Common scab on potato

Identification and characterization of *Streptomyces* species causing common scab in Norway

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Outline

• Introduction
  – Potato common scab

• Studies of *Streptomyces spp* on potatoes in Norway: identification and biology
  – survey of potato common scab in Norway
  – microarray-based comparative genome analysis of *Streptomyces* species

• Questions?
Introduction
Common scab on potato
Common scab

• Occurs worldwide
• Degrades the appearance of the tuber, and thereby marketability
• Caused by a few species in the genus *Streptomyces* (~10)
• Considered a disease of warm, dry, coarse-textured soils
  – Now reported wherever potatoes are grown
    • In wet and dry soils
    • In soils ranging from pH 4.0 to above 9.0
Streptomyces

- Multicellular, filamentous, Gram-positive actinobacteria
- Well known for production of secondary metabolites (antibiotics)
- Seed- and soilborne
- Able to live saprophytically
- *Streptomyces* infects underground portions of a wide range of tap root crops in addition to potato
- Complex morphology
  - Mycelial growth
  - Spore formation
Streptomyces: morphologically diverse
Pathogenicity in the genus *Streptomyces*

- **Thaxtomin A**
  - phytotoxin
  - pathogenicity determinant
  - primary mode of action: inhibition of cellulose biosynthesis in plant cells

- No other factors has yet been proven to be a pathogenicity determinant
Pathogenicity in the genus *Streptomyces*

- Infection is developmentally constrained: the pathogen can only infect plant tissue that is actively growing.

- Molecules of plant origin which is released from expanding cell walls can stimulate/induce thaxtomin production:
  - Cellobiose
  - Cellotriose
  - Suberin
  - Other complex carbohydrates that can break down into simpler oligosaccharides

- The disease does not develop further on tubers in storage.
Symptoms

- Symptoms are ranging from superficial lesions (minor cosmetic damage) to pitted and raised lesions (reduced marketability and loss of crop) with a corky texture.
- The different plant pathogenic *Streptomyces* species cannot be distinguished by symptoms.
*Streptomyces* species found to be pathogenic on potato

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Reported from</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. scabies</em></td>
<td>CS</td>
<td>Finland, Japan, Korea, North America, South Africa, China</td>
<td>(Bukhalid et al. 2002; Lambert and Loria 1989b; Lindholm et al. 1997; Park et al. 2003b; St-Onge et al. 2008; Wanner 2009)</td>
</tr>
<tr>
<td><em>S. europaeiscabiei</em></td>
<td>CS, NS</td>
<td>France, Korea, North America, Norway, Western Europe</td>
<td>(Bouchek-Mechiche et al. 2000a; Dees et al. 2011; Flores-Gonzalez et al. 2008; Song et al. 2004; Wanner 2009)</td>
</tr>
<tr>
<td><em>S. turgidiscabies</em></td>
<td>CS</td>
<td>China, Finland, Japan Korea, North America, Norway, Sweden, UK</td>
<td>(Dees et al. 2011; Kim et al. 1999; Kreuze et al. 1999; Lehtonen et al. 2004; Miyajima et al. 1998; Thwaites et al. 2010; Wanner 2009; Zhao et al. 2008)</td>
</tr>
<tr>
<td><em>S. acidiscabies</em></td>
<td>AS</td>
<td>China, Japan, Korea, North America, UK</td>
<td>(Lambert and Loria 1989a; Song et al. 2004; St-Onge et al. 2008; Thwaites et al. 2010; Tôth et al. 2001; Zhao et al. 2010)</td>
</tr>
<tr>
<td><em>S. stelliscabiei</em></td>
<td>CS</td>
<td>France, North America</td>
<td>(Bouchek-Mechiche et al. 2000a; Wanner 2006)</td>
</tr>
<tr>
<td><em>S. reticuliscabiei</em></td>
<td>NS</td>
<td>France</td>
<td>(Bouchek-Mechiche et al. 2000a; Bouchek-Mechiche et al. 2000b; Pasco et al. 2005)</td>
</tr>
<tr>
<td><em>S. luridiscabiei</em></td>
<td>CS</td>
<td>Korea</td>
<td>(Park et al. 2003a)</td>
</tr>
<tr>
<td><em>S. puniciscabiei</em></td>
<td>CS</td>
<td>Korea</td>
<td>(Park et al. 2003a)</td>
</tr>
<tr>
<td><em>S. niveiscabiei</em></td>
<td>CS</td>
<td>Korea</td>
<td>(Park et al. 2003a)</td>
</tr>
<tr>
<td><em>S. sp. IdahoX</em></td>
<td>CS</td>
<td>North America</td>
<td>(Wanner 2007b)</td>
</tr>
<tr>
<td><em>S. sp. DS3024</em></td>
<td>CS</td>
<td>North America</td>
<td>(Hao et al. 2009)</td>
</tr>
<tr>
<td><em>S. ipomoeae</em></td>
<td>Soil rot on sweet potato</td>
<td>North America</td>
<td>(Clark et al. 1998; Loria et al. 1997)</td>
</tr>
</tbody>
</table>

