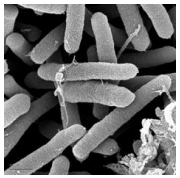
Practical environmental biotechnology

Lecture 03

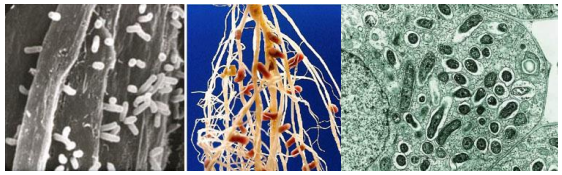
Agriculture biotechnology

Protection of plants from frost damage -symbiotic bacteria

Pseudomonas syringae, P. fluorescens



Nitrogen-fixing bacteria to improve crop yields



Klebsiella pneumoniae - wheat, discovery of genes involved in nitrogen fixation in early 1970s

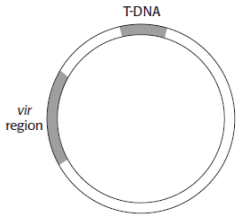
Rhizobium: Sinorhizobium, *Mesorhizobium*, *Bradyrhizobium* (*Leguminosae*, legume), *Rhizobium leguminosarum* – Egypt, rice.

Expand the host-range – *nod*D, *nod*H, *nod*Q bacterial genes responsible for communication with host.

Azospirillum – sugar cane.

Transgenic plants

Agrobacterium tumefaciens – Megaplasmid Ti



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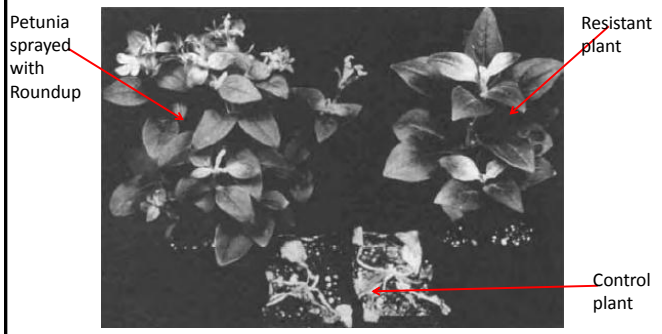
Direct introduction of cloned genes into plants

Incubation of plant protoplasts with DNA.

Introduction of DNA into protoplasts by electroporation.

Bombardment of plant cells with DNA-coated microprojectiles.

Herbicide-Resistant Plants



Glyphosate-resistant plants (Roundup Ready, Monsanto)

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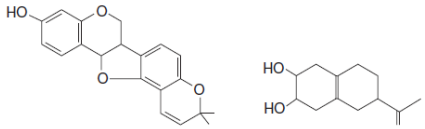
Crop resistant to:

- Insects
- Viruses – viral coat proteins, papaya ringspot virus
- Fungi and bacteria



phytoalexins

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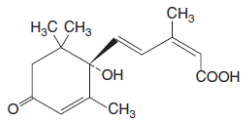
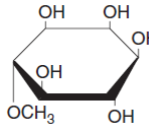
Stress resistant crops

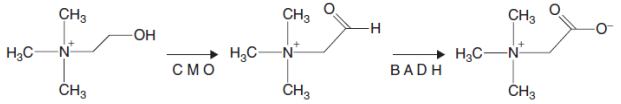
Abscisic acid (ABA)



D-ononitol



Biosynthesis of glycine betaine



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Golden rice

Contains *β*-carotene, the precursor of vitamin A

Three genes, coding for phytoene synthase, phytoene desaturase, and lycopene *β*-cyclase;
Agrobacterium Ti system

Bacillus thuringiensis

Gram-positive soil bacterium.
Saprophytic metabolism - digesting organic matter derived from dead organisms.
Parasitic metabolism - colonization within living insects.

Strains, six pathotypes:
1. lepidopteran-specific (e.g., var. *berliner*);
2. dipteran-specific (e.g., var. *israelensis*);
3. coleopteran-specific (e.g., var. *tenebrionis*);
4. both Lepidoptera and Diptera (e.g., var. *aizawai*);
5. Lepidoptera and Coleoptera (var. *thuringiensis*);
6. no known toxicity in insects (e.g., var. *dakota*).

