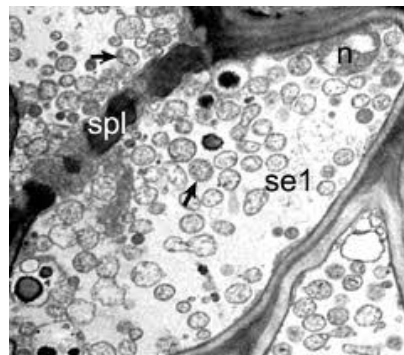
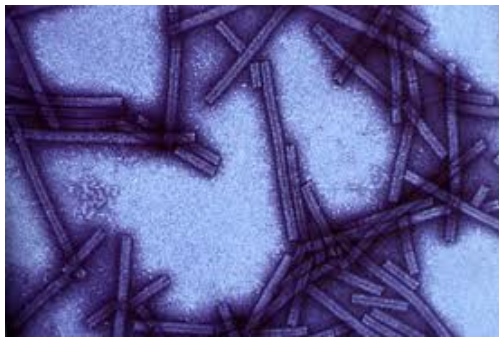




Molecular plant pathology



Assist. Prof. Martina Šeruga Musić

acad. year 2016/17

PHYTOPATHOGENIC BACTERIA

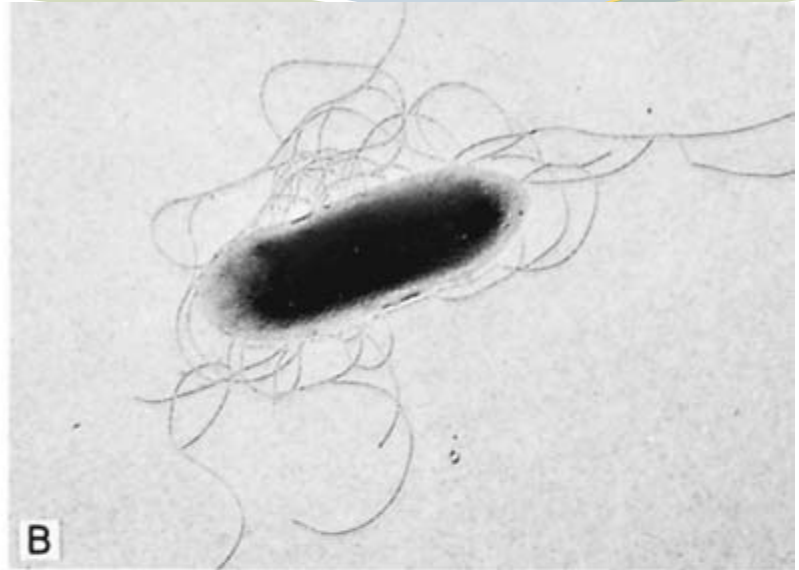
- there are more than 100 species of known phytopathogenic bacteria
- genera *Agrobacterium*, *Erwinia*, *Ralstonia*, *Pseudomonas*, *Xanthomonas*, *Rhizomonas*, *Clavibacter*, *Bacillus*, *Clostridium*, *Streptomyces*, *Xyllela*....
- most of them are classified as Gram-negative
- mostly rod-shaped with flagella, except genus *Streptomyces* – filamentous bacteria





A

genus *Agrobacterium*



B

genus *Erwinia*



C

genus *Pseudomonas*



D

genus *Xanthomonas*









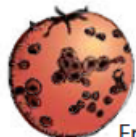






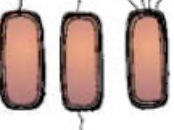

















 <p>Agrobacterium</p>	 <p>Crown gall</p>	 <p>Twig gall</p>	 <p>Cane gall</p>	 <p>Hairy root</p>		
 <p>Clavibacter</p>	 <p>Potato ring rot</p>	 <p>Tomato canker and wilt</p>	 <p>Fruit spot</p>	 <p>Fasciation</p>		
 <p>Erwinia</p>	 <p>Blight</p>	 <p>Wilt</p>	 <p>Soft rot</p>	 <p>Soft rot</p>		
 <p>Pseudomonas</p>	 <p>Leaf spots</p>	 <p>Leaf spots</p>	 <p>Galls (olive)</p>	 <p>Banana wilt</p>	 <p>Blight (lilac)</p>	 <p>Canker and bud blast</p>
 <p>Xanthomonas</p>	 <p>Leaf spots</p>	 <p>Cutting rot</p>	 <p>Black venation</p>	 <p>Bulb rot</p>	 <p>Citrus canker</p>	 <p>Walnut blight</p>
 <p>Streptomyces</p>	 <p>Potato scab</p>	 <p>Soil rot of sweet potato</p>	 <p>Rhizobium Root nodules of legumes</p>			

FIGURE 12-4 The most important genera of plant pathogenic bacteria and the kinds of symptoms they cause.



Xanthomonas phaseoli



Acidovorax citrulli (ex. *A. avenae*
subsp. *citrulli*)





Erwinia amylovora





Pseudomonas syringae pv. *tabaci*

- **INA (ice nucleation-active) proteins** – localized at the outer membrane surface of some Gram-negative bacteria
- Promote nucleation of ice at relatively high temperature above $-5\text{ }^{\circ}\text{C}$ and cause frost damage to many plants
- „ice-plus” and „ice-minus” bacteria

Bacteria that enter the host through leaves

- reach the plants via air, water or insects
- adhesion to the host cell and chemotaxis
- genera *Pseudomonas* and *Xanthomonas*

Bacteria from soil

- genera *Agrobacterium*, *Ralstonia solanaceum* – chemotaxis and motility–
through wounds

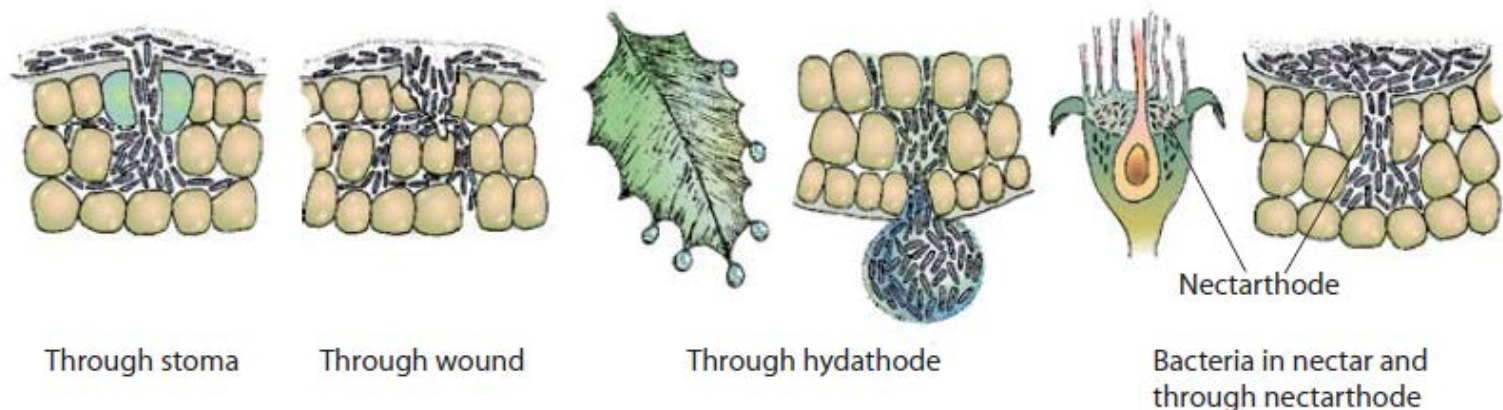


FIGURE 2-7 Methods of penetration and invasion by bacteria.

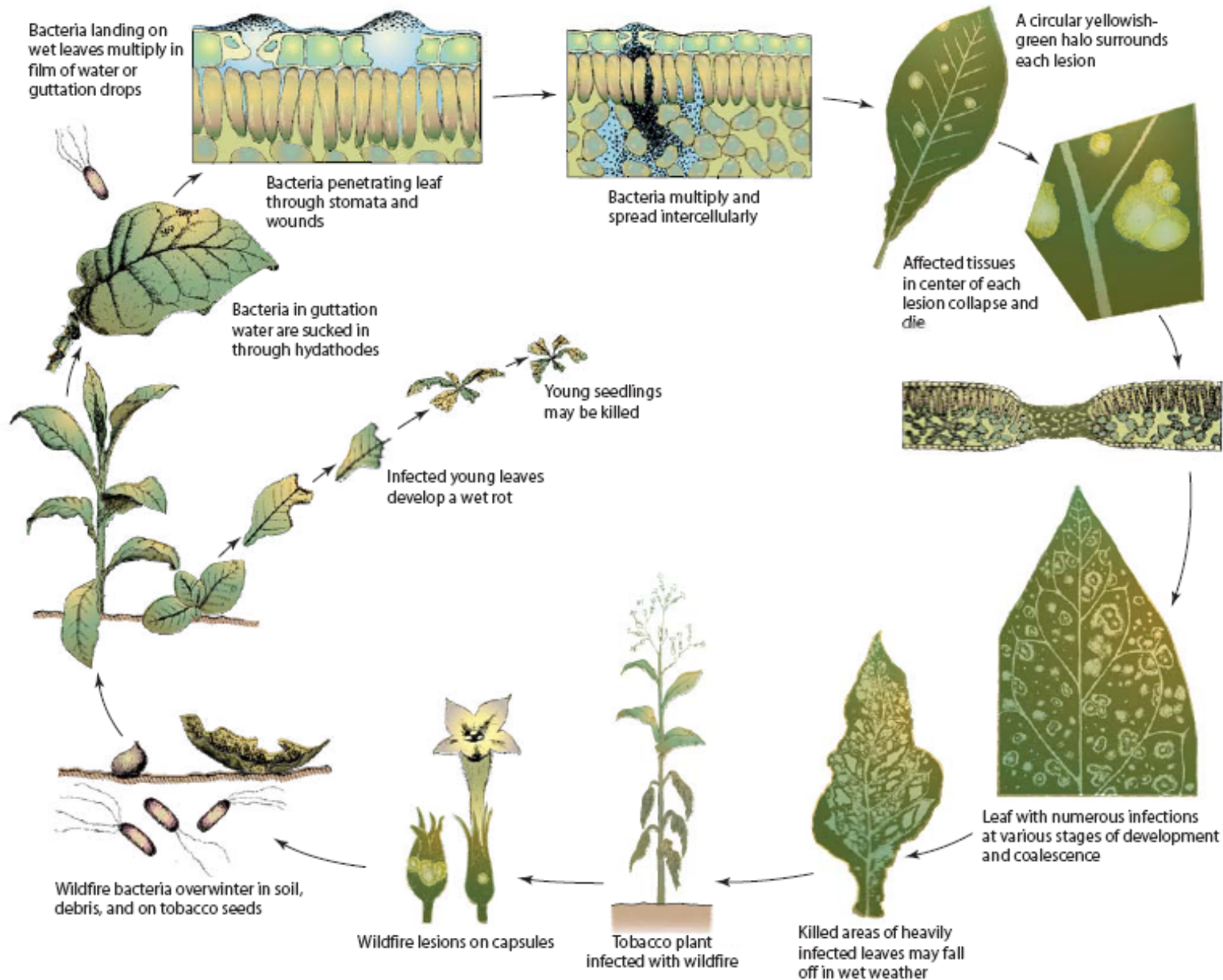


FIGURE 12-10 Disease cycle of a bacterial leaf blight, e.g., wildfire of tobacco or soybeans caused by *Pseudomonas syringae* pv. *tabaci*.

