Detecting ice-nucleating bacteria in environmental samples using PCR of the gene conferring ice nucleation activity

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Detection targets

- Whole, viable cells (culture on nutrient medium)
- INA protein (serology)
- Gene for the INA protein (polymerase chain reaction: PCR)
Click here to see an animated description of the PCR process.

If the link isn’t working, you can go directly to: <http://www.biomultimedia.net/archiv/pcr/pcr.htm>
INA protein

N-terminal: hydrophobic, insertion into membrane

C-terminal: hydrophilic

Core: central domain, repeating units, template for binding water

~ 4000 pairs of bases (A T G C) constitute the code in the DNA (gene) for this protein.

(Kajava and Lindow 1993)
INA gene

The sequence of bases in this gene has been determined for several strains of INA bacteria. These sequences (for a single strand) are available at an open data base (GenBank).

Example

5' end of sequence, corresponding to N-terminal of protein

3' end of sequence, corresponding to C-terminal of protein
Ideal primers target unique sequences that are identical among alleles of the gene.

**Primers**

- **5’**
- **inaZ gene**
- **3’

20 bases:

- N-terminal: 445 bases
- Core: 401 bases
- C-terminal: 255 bases
Primers

Ideal primers target unique sequences that are identical among alleles of the gene.

- **5’**
- **inaZ gene**
- **3’**

20 bases:
- N-terminal
- Core
- C-terminal

445 bases
401 bases
255 bases

Mix and match
Primers: based on the *inaZ* sequence

**C-terminal**

GGG AGA CCA AAG CAG ATT GA

5’ →

CTG CTC CTG CTA CTG ACC TA →3’

**Core**

ACC GCG AGT TAC AGA AGC AT

5’ →

CTG GAA TAG CCC GCA GTA GA
CTG GAA TAG CCC GCA GT C GA
CTG GAA TAG CCC GCA GT G GA
CTG GAA TAG CCC GCA GT T GA
INA bacteria

Species reported to have active strains

*Pseudomonas syringae* ♣

*P. fluorescens*

*P. viridiflava* ♣

*Pantoea agglomerans (Erwinia herbicola)* ♣

*Xanthomonas campestris pv. translucens* ♣

Gram – negative non spore-forming ♣ plant pathogens
INA bacteria

Species reported to have active strains

*Pseudomonas syringae* ♣

*P. fluorescens*

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*Pantoea agglomerans (Erwinia herbicola)* ♣

*Xanthomonas campestris pv. translucens* ♣

Test strains

> 50 from the above species

plants, water, snow, rain

US, Europe, Asia, Antarctica

10 ‘negative’ strains: no INA gene or other species
### Primer specificity

#### Strains without an ice nucleation gene

<table>
<thead>
<tr>
<th># strains</th>
<th>Strain</th>
<th>C-terminal</th>
<th>Core</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Agrobacterium radiobacter</em></td>
<td>-</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>A C G T</td>
</tr>
<tr>
<td>2</td>
<td><em>Erwinia carotovora</em></td>
<td>+ (2 bands)</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>Erwina chrysanthemi</em></td>
<td>+</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>Flavobacterium sp.</em></td>
<td>+</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>Aureobacterium sp.</em></td>
<td>-</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>Micrococcus sp.</em></td>
<td>+</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae 1448A</em></td>
<td>-</td>
<td>A C G T</td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae DC3000</em></td>
<td>-</td>
<td>A C G T</td>
</tr>
</tbody>
</table>
## Primer specificity

### Ice nucleation active strains (-2°C to -8°C)

<table>
<thead>
<tr>
<th># strains</th>
<th>Strain Details</th>
<th>C-terminal</th>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. viridiflava</em> (China)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>P. viridiflava</em> (Montana)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>P. fluorescens</em> (Antarctica)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Pantoea agglomerans</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Pantoea agglomerans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td><em>Pantoea agglomerans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td><em>Pantoea agglomerans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>33</td>
<td><em>P. syringae</em> (diverse)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em> (irrigation lake)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em> (pv. <em>pisi</em>)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em> (pv. <em>melea</em>)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>X. campestris</em> pv. <em>transluscens</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>X. campestris</em> pv. <em>transluscens</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>X. campestris</em> pv. <em>transluscens</em></td>
<td>+</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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Biogenic Ice Nucleators in the Atmosphere – at the Crossroads of Physics and Biology

IUGG 2007, Perugia, Italy
**Primer specificity**

**Inactive strains of ice nucleation species (< - 8°C)**

<table>
<thead>
<tr>
<th># strains</th>
<th>Species</th>
<th>C-terminal</th>
<th>Core</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. fluorescens</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>P. fluorescens</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>P. syringae</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>P. syringae</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td><em>P. syringae</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td><em>P. syringae</em></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Applications

Limits of detections, and direct detection of INA bacteria in environmental samples

Validation

1 L of ‘test water’ filtered on 0.22 µm membrane
DNA extracted in 50 µl solvents
PCR (core) with 2 µl of extracted total DNA
‘test water’ =

*P. syringae (CC94)* at $10^6$ and $10^7$ bacteria/ L in river water
*P. syringae (CC94)* in sterile distilled water at $10^{-10^8}$ bacteria/ L
River sample from which *P. syringae* was isolated at $1.5 \times 10^4$ bacteria/ L
Applications

Water naturally containing *P. syringae* at $1.5 \times 10^4$ bacteria/L

*P. syringae* CC94 in sterile water (bacteria/L):

$10^2$ $10^3$ $10^4$ $10^5$ $10^8$

Negative control

**P. syringae** seeded into river water at $10^7$ and $10^6$ bacteria/L was detected (lower concentrations not yet tested).
Applications

Perspectives

Pursue development of methodology for detection in environmental samples – snow, rain, cloud water, lake and river water, ice cores (qualitative measures).

Concentrations of INA P. syringae in environmental samples are generally above the limit of detection by PCR.
### P. syringae in non-agricultural substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ps bacteria / g or / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snow, fresh</td>
<td>$150 - 10^5$</td>
</tr>
<tr>
<td>Snow, firn</td>
<td>$100 - 5000$</td>
</tr>
<tr>
<td>Lakes and streamheads</td>
<td>$100 - 10^4$</td>
</tr>
<tr>
<td>Rain</td>
<td>$10^4$</td>
</tr>
<tr>
<td>Cloud water</td>
<td>ca. $10^4$</td>
</tr>
</tbody>
</table>
Applications

Perspectives

Develop **quantitative-PCR** method for use with environmental samples.

- quantify
- enhance sensitivity of detection
Applications

Perspectives

Use primers to amplify large regions of the genes to determine the sequence of bases:

- gene evolution

- relationship between gene sequence and expression of ice nucleation activity.
Summary

PCR detection of INA bacteria in environmental samples

Development of this tool in progress:

Primers available for ‘core’ region of gene for diverse INA bacteria.

Additional primers needed for *P. fluorescens*, etc.

Fine-tuning of sample preparation and PCR conditions for environmental samples in progress.