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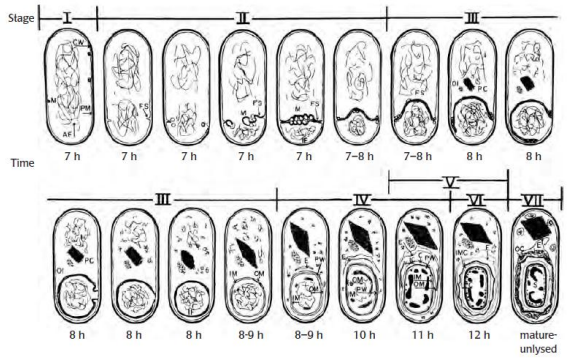
Sporulation of *Bacillus thuringiensis*



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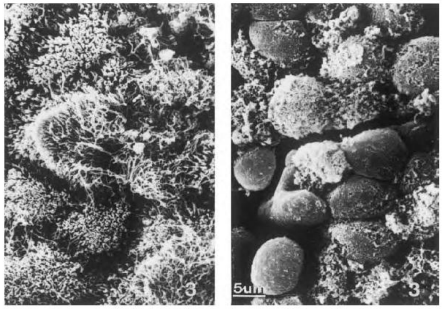
Mechanism of action of *Bacillus thuringiensis*

Endotoxin
alkaline midgut
↓
Prototoxin
proteases
↓
Active toxin



white butterfly (*Pieris brassicae*) larva

healthy midgut epithelium

after endotoxin ingestion (disappearance of microvilli)

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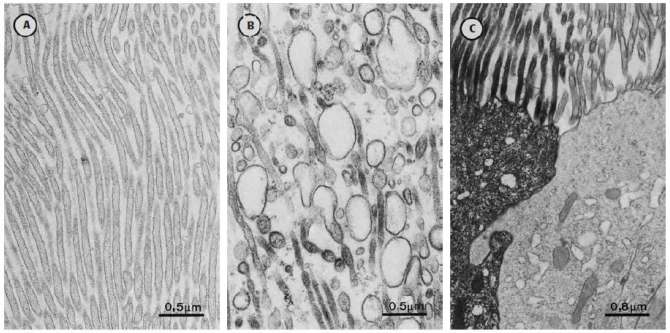
Damage of midgut epithelium



Intact

10 min after endotoxin ingestion

cells become permeable to the indicator stain

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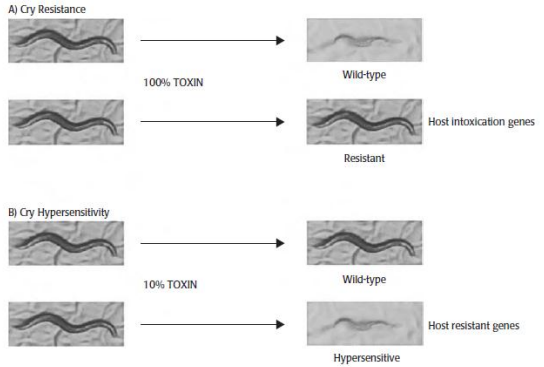
Resistance of *Caenorhabditis elegans to* Cry toxins



Resistance: lack of receptors (glycolipids) of Cry toxin

Insects sensitive to Bt toxin

TABLE 7.1 Main target insect pests of corn, cotton, and potatoes, susceptible to particular *B. thuringiensis* Cry toxins

Crop	Common name of pest	Scientific name of pest
Corn	Black cutworm	*Agrotis ipsilon* (Hufnagel)
(Zea mays)	Corn earworm	*Helicoverpa zea* (Boddie)
Cotton	Common stalk borer	*Papaipema nebris* (Guen.)
(Gossypium hirsutum)	European corn borer	*Ostrinia nubilalis* (Huebner)
	Fall armyworm	*Spodoptera frugiperda* (J. E. Smith)
Potato	Southern corn stalk borer	*Diatraea crambidoides* (Grote)
(Solanum tuberosum)	Southwestern corn borer	*Diatraea grandiosella* (Dyar)
	Cotton bollworm	*Helicoverpa zea* (Boddie)
	Pink bollworm	*Pectinophora gossypiella* (Saunders)
	Tobacco budworm	*Heliothis virescens* (Fabricius)
	Colorado potato beetle	*Leptinotarsa decemlineata* (Say)