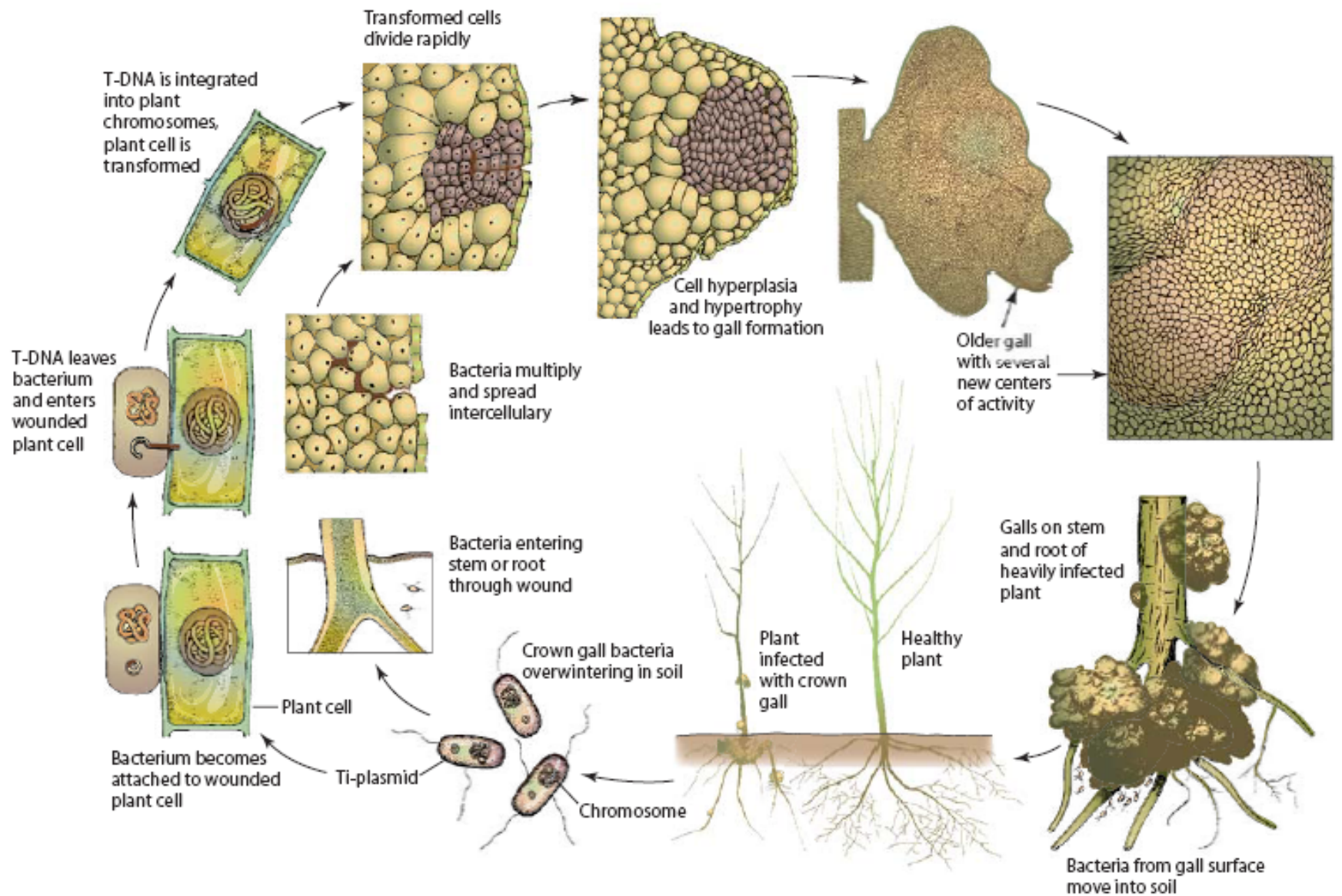
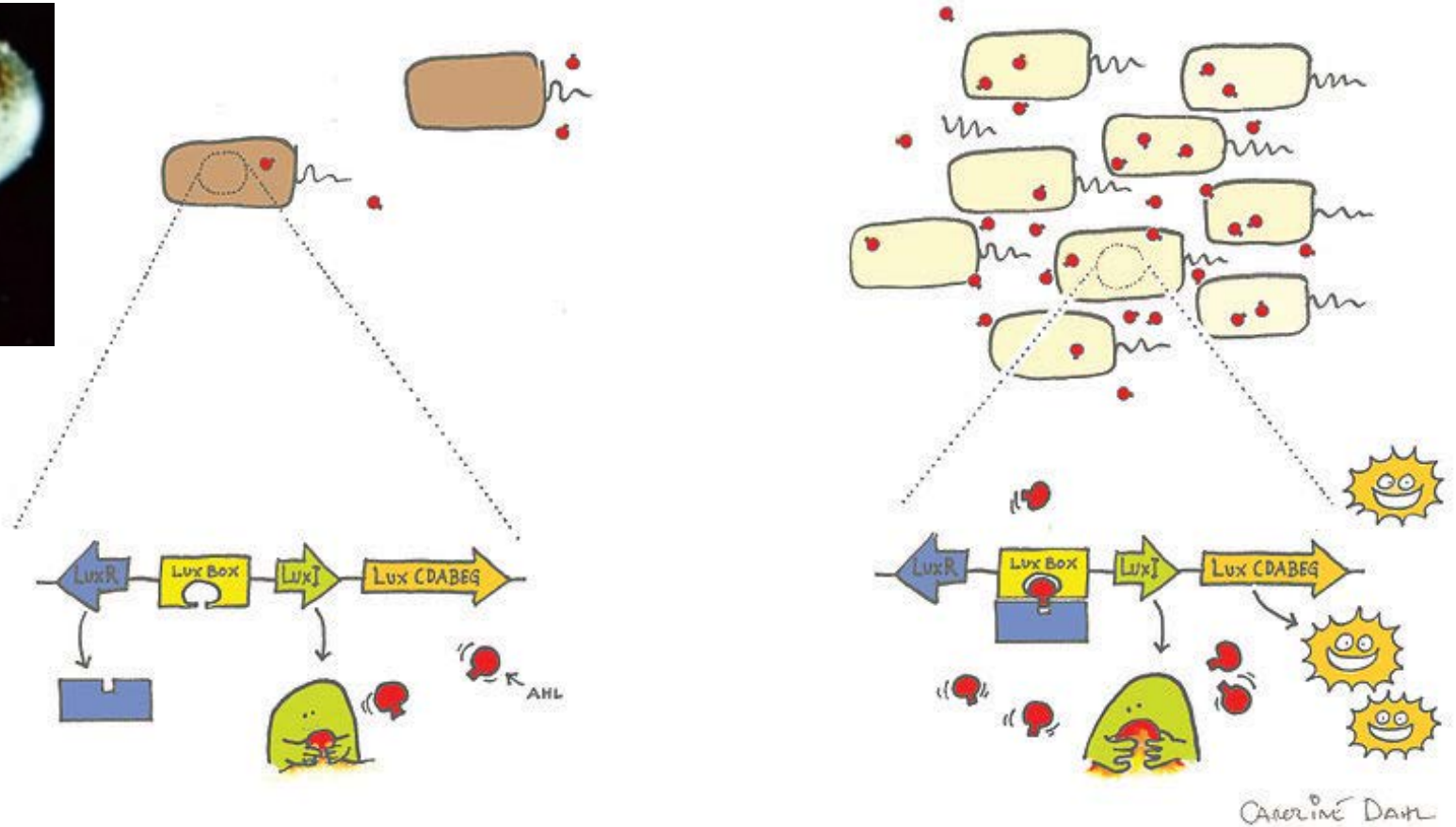
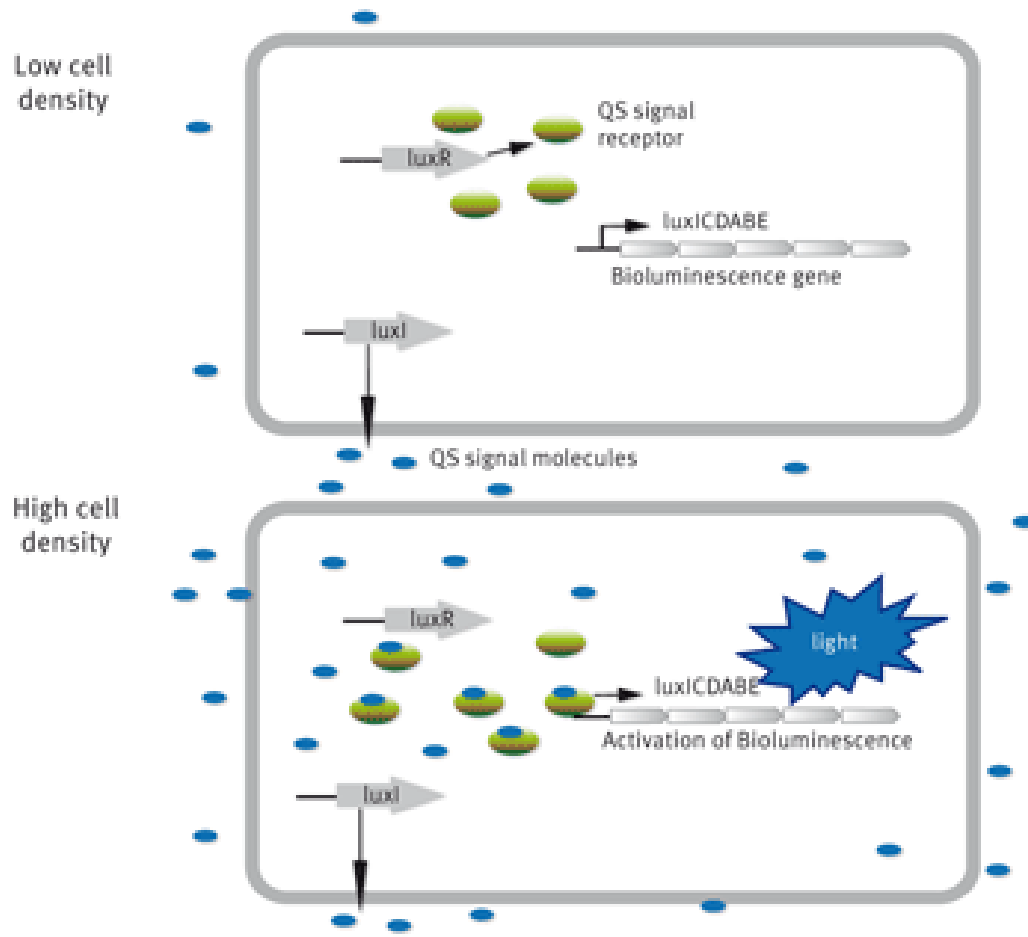