1 CS=common scab; NS=netted scab; AS=acid scab
What causes common scab?

- Interactions between the plant, pathogen and the environment

**Plant**
(genetic differences between potato varieties)

**Environmental conditions**
(soil humidity, pH and other microorganisms)

**Pathogen**
(genotype)
Control practices for common scab and their effectiveness

<table>
<thead>
<tr>
<th>Control practice</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation during tuber formation</td>
<td>Inconsistent, can fail</td>
</tr>
<tr>
<td>Lowering of soil pH to &lt;5.2</td>
<td>Limits crop rotation, can fail</td>
</tr>
<tr>
<td>Crop rotation</td>
<td>Inconsistent, can fail. The bacteria can survive as saprophytes or on other host plants</td>
</tr>
<tr>
<td>Biological control</td>
<td>Can work under controlled conditions, but variable in the field. More investigation needed</td>
</tr>
<tr>
<td>Disease-free seed tubers</td>
<td>Not sufficient, Streptomyces is also soil-borne</td>
</tr>
<tr>
<td>Chemical fumigation</td>
<td>Can work for a season; expensive, environmentally unfriendly</td>
</tr>
<tr>
<td>Resistant potato cultivars</td>
<td>Most desirable and reliable method</td>
</tr>
</tbody>
</table>

- Best option for controlling CS (breeding):
  Choose plant material with broad-based, durable response to the pathogen to develop varieties showing less scab damage
In Search of Better Management of Potato Common Scab

Merete W. Dees • Leslie A. Wanner

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Abstract Common scab (CS) is an important disease and quality problem in potato crops worldwide. CS degrades the appearance of the potato tubers, thereby diminishing market value. Knowledge of CS has expanded considerably over recent years, enabling improved detection of the causal pathogens and increased understanding of mechanisms of pathogenicity, and providing potential methods of modulating pathogen response for disease resistance. However, effective control of this disease remains elusive, and will
Studies of *Streptomyces spp* on potatoes in Norway; identification and biology
PhD-project:

- part of the project “Improved potato quality by reduced skin blemish diseases (scab and scurf) in Norwegian potato production” funded by the Norwegian Research Council

- focus on common scab
Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway

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Bioforsk, Norwegian Institute for Agricultural and Environmental Research, N-1432 Ås, Norway

The present survey was conducted to isolate and characterize *Streptomyces* species from common scab lesions of potato in Norway. Bacteria were isolated from scab lesions on tubers sampled in two consecutive years at different locations in Norway spanning ~1400 km from south to north. In total, 957 independent isolations from individual tubers were performed, with 223 putative pathogenic isolates obtained from 29 different potato cultivars and 130 different fields. *Streptomyces europaescabiei* was the most abundant species isolated from common scab lesions (69%), while 31% of the isolates obtained were *S. turgidiscabies*. *Streptomyces scabies* was not found. Pathogenicity of selected *Streptomyces* isolates was tested on potato. The ability of the bacterial isolates to infect potato was consistent with the presence of the *txtAB* operon. The results revealed no pattern in geographical distribution of *S. europaescabiei* and *S. turgidiscabies*; both could be found in the same field and even the same lesion. Four different pathogenicity island (PAI) genotypes were detected amongst the *txtAB* positive isolates: nec1+/tomA+, nec1−/tomA+, nec1+/tomA− and nec1−/tomA−. The current findings demonstrate that there is genetic variability within species and that the species are not spread solely by clonal expansion. This is thought to be the most comprehensive survey of *Streptomyces* species that cause common scab of potato in a European country.
Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway

- Tubers sampled from 15 counties of Norway spanning ~1400 km from north to south
- Two years of sampling
- In total, 957 independent isolations (tubers) were performed

A small piece of potato tissue is cut from under the surface of a single lesion
Isolation and characterization of \textit{Streptomyces} species from potato common scab lesions in Norway

The piece of potato tissue is homogenized in 200 µl of sterile distilled water (SDW). An aliquot of the homogenate is plated out on water agar and incubated at 28 °C in dark.
Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway

Hundreds of *Streptomyces* colonies can appear on water agar after isolation from potato.

Different species of *Streptomyces*, both pathogenic and non-pathogenic, can be found on the plate.