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Bt crops

TABLE 7.2 Some crops for use in human food and animal feed that express *B. thuringiensis (Bt)* insecticidal proteins

Crop	Protein	Source	Intended effect
Cotton	Cry1Ac	*Bt* subsp. *kurstaki*	Resistance to cotton bollworm, pink bollworm, tobacco budworm, and European corn borer
Cotton	Cry1Ab	*Bt* subsp. *kurstaki*	Resistance to European corn borer
Cotton	Cry2Ab and Cry1Ac	*Bt* subsp. *kumamotoensis*	Resistance to lepidopteran insects
Corn[a]	Cry9C	*Bt* subsp. *tolworthi*	Resistance to certain lepidopteran insects
Corn	Cry1F	*Bt* subsp. *aizawai*	Resistance to certain lepidopteran insects
Corn	Cry3Bb1	*Bt* subsp. *kumamotoensis*	Resistance to coleopteran insects, including corn rootworm
Corn	Cry34Ab1 and Cry35Ab1	*Bt* strain PS149B1	Resistance to coleopteran insects
Potato	Cry3A	*Bt* subsp. *tenebrionis*	Resistance to Colorado potato beetle

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Benefit and risk assessment of *Bt* crops

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Biological control, BC

Use of an organism to reduce the population density of another organism and thus includes the control of animals, weeds and diseases.

Against animals - study and uses of parasites, predators and pathogens for the regulation of host (pest) densities.

BC reduces rather than eradicates pests - natural enemy remain in the agro-ecosystem at low densities.

BC Agents (BCA):
„Macrobial": insects, mites.
„Microbial": viruses, bacteria, fungi

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Three main techniques of BC

Classical („natural", „inoculative"), ladybird *Rodolia cardinalis* against *Icerya purchasi*
Parasitoid (wasp) *Aphelinus mali* angainst aphid *Eriosoma lanigerum*

Augmentative (mass scale, comercial)
Parasytoid (wasp) *Trichogramma* against Lepidoptera (cotton bollworm *Heliothis virescens*, sugar cane borer *Diatraea saccharalis*, European corn borer *Ostrinia nubilalis*)
Other „pests": whiteflies, leafminers, thrips, aphids and mites.

„Conservation control" - use of indigenous predators and parasitoids, usually against native pests.

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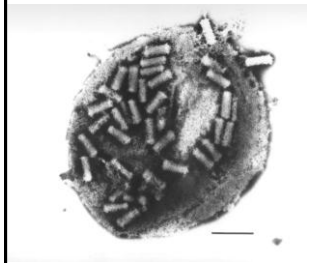
Advantages and limitations of

Agains – slow action
Pro – specificity

	chemical control	biological control
number of 'ingredients' tested	>3.5 million	3000
success ratio	1 : 200 000	1 : 20
developmental costs	180 million US$	2 million US$
developmental time	10 years	10 years
benefit per unit of money invested	2.5–5	30
risk of resistance	large	nil/small
specificity	low	high
harmful side effects	many	nil/few

Viruses, family Baculoviridae



Genus: Nucleopolyhedrovirus (PV), either single (SNPV) or mutliple (MNPV) nucleocapsid per envelope, eg. Autographa californica nucleopolyhedrovirus, AgMNPV;
Enveloped virions are further occluded in polyhedrin matrix.

AgMNPV in Brazil – BC of soya beans

Genus: Granulovirus, GV, one nucleocapsid per envelope, eg. Cydia pomonella granulovirus, CpGV.
Enveloped virions are further occluded in granulin matrix.