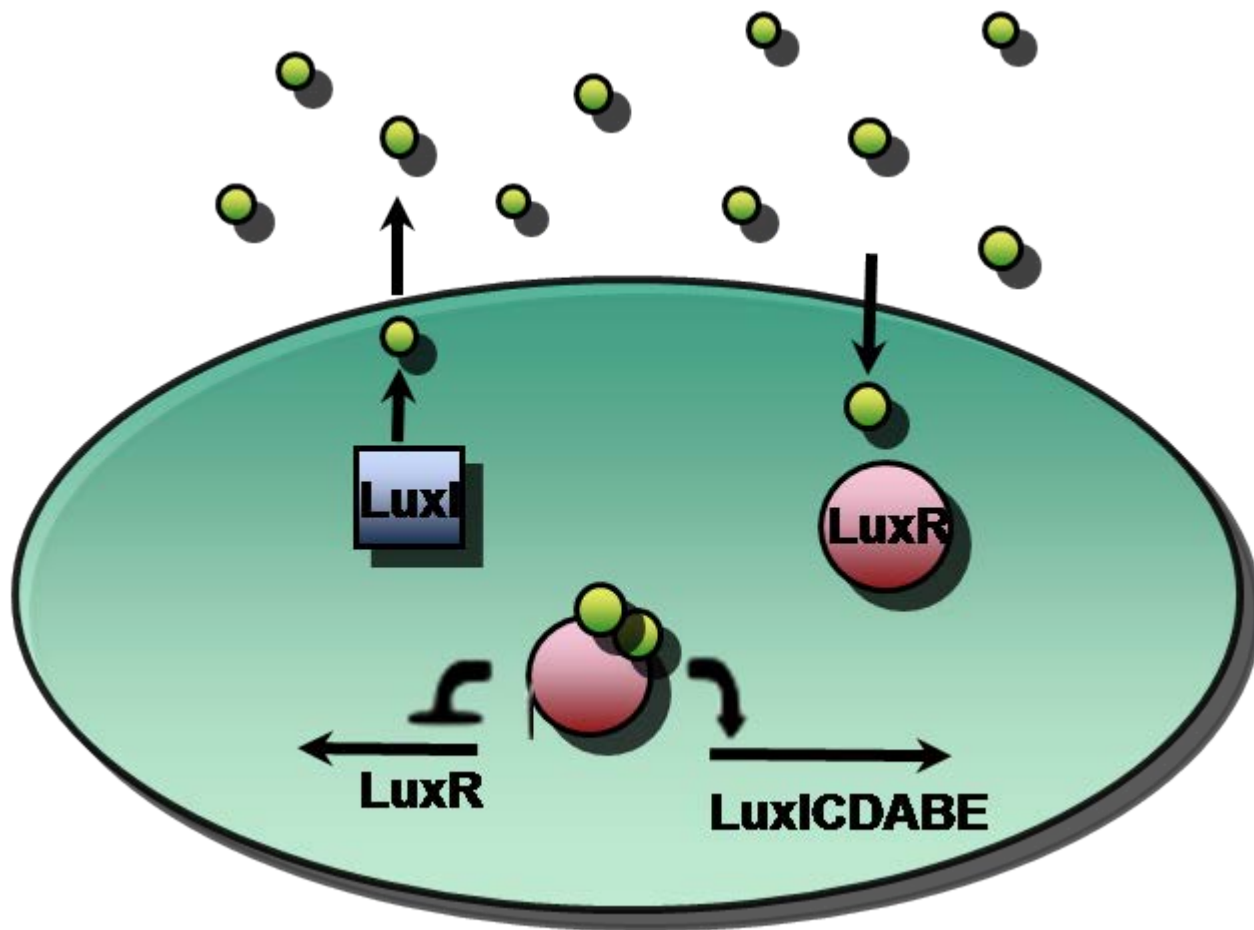
FIGURE 12-36 Disease cycle of crown gall caused by *Agrobacterium tumefaciens*.

- **quorum sensing** - a system of stimuli and response correlated to population density – it was first observed in bacteria *Aliivibrio fischeri* (*Vibrio fischeri*) – a bioluminescent bacterium that lives as a mutualistic symbiont in the photophore (or light-producing organ) of the Hawaiian bobtail squid (*Euprymna scolopes*) – **bioluminescence**



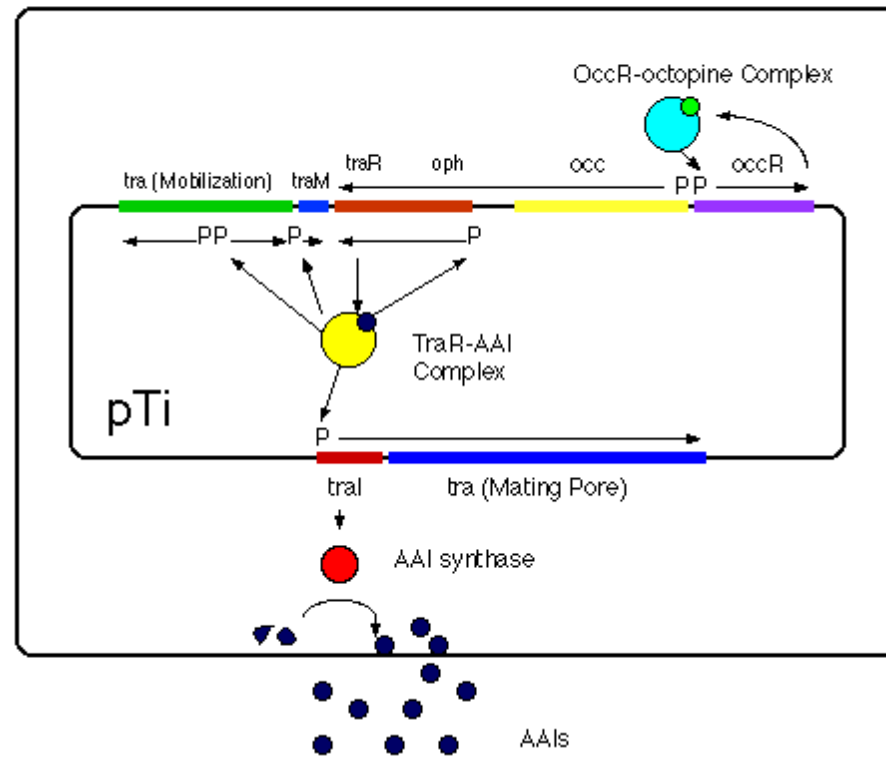
- various mechanisms in Gram-positive and Gram-negative bacteria
- Gram-positive bacteria - oligopeptides - via the ABC transport system
- Gram-negative bacteria - AHL (N-acyl-homoserine-lactone) - *LuxI/LuxR* system





- *quorum sensing*
- *Ralstonia solanacearum*, *Erwinia carotovora*, genera *Xanthomonas*, *Pantoea* – regulation of secretion of cell-wall- degrading enzymes (CWDE)
- *Agrobacterium tumefaciens* – regulation of conjugational transfer of Ti-plasmid – activation of TraR transcription factor

Ti Plasmid



How pathogen attacks a host cell?

- **EFFECTORS**

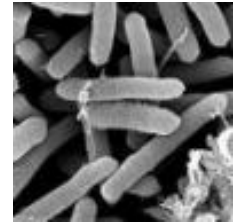
- cell-wall- degrading enzymes (CWDE)

- genus *Erwinia* – pectinases, pectate-liases – *pel* – genes – mutation in single *pel* –genes cannot eliminate symptoms completely, but have an influence on infectivity
- very complex regulation involving a lot of signaling pathways – *quorum sensing*



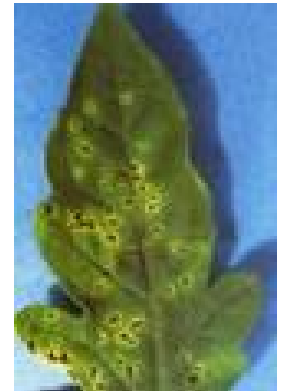
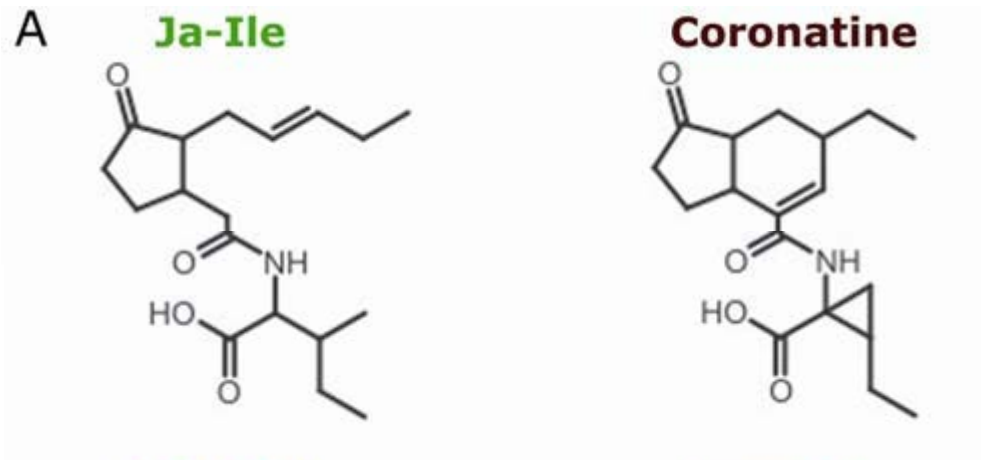
- toxins

- particularly important in *Pseudomonas syringae* – coronatine, tabtoxin, phaseolotoxin, syringomycin...

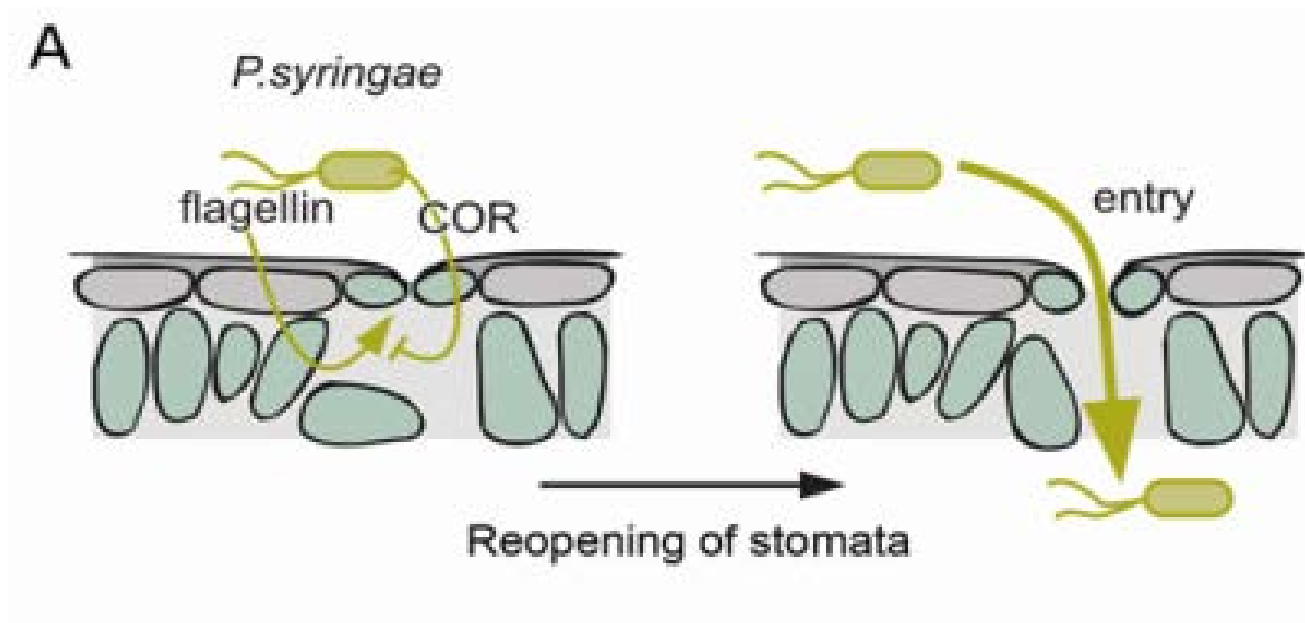


- **coronatine** – molecular mimicry – a molecule mimicking plant Ja-Ile and thus suppressing the induction of plant defense genes

