow-cytometry histograms (17 rounds)
  - 20,000 cells

Target cells (UW485)  |  Negative cells (Rs5)
Results

- Sequencing of evolved DNA pools (454)

**SP10**

353 repeated sequences (out of 3263)

One homologous family: **12 repeated sequences (0.4% total)**

**SP16-17**

769 repeated sequences (out of 8044)

Several homologous family: **22 repeated sequences (0.3% total)**

Only one conserved DNA family

For Primer – `cagggtcgagatgtttgcgatcctgttgcgtccgtgga` - Rev Primer
Conclusion and perspectives

■ Conclusion

- Efficiency of flow-cytometry to differentiate cells of *R. solanacearum* after 10 rounds of selection.
- Enrichment of DNA sequences with cell-SELEX.
- Low enrichment due to variability in target molecules (could be EPS ?).
- Amplification of potential aptamers not shown.

■ Perspective

- **Increase the number of repeated sequences.**
  More rounds of selection.
- **Test the affinity of the repeated sequences.**
  Amplification and testing these sequences as potential aptamers.
- **Control the specificity of the repeated sequences.**
  Test with target cells and heterologous bacteria.
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