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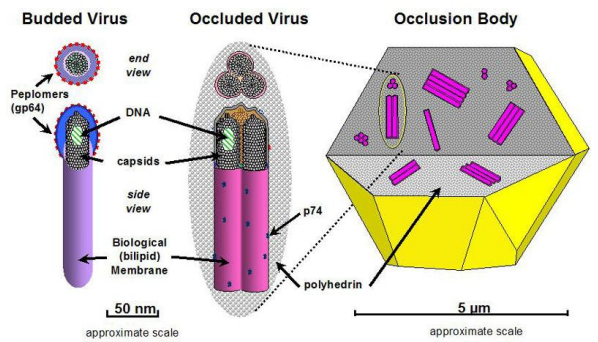
Structure of *Autographa californica* (AgMNPV)



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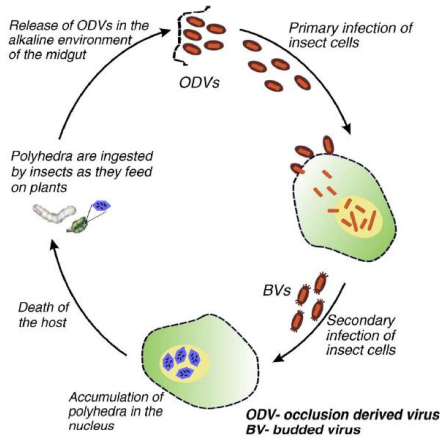
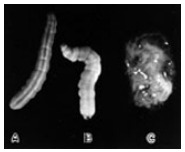
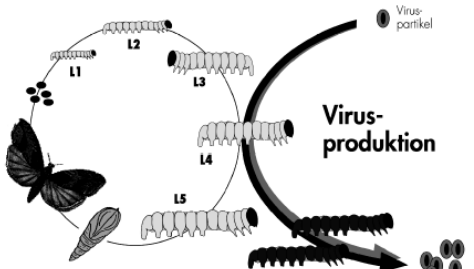
Cycle of AcMNPV infection





Production of CpGV, MADEX



Cydia pomonella after infection with CpGV

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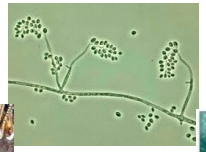
Entomopathogenic fungi

Fungi (BCA): Beauveria, Metarhizium, Nomuraea, Aspergillus, Verticillium, Paecilomyces, Isaria, Fusarium, Cordyceps, Entomophthora

Hosts: Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera, Homoptera, Diptera

Infected grasshoopers



Beauveria bassiana

Infected Colorado beetles

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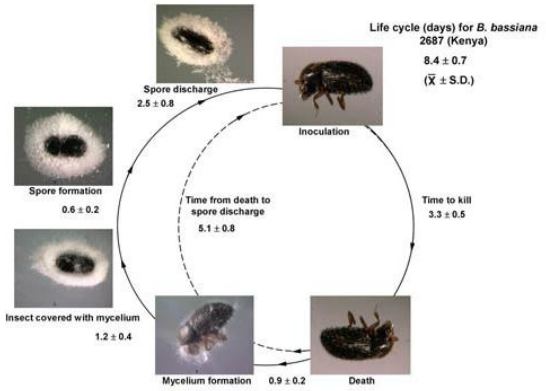
Life cycle of *Beauveria bassiana*



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Application of *Beauveria bassiana*

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Metarhizium anisopliae

Locust

Rice covered with fungi spores

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Nematophagous fungi

Mode of infection: nematode-trapping, endoparasitic, egg and female-parasitic, toxin-producing

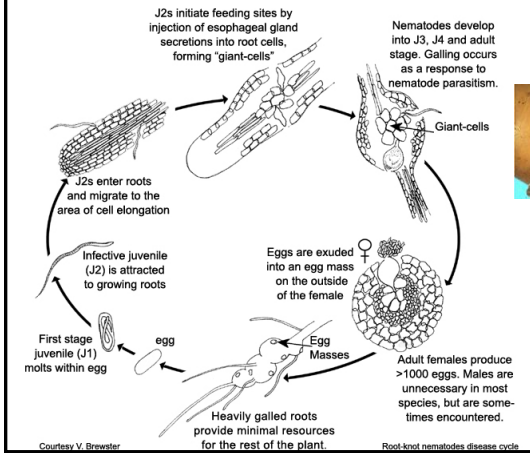
Structure of the traps (formed from vegetative hyphae): adhesive nets, adhesive knobs, adhesive branches, and constricting rings;

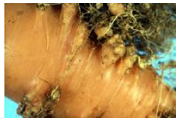
Endoparasites do not form trapping organs, but use their spores to infect.