Jasmonoil-
isoleucine;
regulating defense
mechanisms of
plants



- coronatine inhibits closing of the stomata that occur when the plant detects bacterial flagellin



- phytohormones (plant hormones)

- significant in pathogens that cause the uncontrolled proliferation of plant tissues, resulting in the appearance of tumors and nodes
- *Pseudomonas syringae*, *Agrobacterium tumefaciens*, *Pantoea herbicola*
- pathogenesis related mainly with the excessive production of IAA (indole acetic acid)



Pseudomonas syringae

• avirulence factors – type III effectors (T3E)

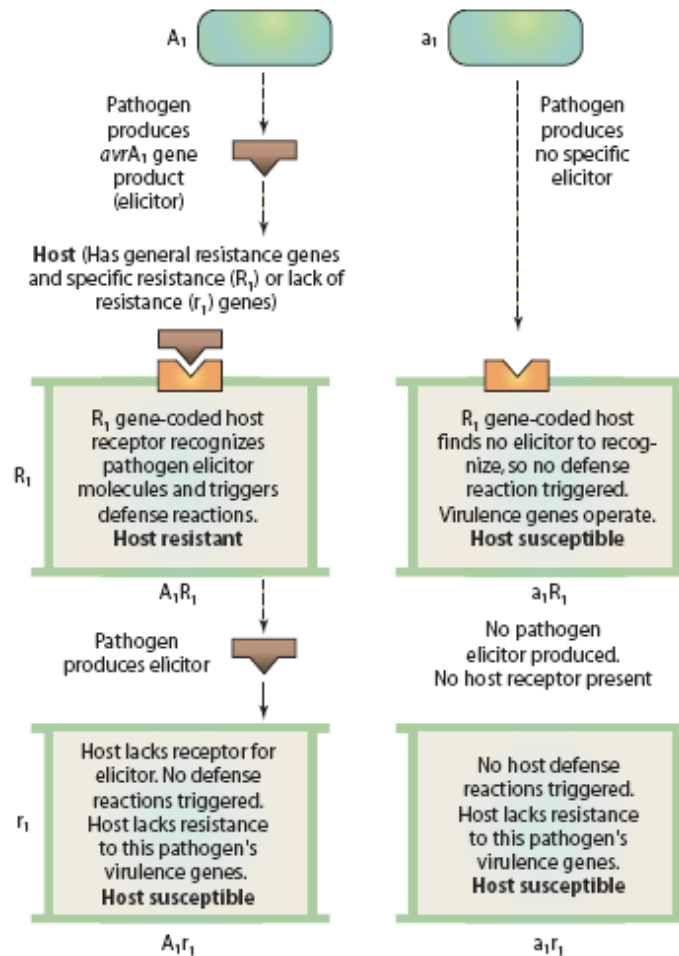
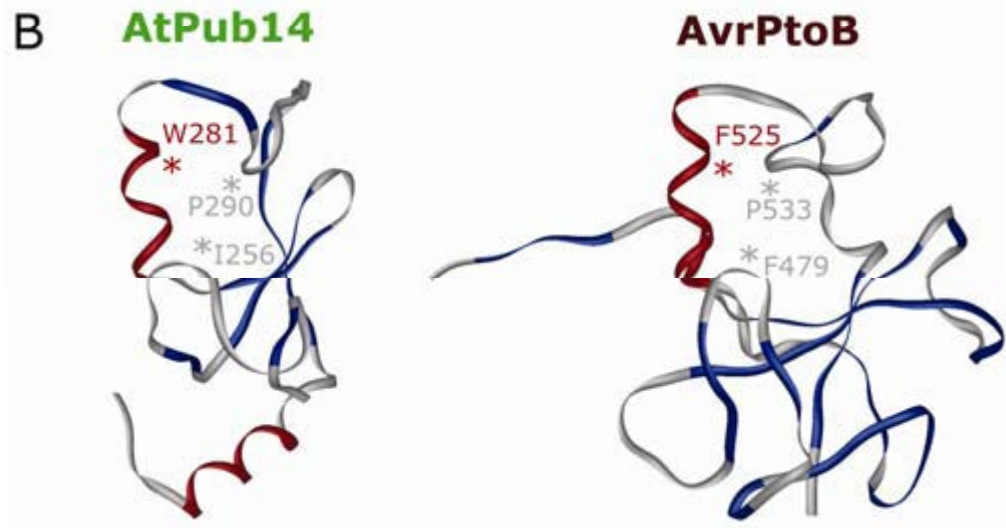


FIGURE 4-11 Basic interactions of pathogen avirulence (A)/virulence (a) genes with host resistance (R)/susceptibility (r) genes in a gene-for-gene relationship and final outcomes of the interactions.

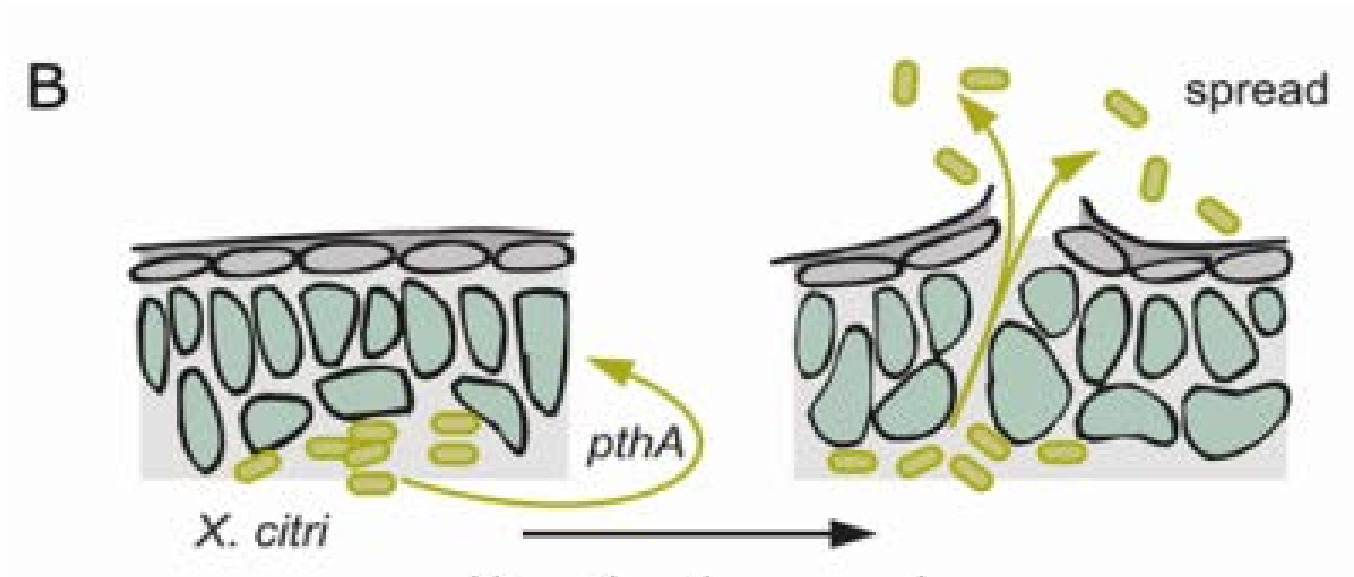
- numerous virulence factors of infectious bacterial species are known
- more than 50 genes for avirulence factors (*avr*) are known in *Pseudomonas syringae* and *Xanthomonas campestris*
- AvrPtoB - mimicry - mimics the E3 ubiquitin ligase that is involved in the degradation of proteins - the inhibition of programmed cell death of the host cell

E3 ubiquitin ligase;
regulation of
protein
degradation in
plants



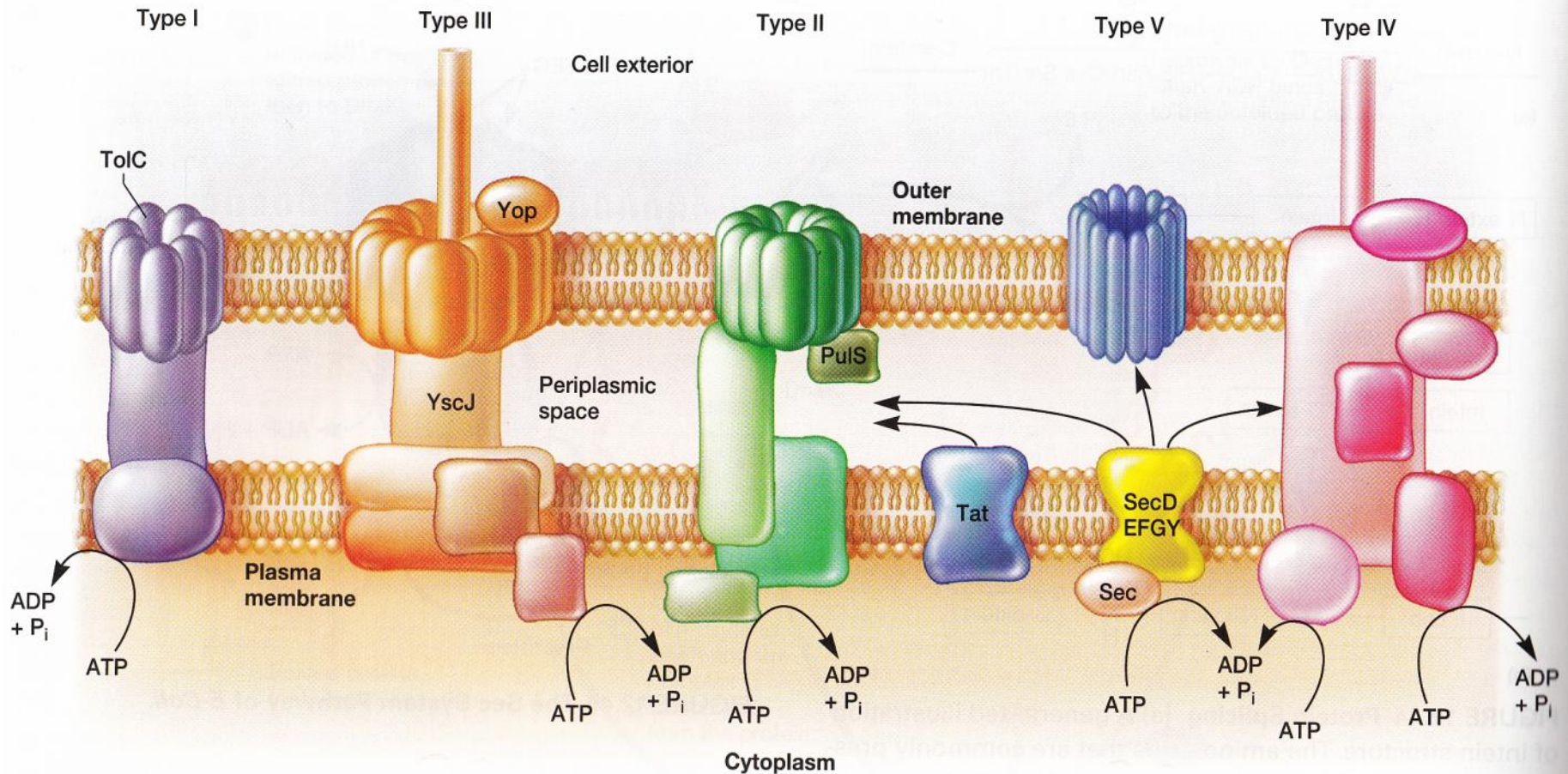
Pseudomonas syringae

- *Xanthomonas citri* - PthA effector (AvrB3) through mimicry of transcription factor induces hypertrophy, hyperplasia and necrosis resulting in the appearance of cracks on the surface of leaves that allows the spread of bacteria



How bacteria deliver their effectors in the host cells?

