**AGROBACTERIUM**

First described by Smith and Townsend (1907)

Responsible for crown gall

Performed Koch’s postulates

The disease is worldwide in distribution

Speciation was based on pathogenicity

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*Agrobacterium tumefaciens*

causes tumors

hyperplastic

hypertrophic

badly organized groups of elements

Bacterium infects:

- 124 genera of plants
- 57 of 131 families of dicots
- 16 genera representing 2 families of gymnosperms
- 2 genera representing 2 families of monocots
  - Liliales and Arales

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*A. radiobacter*

nontumorigenic

*A. rhizogenes*

causes hairy-root disease

*A. rubi*

tumors on blackberries, raspberries

*A. vitis*

biovar 3 strains
Agrobacteria
Mesophilic soil inhabitants
Short Gram-negative rods
Commonly motile- one to six peritrichous flagella
Two chromosomes
   One circular and one linear
Each biovar has a specific genome size

Biovars 1, 2, and 3
Biovar 1
   includes most *A. tumefaciens* and *A. radiobacter*
   production of 3-ketoglycosides
Biovar 2
   contains all *A. rhizogenes* and probably most *A. rubi*
Biovar 3
   *A. vitis*
Selective media

Taxonomy at the general level
Agrobacterium species are related to *Rhizobium*
   based on numerical taxonomy
DNA composition and hybridization
DNA hybridization -- DeLey et al.
Biovar 2
   97% internal homology
   less than 20% homology with biovar 1
Biovar 1
   seven genetic subgroups
   each subgroup had 80% or greater homology
   at least 45-55% homology between groups
Pathogenicity

Characteristics of Crown Gall

1. Wounds are necessary
2. Bacteria spread intercellularly
3. 30°C and above - nonpermissive for transformation
4. Autonomous (tumors)
5. Only one cell is needed for tumor initiation
6. A 4 to 8 h time interval is needed for host cells

Characteristics of Crown Gall continued….

7. Site attachment of bacterial cell
8. Degree of wounding is proportional to tumor size
9. Secondary tumors are formed, but are not interconnected with primary tumors
10. Spread through tracheids
11. Plant hormonal changes
12. Unusual amino acids
13. TIP -- plasmid

1. Wounds are essential
   all parts of a susceptible host
   younger tissues are more susceptible
   Direct infiltration?

   A wound is essential because:
   a. It provides a site of attachment
   b. It supports the metabolic activities of the bacterium
   c. Changes in cells surrounding the wound leads to a susceptible state
2. Bacteria remain intercellular in the host

Exchange of DNA (TIP) between the bacterial cell and the host cell

3. Crown gall formation occurs readily at 27°C but is prevented at 30°C and above

4. The tumorous phenotype is a stable heritable state.

Sterile cultured tumors maintain their abnormal growth

Suggestion that the TIP may be a nucleic acid

Tumor Induction
   a. Attachment to a specific plant cell site
   b. Induction of the vir genes by phenolics.
   c. Transfer of T-DNA
   d. Auxin is an essential participant

5. Pinto bean leaves

# tumors is proportional to the concentration of bacteria noted that 1 bacterial cell is all that is needed for tumor initiation

In practice ~ 10⁴ cells are required for each tumor formed

This is because:
   a. The probability that a bacterium will reach an appropriate site in the small surface wounds
   b. Possible variation in host cell sensitivity
   c. Specific virulence of the pathogen
6. The time required for tumor induction range from 4 to 8 h

7. Tumor formation can be inhibited by:
   a. Avirulent *A. tumefaciens*
   b. Heat-killed virulent bacteria
   c. UV-killed virulent bacteria

Attachment to some site on the wound is essential
   LPS binds to plant polysaccharide in the cell wall
   Site attachment itself is complete in bean leaves within 15 min after inoculation

8. Degree of wounding is proportional to tumor size
   Within 48 h, the numbers of bacteria decrease

9 and 10. Secondary tumors appear at points distant from inoculated wound
   often free of bacteria
   *A. tumefaciens* also can move in tracheids
   The xylem, parenchyma and ray cells in grape are injured by cold temperatures

11. The various growth characteristics of crown gall tumors suggest hormonal changes in cells
    *A. tumefaciens* can synthesize:
    a. β-indoleacetic acid
    b. Cytokinin
    c. N-88-dimethylallylaminopurine
    d. Gibberelin-like substances

To grow normal plant callus tissue one needs to include auxin and cytokinin for growth
12. Crown gall tissue contains unusual amino acids that are derivatives of lysine, arginine or glutamine

lysopine = $N^2$-(D-1-carboxyethyl)-L-lysine
octopine = $N^2$-(D-1-carboxyethyl)-L-arginine
octopinic acid = $N^2$-(D-1-carboxyethyl)-L-ornithine
nopaline = $N^2$-(1,3-dicarboxypropyl)-L-arginine
mannopine = $N^2$-(1′-deoxy-D-mannohexitol-1′-yl)-L-glutamine
cucumopine = L-histidine and $\alpha$-ketoglutaric acid

Six types of crown gall tumors:

a. Produce lysopine, octopine, histopine, noroctopine, homoctopine and octopinic acid.
b. Produce nopaline and nopalinic acid (ornaline).
c. Agropine (null-type, formerly).
d. Produce cucumopine
e. Those that produce vitopine
f. Those that produce chrysopine

Control

a. Sanitation
b. Soil fumigation (chloropicrin)
c. Bacticin
d. Biological control
Ecological Distribution

28 soil samples in California - all contained agrobacteria

17 yielded phytopathogenic forms

Recover *A. tumefaciens* and *A. radiobacter*

- grass pasture never cultivated
- cotton fields, tomato fields and even fallow fields

Widespread distribution - reside in soil indefinitely

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Natural oak savanna and tallgrass prairie in Minnesota

Recovered $10^5$ cfu/g of agrobacteria

- 12 soil and root samples
  - never been cultivated

Most were biovar 2 with a few in biovar 1 - no biovar 3

- true inhabitant of the Midwest savanna soils and resides on the roots of native plants

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Crown gall of grapevines

The bacterium lives in the rhizosphere of grapevines

- Enter into the vascular system
  - injuries or root decay

*A. vitis* specifically causes a root decay of grapevines

- Gain entrance to the vascular system

High populations found in the grapevine rhizosphere