CROWN GALL AND THE DIVERSITY AND DETECTION OF ITS CAUSAL AGENT

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CROWN GALL
Caused by tumorigenic Agrobacterium/Rhizobium spp on over 640 plant species
• Genetic character of the disease

http://rasbinbasnet.blogspot.com/
Economic significance

- The disease seldom kills plants, but it can elicit lack of vigour and reduced growth and crop.

- Highest losses occur in young plants.

- Infected plants, especially those with tumors on the main roots and collar, are unfit for marketing.

- Losses of 10-30% of nursery stock of fruit trees caused by crown gall are common - In exceptional cases they can reach 80% (Schroth et al., 1971; New and Kerr, 1972; Garret, 1987)
Crown gall is caused NOT by ONE bacterial species but by up to now by 8 species:

- *Agrobacterium radiobacter*
- *Agrobacterium rhizogenes*
- *Agrobacterium larrymoorei*
- *Agrobacterium rubi*
- *Agrobacterium vitis*
- *Rhizobium skierniewicense* (Puławska et al. 2012)
- *Rhizobium nepotum* (Puławska et al. 2012)
- *Rhizobium pusense*
- + 9 genomospecies within *A. tumefaciens* complex (biovar 1)
AIM: detection, identification and characteristics of bacteria causing crown gall on stone fruits in Poland

- Phenotypic characteristics
- 16S rRNA, atpD, glnA, gyrB, recA i rpoB;

Isolation on media: MG+Te, 1A+2E


PCR with primers tms2F1 i tms2R2 complementary to gene tms2 on pTi

Results

1200 isolates

418 pre-identified as *Agrobacterium (Rhizobium) spp*

380 – biovar 2/*Rhizobium rhizogenes*

18 – biovar 1/*Agrobacterium tumefaciens* complex

4 – *Rhizobium nepotum*

1 – *Rhizobium skierniewicense*

15 – not classified

318 pathogenic in plant test or PCR

307 – PCR = test on sunflower

9 – PCR “+” but sunflower “-”

2 – PCR “-” but sunflower “+”
1200 isolates
418 pre-identified as *Agrobacterium (Rhizobium)* spp
309 pathogenic in plant test or PCR

**Nopaline** type pTi
12 T-DNA RFLP groups

307 – PCR = test on sunflower
9 – PCR “+” but sunflower “-”
2 – PCR “-” but sunflower “+”

Mutations?
R. giardinii/R. herbae relatives

recA ML tree

F5.1, AL5.1.8
T-DNA PCRs – negative
virC - positive
test on plants - positive

lack of symbiotic genes
F5.1 genome sequencing

- ANI between F5.1 and *R. giardinii* H152 ~90%

No mapping of sequencing reads to any known T-DNA sequence
Conclusions

- In Poland crown gall of stone fruit is caused mostly by nopaline strains of \textit{R. rhizogenes} (biovar 2)

- Two new species – \textit{R. skierniewicense} and \textit{R. nepotum} of tumorigenic bacteria were discovered and validly published

- \textbf{Novel species} of bacteria causing crown gall will be described

- Unknown mechanism of pathogenicity of F5.1
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