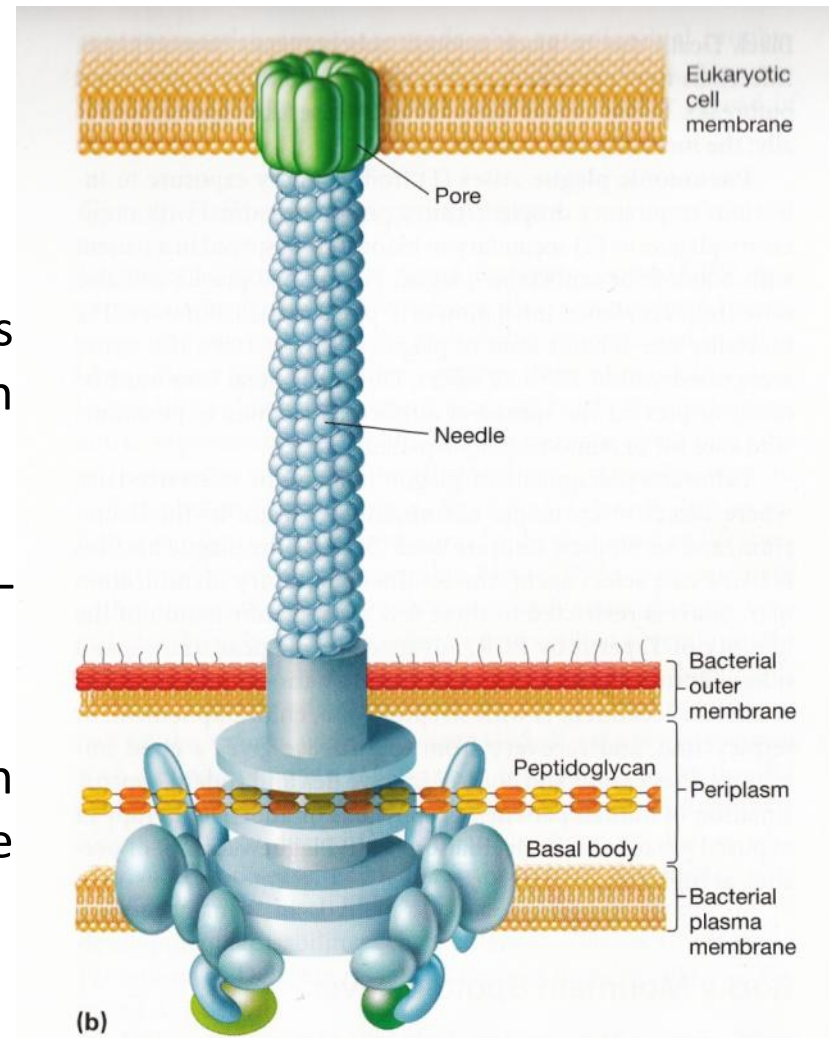
Secretion systems (pathways)



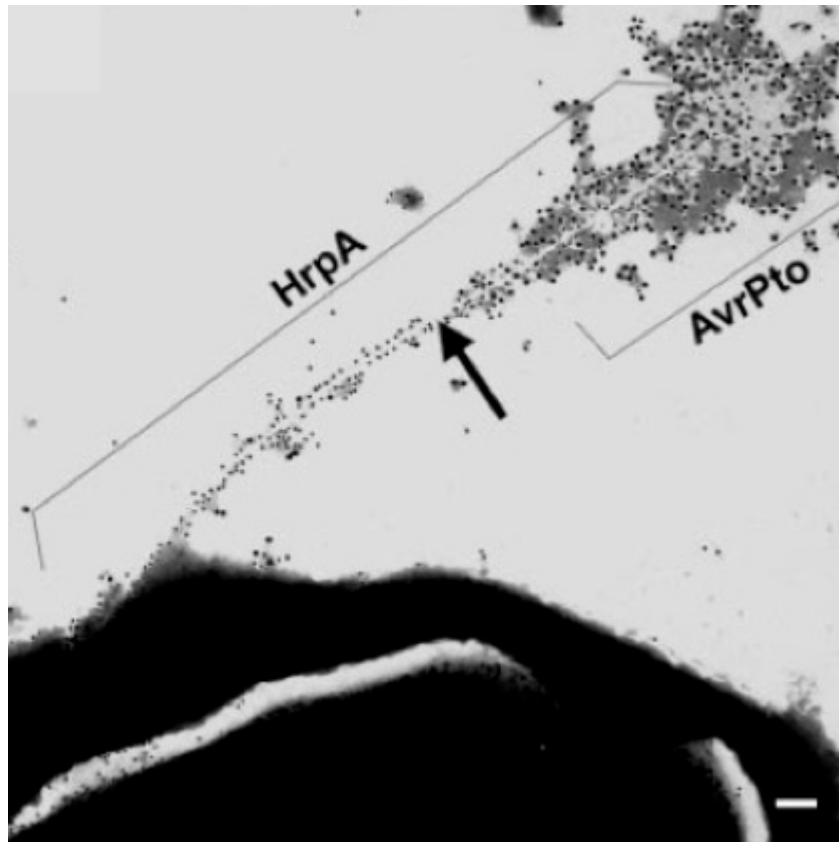
- type I – secretion of proteases - *Erwinia chrysanthemi*
- type II – secretion of pectinases and cellulases– *Erwinia*
- **type III (T3SS; TTSS)** – the most significant in the majority of pathogenic bacteria – many of Avr and Hrp proteins
- type IV – *Agrobacterium tumefaciens* - T-DNA transfer; *Acidovorax citrullii* – pilli of type IV important for pathogenicity

Type III secretion system (TTSS; T3SS)

- conserved among Gram-negative bacteria
- necessary for pathogenicity
- clusters of genes organized in operons present on the bacterial chromosome or on the plasmid
- complex structure – approx. 30 proteins – structural proteins, effectors and chaperons
- “needle” (“injectosome”) through which the bacterial proteins are delivered to the host cells



- the best characterized for *Yersinia pestis* (causative agent of plague) – **Ysc-Yop** virulence apparatus
- homologues i plant pathogens – **Hrp and Hrc proteins**



Mechanisms of genetic variability in bacteria

Pathogenicity islands (PAI)

- genomic regions (clusters) of **10-200 kb** found in **pathogenic Gram-negative bacteria**, carrying virulence and pathogenicity genes (TTSS, effectors – adherence factors, toxins...)
- can be situated on a plasmid, but usually located also on a few loci in the bacterial chromosome – mobile genetic elements containing integrase and transposase genes
- transferred via horizontal gene transfer (HGT), even between different species – conjugation via pilli of type IV

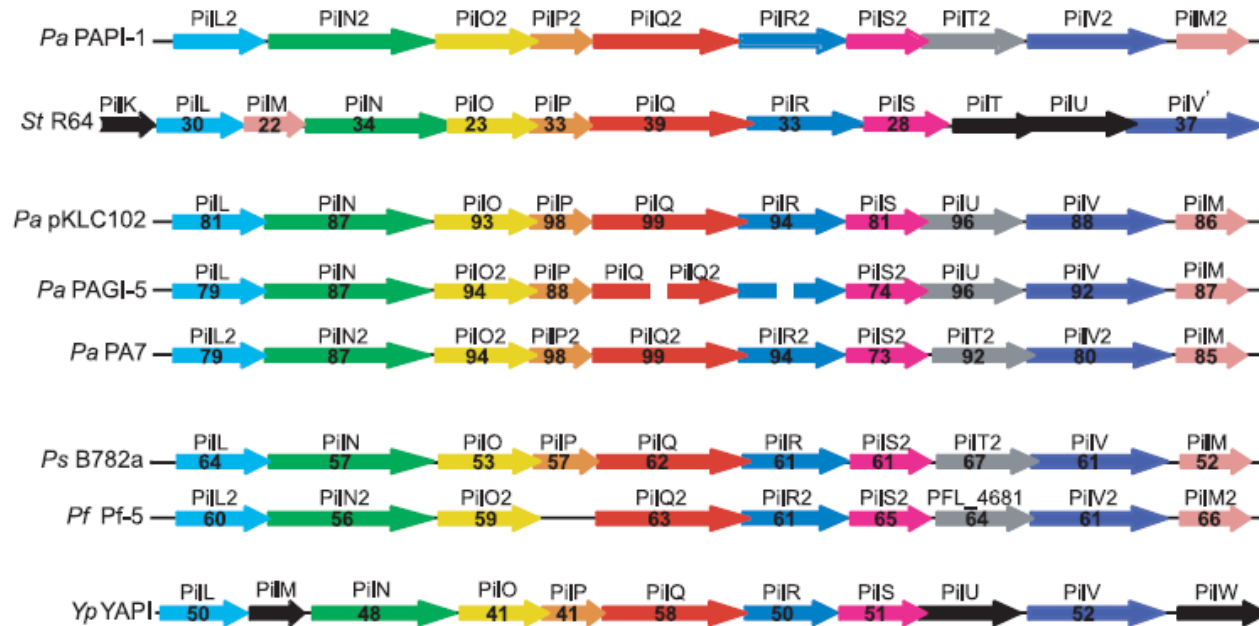
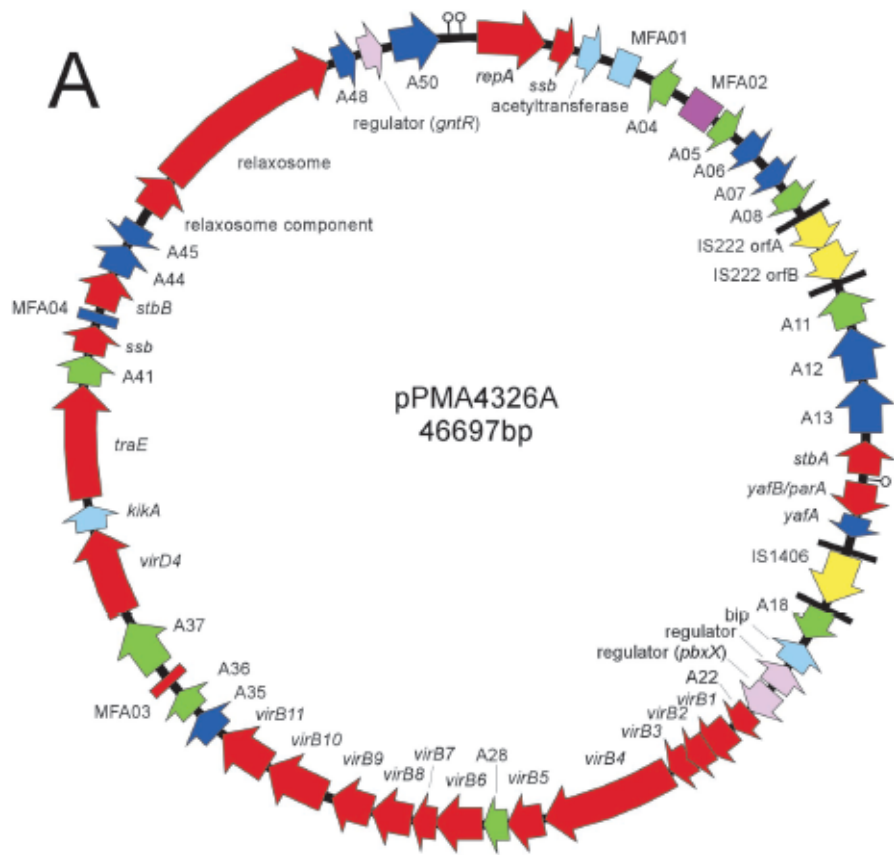