Little host specificity, can also live at varying degrees of saprophytism in soil.

The largest group of antagonists against plant-parasitic nematodes.

Herbivorous nematodes - eg. genus *Meloidogyne*





Constricting rings

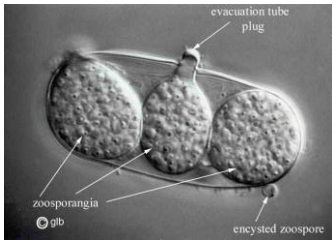
Arthrobotrys dactyloides, A. brochopaga

Adhesive hyphae

Arthrobotrys oligospora, Meria spp. *Hirsutella rhossiliensis*.

Infecting spores

Cysts of *Catenaria anguillulae* in *Xiphinema americanum*

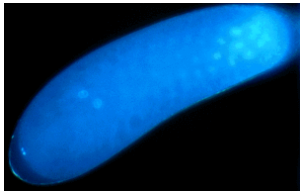


Nematodes eggs infecetd with spores, eg.: *Paecilomyces lilacinous, Dactylella oviparasitica*

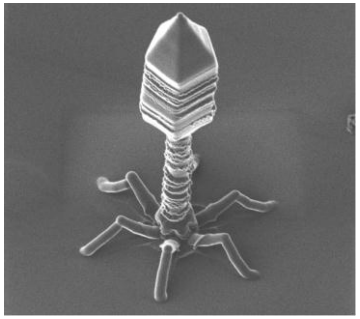
Highly specific endoparasites

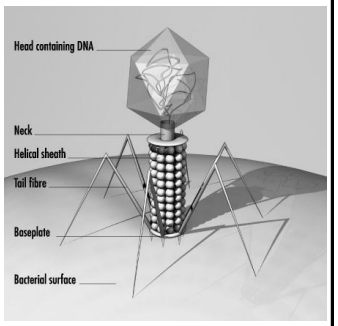
Nematophthora, Nematoctonus

Endosymbionts - *Wolbachia*

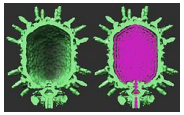


Bakteriophages





Bacterial pathogens of crops

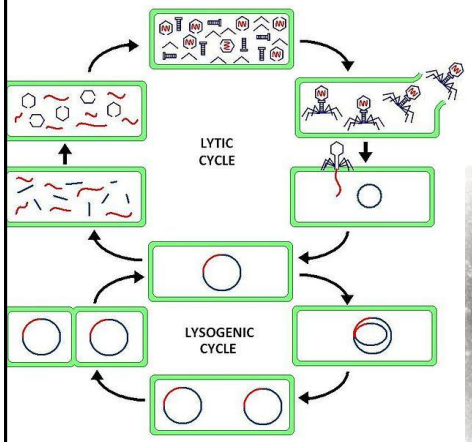


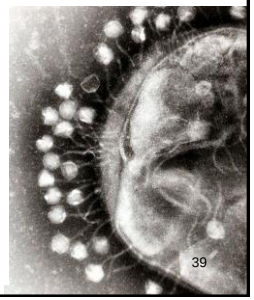
Xanthomonas campestri infecting tomato

Bacterial pathogens of crops

Host	Disease	Pathogen
Cabbage	black rot	*Xanthomonas campestris pv. campestris*
Calla Lily	bacterial soft rot	*Erwinia carotovora subsp. carotovora*
Citrus	citrus canker	*Xanthomonas citri subsp. citri*
Citrus	citrus bacterial spot	*Xanthomonas fuscans* subsp. *citrumelonis*
Mungbean	bacterial leaf spot	*Xanthomonas axonopodis pv. vignaeradiatae*
Mushroom	bacterial blotch	*Pseudomonas tolaasii*
Onion	xanthomonas leaf blight	*Xanthomonas axonopodis pv. allii*
Pepper	bacterial spot	*Xanthomonas campestris pv. vesicatoria*
Pomefruits	fireblight	*Erwinia amylovora*
Potato	potato scab	*Streptomyces scabies*
Stonefruits	bacterial spot	*Xanthomonas arboricola pv. pruni*
Tobacco	bacterial wilt	*Ralstonia solanacearum*
