

CROWN GALL AND THE DIVERSITY AND DETECTION OF ITS CAUSAL AGENT

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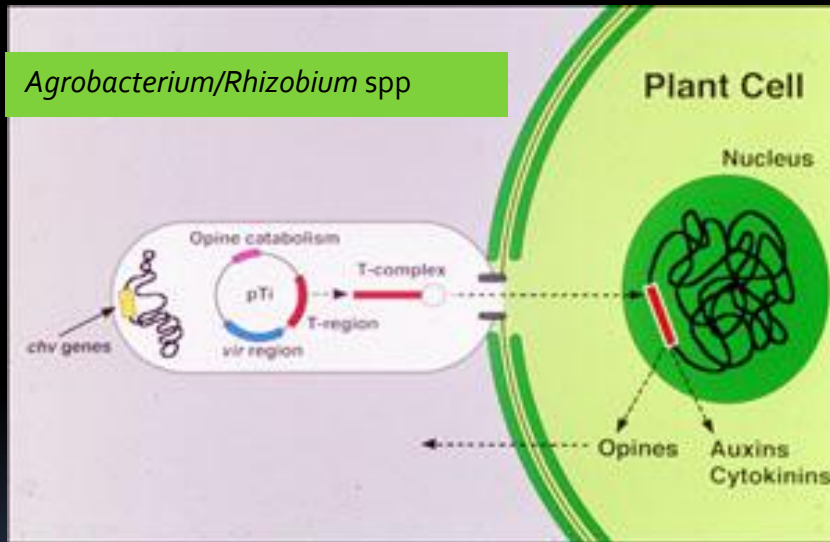
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[COST FA1104 Meeting 13-15. 10.2014 Bordeaux, France](#)

CROWN GALL

Caused by tumorigenic *Agrobacterium/Rhizobium* spp on over 640 plant species

- Genetic character of the disease



- <http://rasbinbasnet.blogspot.com/>

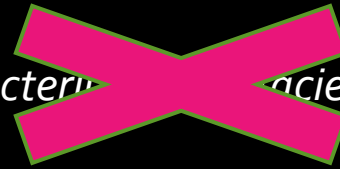


Economic significance

- 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)

Causal agent

Agrobacterium tumefaciens



- 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)

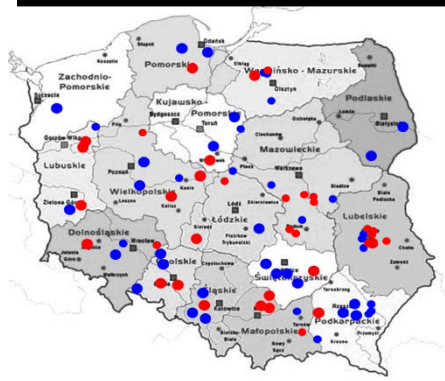
- *Rhizobium pusense*

Rhizobium

Allorhizobium

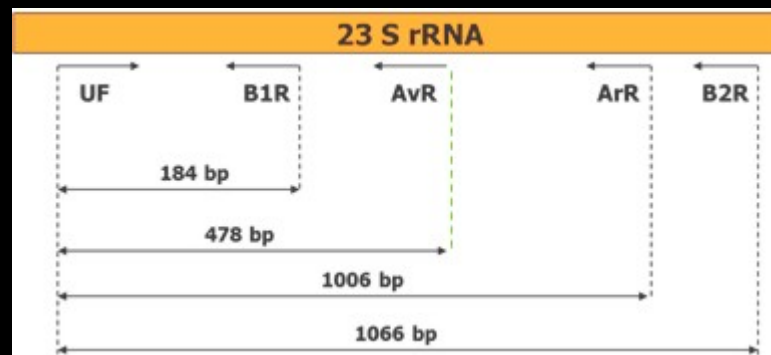
- + 9 genomospecies within *A. tumefaciens* complex (biovar 1)

AIM: detection, identification and characteristics of bacteria causing crown gall on stone fruits in Poland



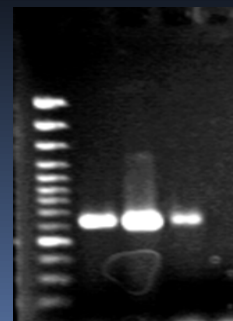
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Isolation on media: MG+Te, 1A+2E



Puławska et al., System. Appl. Microbiol. 2006

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



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

Puławska & Sobiczewski, J. Appl. Microbiol. 2005

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 „+“

pTi

1200 isolates



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



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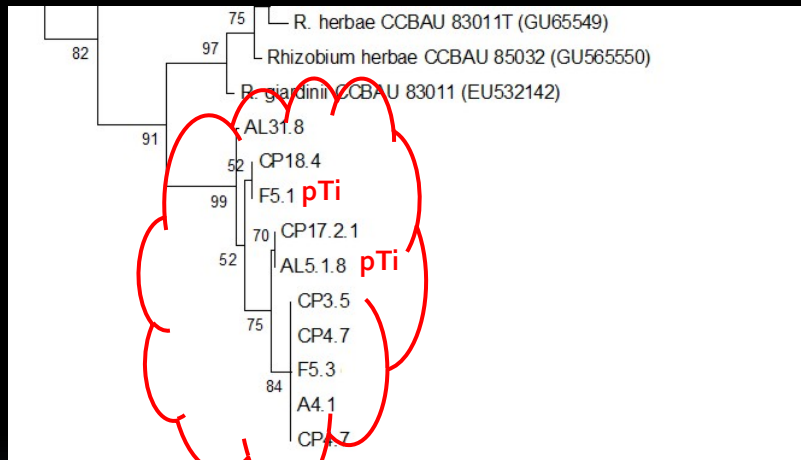


Nopaline type pTi

12 T-DNA RFLP groups

Mutations?

R. giardinii/*R. herbae* relatives



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%



different species

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 *R. rhizogenes* (**biovar 2**)
- Two new species – *R. skierniewicense* and *R. nepotum* of tumorigenic bacteria were discovered and validly published
- **Novel species** of bacteria causing crown gall will be described
- Unknown mechanism of pathogenicity of F5.1

ACKNOWLEDGEMENTS



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THANK YOU!