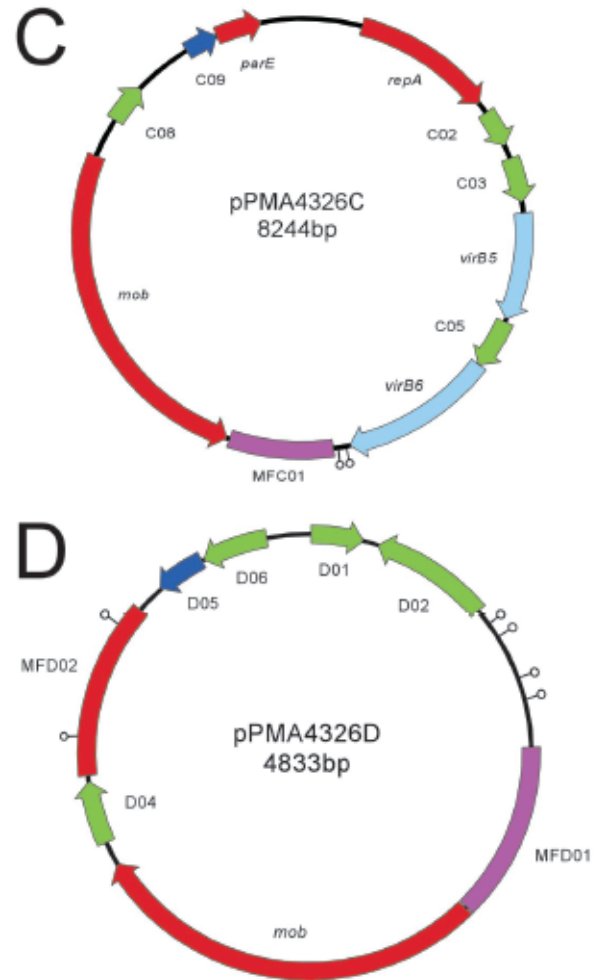
FIG. 1. Conservation of the PAPI-1 *pil* gene clusters in different microorganisms. The putative function of each PAPI-1 pilus protein is presented in Table 2. Homologous proteins in various bacteria are indicated by the same color. The numbers indicate the sequence similarity of each protein with its PAPI-1 homolog. The black arrows represent those genes lacking homologs in the PAPI-1 island. The gaps in PilQ2 and PilR2 of the *P. aeruginosa* (Pa) PAgI-5 represent the disruptions of ORFs by insertions. The *pil* cluster sequences were retrieved from GenBank: PAPI-1, accession no. AY273869; pKLC102, AY257538; PAgI-5, EF611301; R64 from *Salmonella enterica* serovar Typhimurium (*St*), NC_005014; and YAPI from *Yersinia pseudotuberculosis* (*Yp*), AJ627388. The *pil* cluster sequence of *P. aeruginosa* strain PA7, *P. syringae* (*Ps*) strain B782a, and *P. fluorescens* (*Pf*) strain Pf-5 were obtained from the *Pseudomonas* Genome Database (38).

The role of plasmids

- important role in pathogenicity – very often pathogenicity islands
- *Pseudomonas syringae* – TTSS genes – avirulence genes (*avrD*, *avrPphF*, *hrp* geni...), genes for the synthesis of coronatine, resistance to copper...
- *Pseudomonas savastanoi* – genes for IAA and cytokinines biosynthesis
- *Xanthomonas campestris* - avirulence genes (*avrBs1*, *avrBs3*) – type III effectors
- *Ralstonia solanacearum* – *hrp* – genes - type III effectors

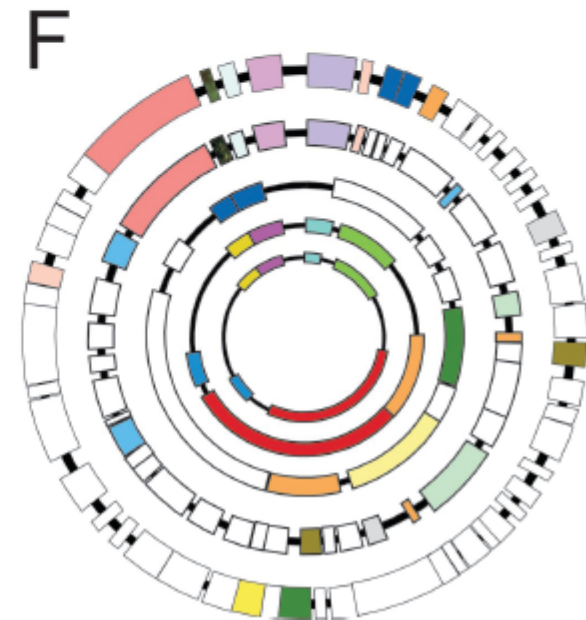
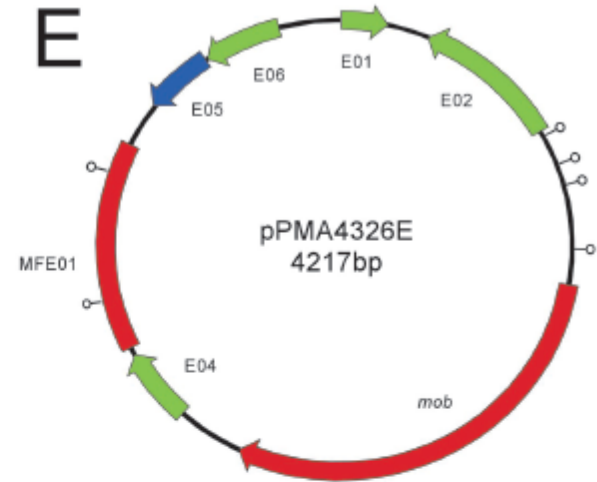
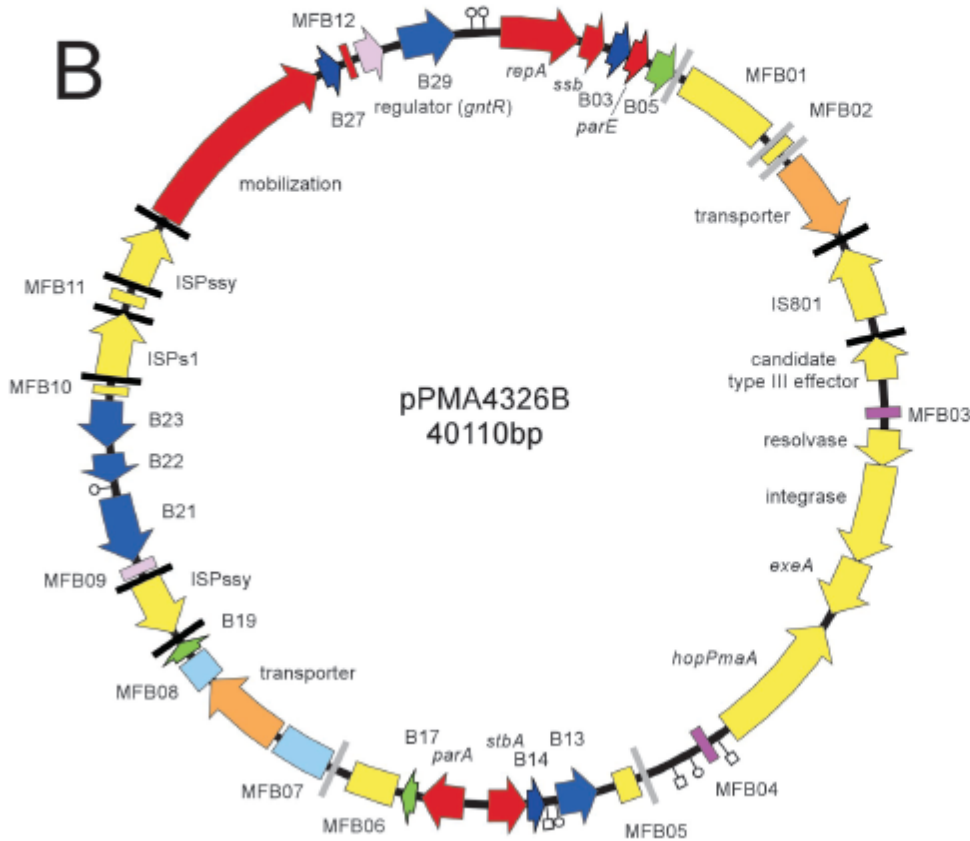


- Maintenance
- Virulence-associated and Mobile Elements
- Transporters
- Regulators
- Conserved Hypothetical Proteins
- New Hypothetical Proteins
- Miscellaneous Proteins
- Other Feature



Pseudomonas syringae

- Virulence-associated and Mobile Elements
- Transporters
- Regulators
- Conserved Hypothetical Proteins
- New Hypothetical Proteins
- Miscellaneous Proteins
- Other Feature