- Problem: they cannot be distinguished by visual inspection.
Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway

- Single colonies, phenotypically characteristic of *Streptomyces*, were transferred to yeast malt extract agar (YME) after ~6 days.
- DNA is extracted from each isolate. The isolates are classified as putative pathogenic isolates based on presence of the thaxtomin operon.
**Streptomyces** species determination

- **Streptomyces** species determination is based on sequence-variable regions in the 16s ribosomal RNA gene

- **S. scabies** and **S. europaeiscabiei** - almost identical in the 16S rDNA sequence
  - parts of the internal transcribed spacer region (ITS) is amplified and subsequently cut with the restriction endonuclease *Hpy99I* to distinguish between the two species

![Image of gel electrophoresis](image.png)

- S.eu = Hpy99I-
- S.s = Hpy99I+
## Putative pathogenic Streptomyces isolates characterized in the study

<table>
<thead>
<tr>
<th>Geographic origin</th>
<th>Fields</th>
<th>S. europaeiscabiei</th>
<th>S. turgidiscabies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isolates</td>
<td>TxN1Tx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tx</td>
</tr>
<tr>
<td>Akershus</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Aust-Agder</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Buskerud</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Finnmark</td>
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<td>4</td>
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</tr>
<tr>
<td>Hedmark</td>
<td>27</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Møre og Romsdal</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nord-Trøndelag</td>
<td>16</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Oppland</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Rogaland</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sør-Trøndelag</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Telemark</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Troms</td>
<td>15</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Vest-Agder</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vestfold</td>
<td>5</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Østfold</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Unknown</td>
<td>29</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>130</strong></td>
<td><strong>152</strong></td>
<td><strong>57</strong></td>
</tr>
</tbody>
</table>

a PAI genotype: Tx = presence of txtAB-operon; N1= presence of Nec1 gene; To = presence of tomA gene
Pathogenicity test on potato
- Symptom variation within *S. turgidiscabies*

a) potato, cv Blue Congo, grown with a *txtAB*-negative isolate was completely free of symptoms characteristic of common scab.

b), c), d) Tubers grown with *txtAB*-positive *S. turgidiscabies* isolates 1B, 10-129-3-1 and 08-54-05-1, respectively, showing a range of symptoms characteristic of common scab.
Species' identification and microarray-based comparative genome analysis of *Streptomyces* species isolated from potato scab lesions in Norway

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Species identification and microarray-based comparative genome analysis of *Streptomyces* spp.

- 28 putative pathogenic *Streptomyces* strains isolated from CS lesions of potato tubers from a wide geographic range in Norway were selected for:
  - microarray-based comparative genome analysis (CGH)
  - species identification by PCR
  - 16S rRNA phylogenetic analysis

- Microarray based on:
  - The whole genome of S. scabies, 12766 probes for 8848 ORFs
  - The PAI of *S. turgidiscabies*, 133 probes
Clustering of the strains based on microarray analysis and 16S rRNA analysis

Based on the whole genome sequence of *S. scabies* (~10 000 000 basepar)

Based on a short sequence (~1500 bp)

16S rRNA

microarray
Species determination by using microarray based analysis

• **Clade I**, 14 pathogenic strains of *Streptomyces*:
  – Not melanin producing
  – Detectable with primers specific for 16S rRNA gene of *S. turgidiscabies*
  – Clustered with a Swedish strain of *S. turgidiscabies* in microarray analysis and the type strain of *S. turgidiscabies* in 16S rRNA phylogenetic analysis
    ➢ *S. turgidiscabies*

• **Clade II**, 14 pathogenic strains of *Streptomyces*:
  – Melanin producing
  – Detectable with primers specific for 16S rRNA gene of *S. scabies*, but did not cluster with the type strain of *S. scabies* in microarray analysis
  – Clustered with the type strain of *S. scabies* and the type strain of *S. europaeiscabiei* in 16S rRNA phylogenetic analysis
  – Amplicons from ITS sequence were not restricted by *Hpy99I*
    ➢ *S. europaeiscabiei*
Genetic differences between *S. scabies* and *S. europaeiscabiei*

- Significant differences between the genome sequences of *S. scabies* and *S. europaeiscabiei* based on 762 genes
- Underrepresentation of genes related to primary metabolism among the divergent genes
Summary

• First report of occurrence of *S. turgidiscabies* and *S. europaeiscabiei* in Norway
  – 69 % *S. europaeiscabiei*
  – 31 % *S. turgidiscabies*
  – Surprisingly, *S. scabies* was not found in this survey

• *S. europaeiscabiei* most common in Europe

• The results provided novel information about:
  – genetic variability of *S. europaeiscabiei*
  – gene-specific variability between the genomes of *S. europaeiscabiei* and *S. scabies*

• On-going work: To study the response of *S. scabies* to potato microtubers added to liquid culture and to identify genes involved in early stages of plant pathogen interactions
Thank you for your attention

Potato cv Blå Kongo