Tomato	bacterial spot	*Xanthomonas campestris pv. vesicatoria*
Tomato	crown gall	*Agrobacterium tumefaciens*
Walnut	walnut blight	*Xanthomonas campestris pv. juglandis*

Mode of action





Resistance of bacteria to phages

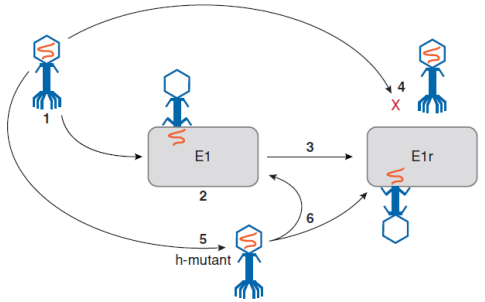


Figure 1

Development of phage-resistant bacterial strains and host-range mutant phages (h-mutant) in nature. 1, wild-type phage; 2, wild-type, specific bacterial host (E1); 3, 1 per 10^6 mutate to phage-resistance (E1r); 4, wild-type phage cannot kill phage-resistant mutant (E1r); 5, 1 per 10^7 to 10^9 spontaneously mutate to h-mutant; h = host-range (extended host range); 6, h-mutant kills both wild-type parent bacteria and phage-resistant mutants.

Phage-propagating by nonpathogenic bacterial strains


Erwinia amylovora, fire blight, (Rosaceae)

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Plant diseases caused by soil pathogens



Gaeumannomyces graminis, *Fusarium oxysporum,* *Ralstonia solanacearum*

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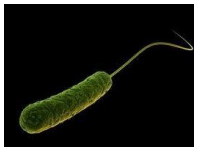
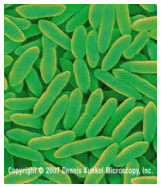
Pseudomonas – produce pseudobactin







Pseudomonas aeruginosa, *P. putida*, *P. fluorescens, P. syringae*; Cedomon; BioAgri

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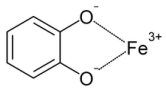
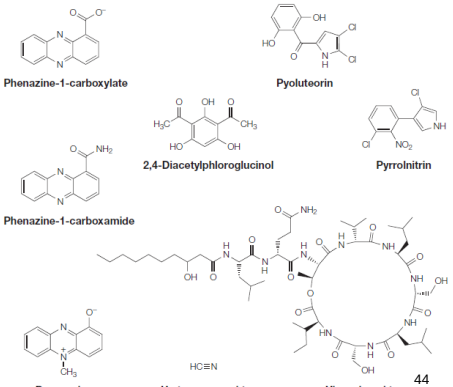
Antybiotic produced by *Pseudomonas*

Fluorescent pigment: pseudobactin, siderophore





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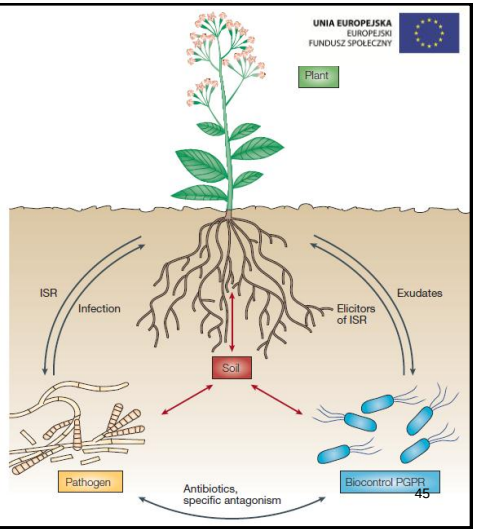
Interactions between biocontrol plant growth-promoting rhizobacteria (PGPR), plants, pathogens and soil. These elements interact with one another through biotic and abiotic signals, many of which are still unknown.