Pseudomonas syringae

Agrobacterium tumefaciens
 -Ti (tumor inducing) – plasmid

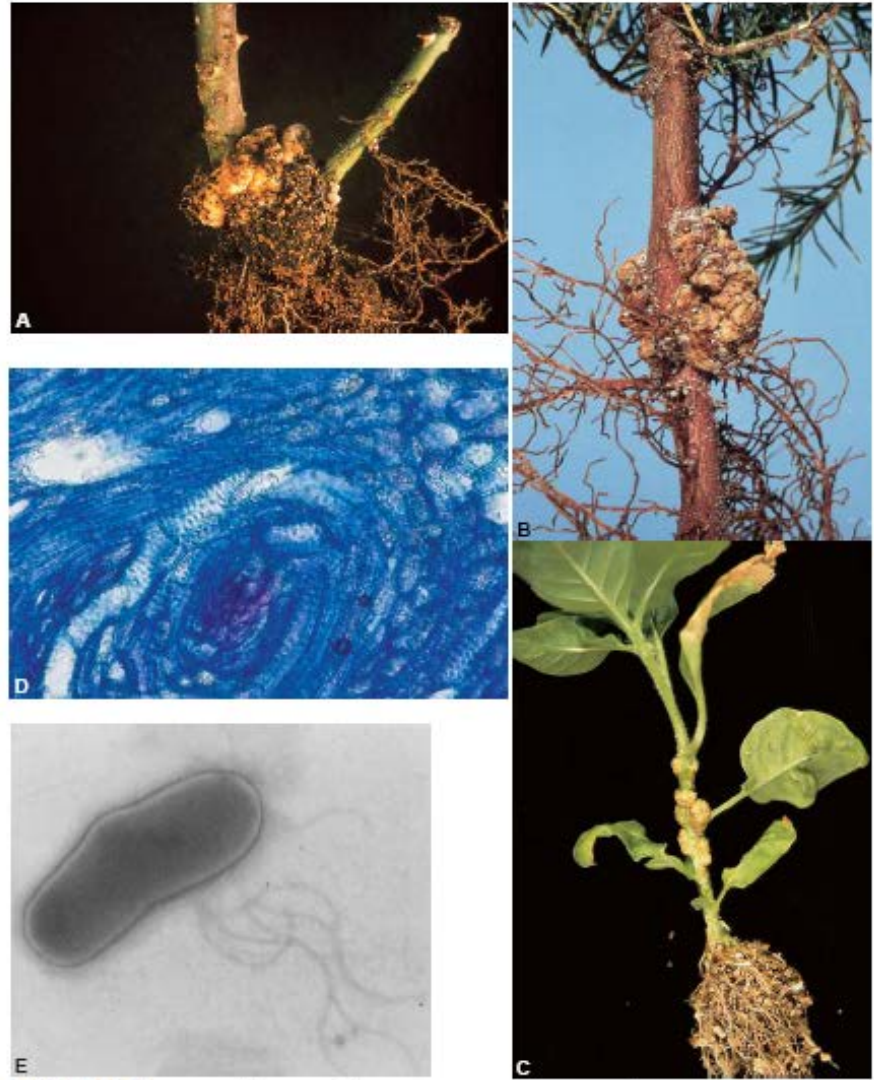
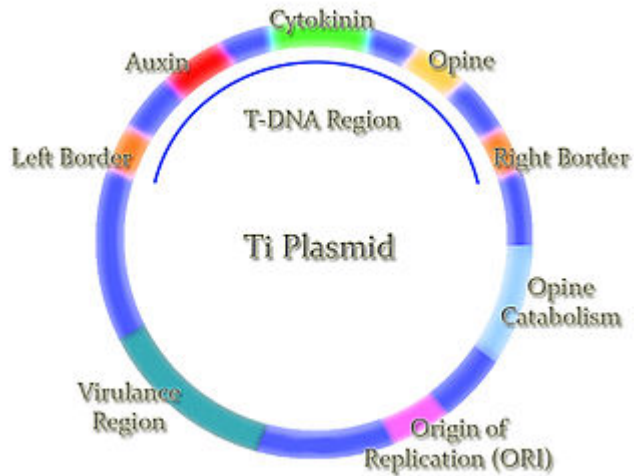
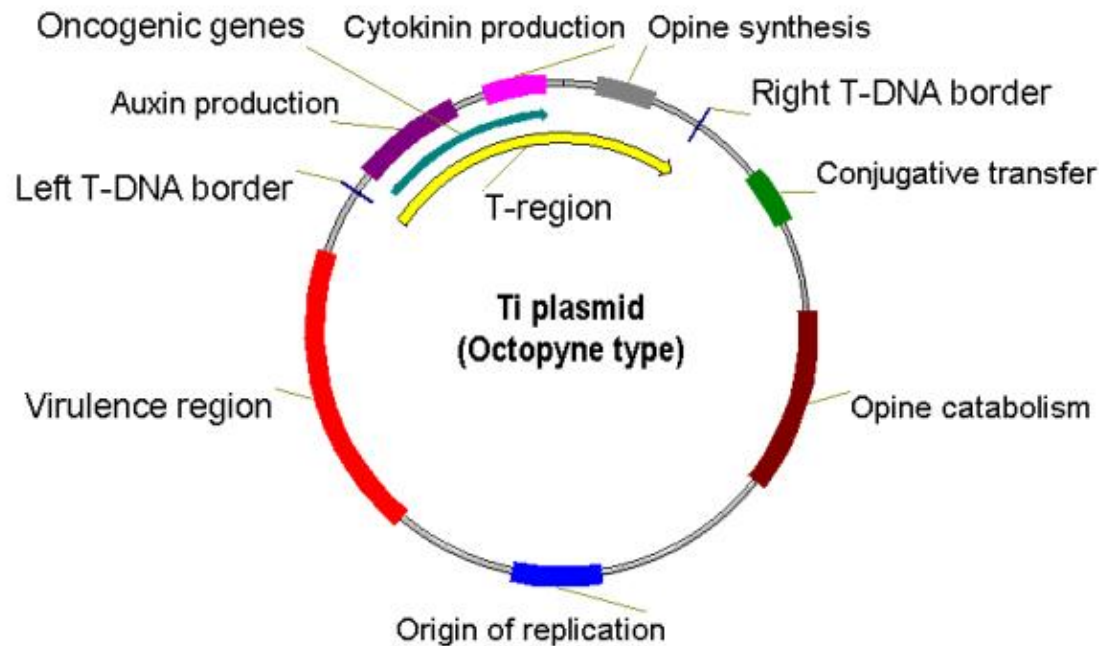
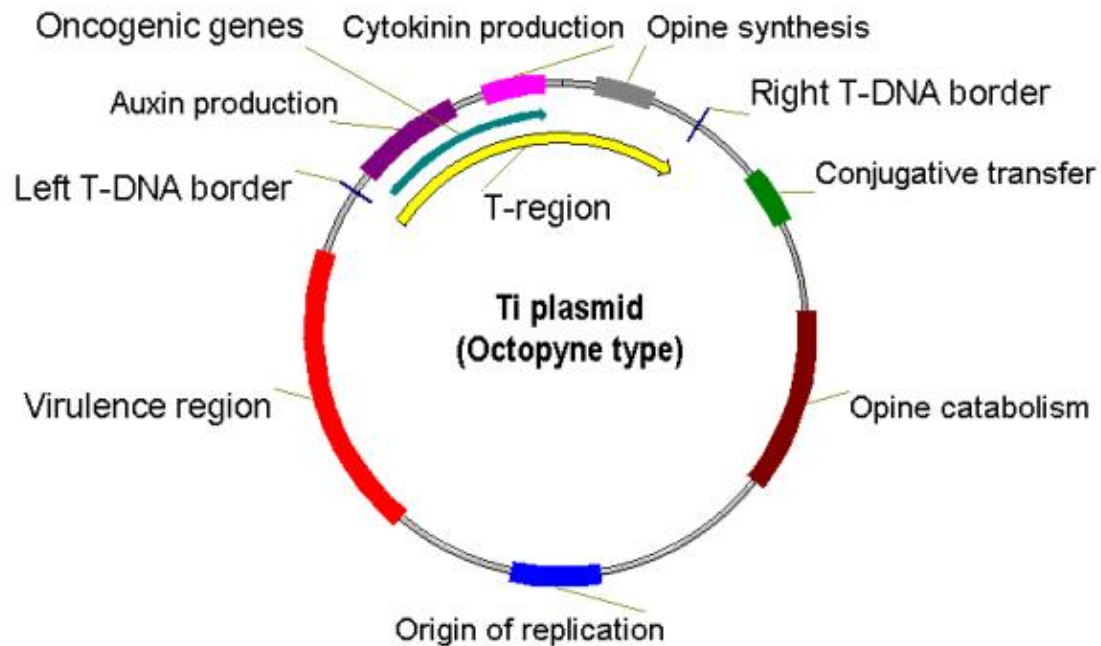


FIGURE 12-35 Crown gall disease caused by *Agrobacterium tumefaciens*. Naturally occurring crown galls on rose (A) and on spruce seedling (B). (C) Artificially induced galls in young tobacco plant by injecting crown gall bacteria into the stem. (D) Abnormal arrangement of tissues within a young crown gall. (E) The crown gall bacterium. [Photographs courtesy of (A) D. R. Cooley, University of Massachusetts, (B) E. L. Barnard, Florida Division of Forestry, and (E) R. E. Wheeler and S. M. Alcorn.]

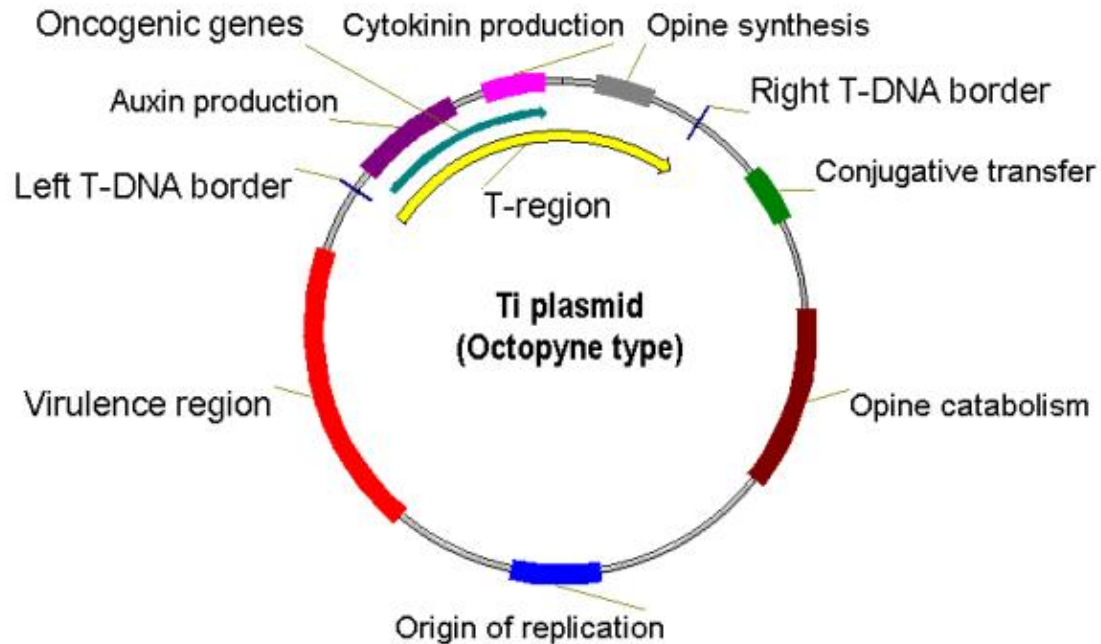
- size – **180-250 kb** – encoding 195 proteins
- virulence region – ***virABCDEFGH*** – approx. 21 *vir*-genes in 6-8 operons
- induction of expression by plant phenolic compounds and monosaccharides from the soil
- **VirA** – membrane receptor – a kinase – phosphorylation of **VirG** – transcription factor activating transcription of other *vir*-genes



- VirD1 and VirD2 – endonucleases that cut the T-DNA that is transferred to a plant cell
- VirE2 – binds the T-DNA and forms a complex similar to ssDNA of bacteriophages
- 11 proteins from *virB* operon – formation of apparatus for T-DNA transfer – pilus - secretion system type IV



- Integration of the T-DNA into the plant genome - gene expression of plant hormones - the uncontrolled proliferation and tumor/gall promotion
- expression of the genes for the synthesis of opines – a source of nutrients for *Agrobacterium*



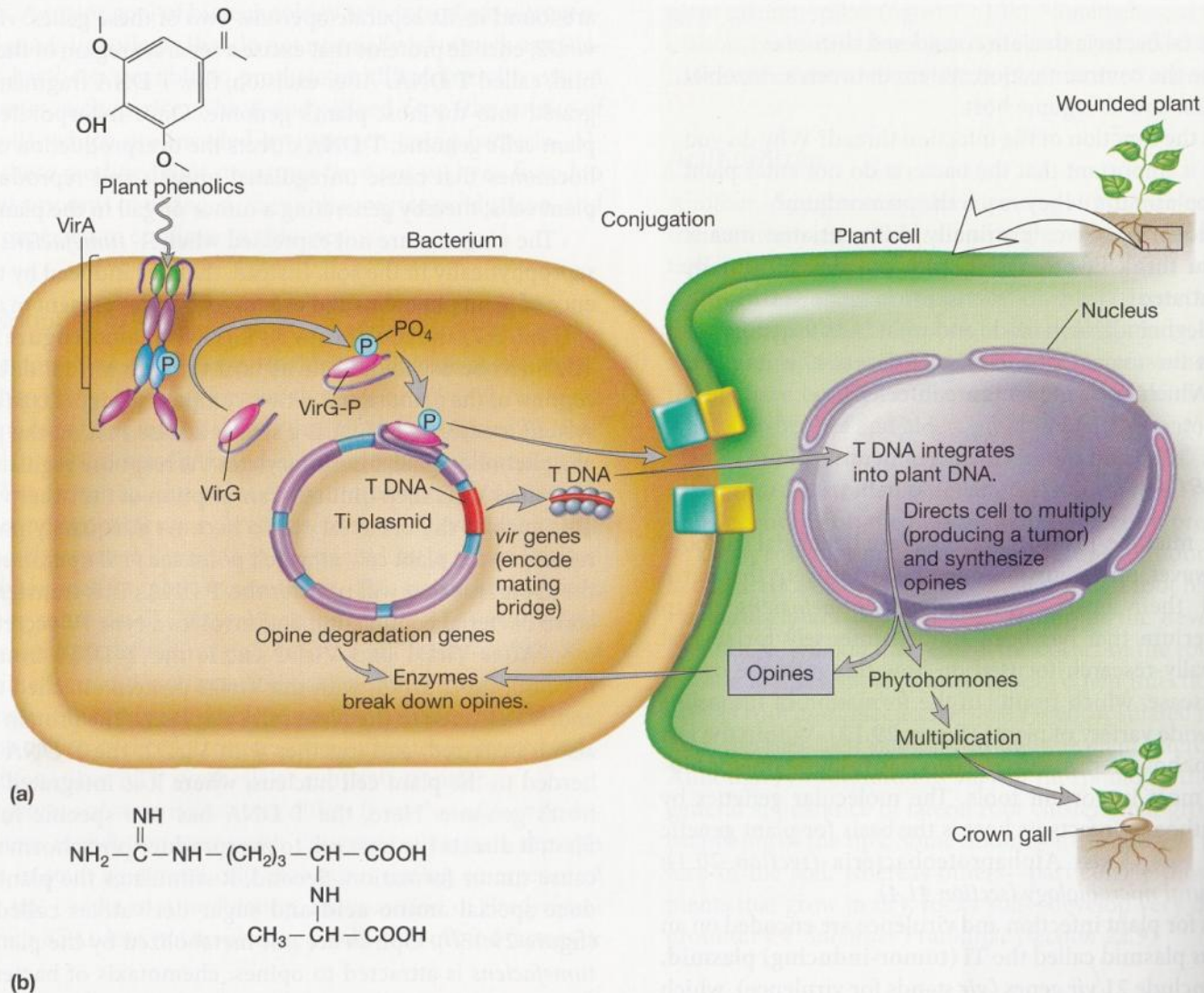
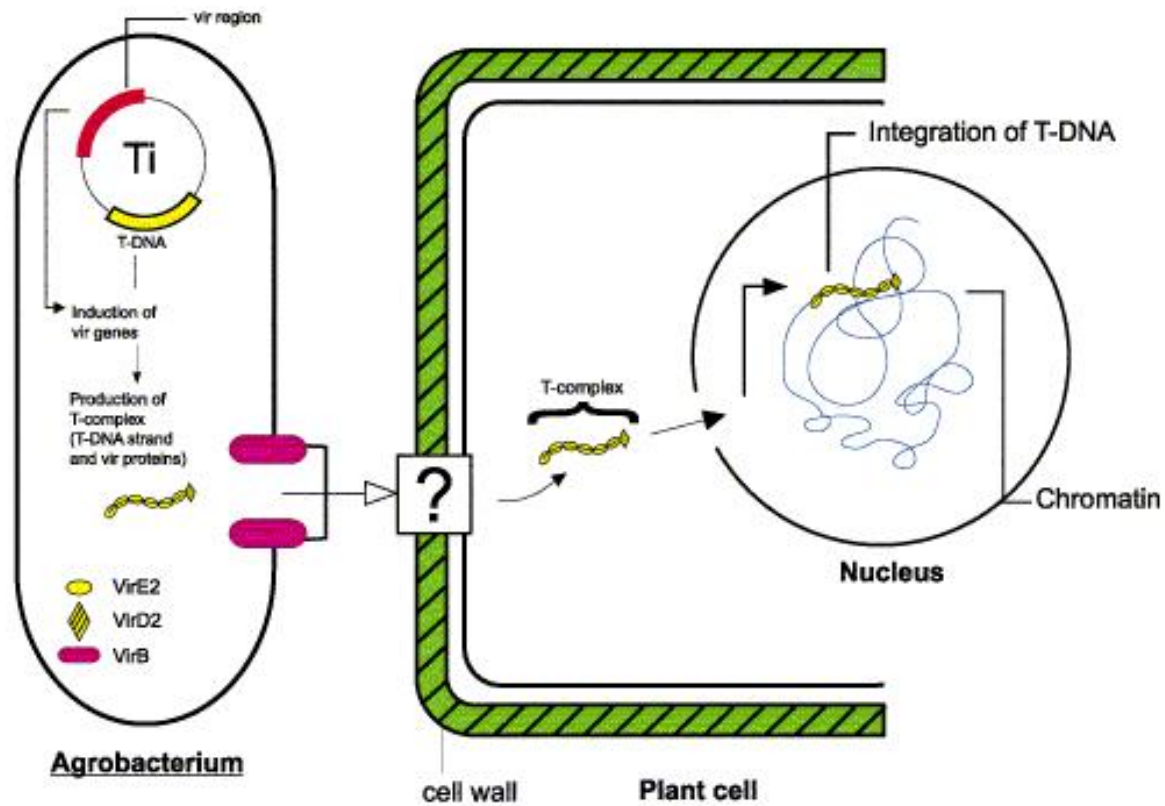


FIGURE 29.13 Functions of Genes Carried on the *Agrobacterium* Ti Plasmid. (a) Genes carried on the Ti plasmid of *Agrobacterium* control tumor formation by a two-component regulatory system that stimulates formation of the mating bridge and excision of the T DNA. The T DNA is moved into the plant cell, where it integrates into the plant DNA. T DNA induces the production of plant hormones that cause the plant cells to divide, producing the tumor. (b) The tumor cells produce opines that can serve as a carbon source for the infecting *Agrobacterium*. Ultimately a crown gall is formed on the stem of the wounded plant above the soil surface.



T-DNA transfer into the Plant's Genome

Adapted from Zupan et al 2000

Agrobacterium tumefaciens – “natural genetic engineer”
 - the main tool in genetic engineering of plants

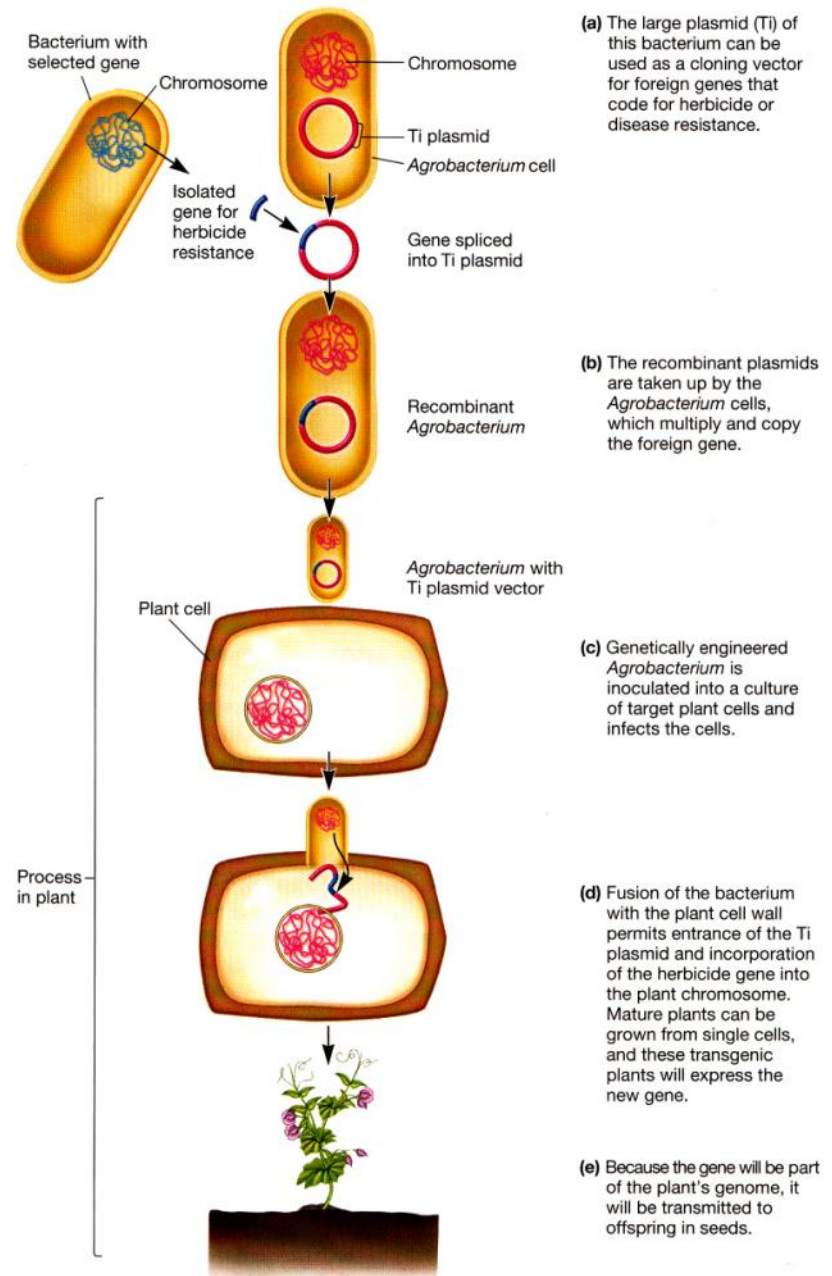


FIGURE 41.10 Bioengineering of Plants. Most techniques employ a genetically modified strain of the natural tumor-producing bacterium called *Agrobacterium tumefaciens*.