ISR, induced systemic resistance.



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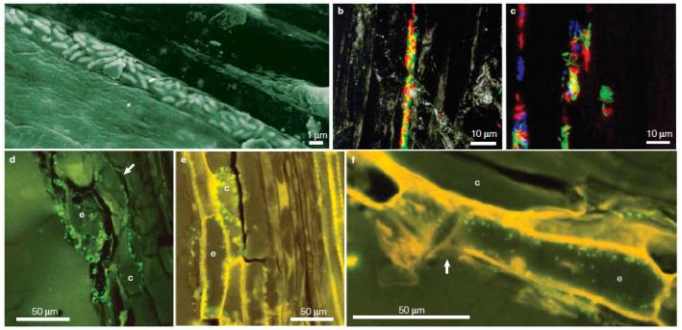
Root colonization by fluorescent pseudomonads



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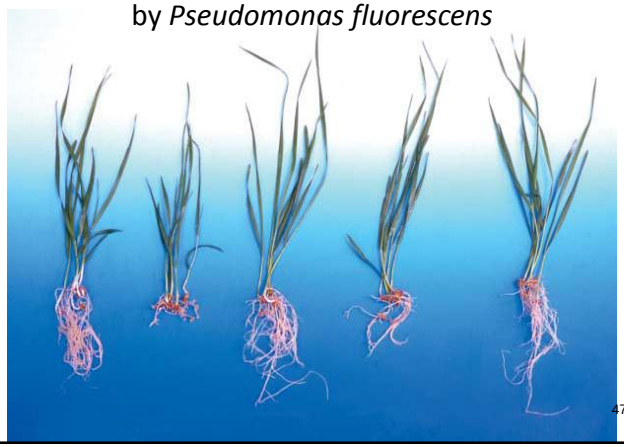
Protection of wheat from *Pythium ultimum* by *Pseudomonas fluorescens*



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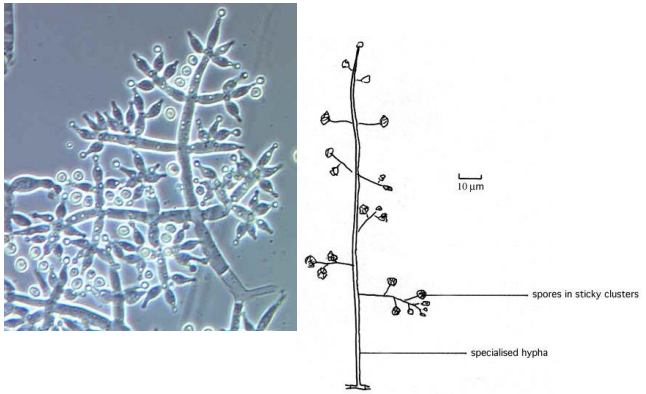
Trichoderma



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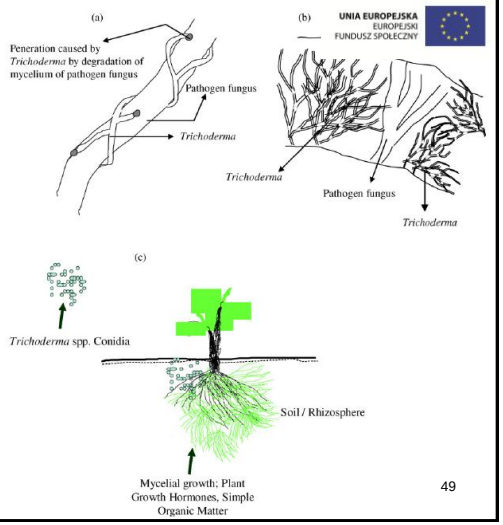
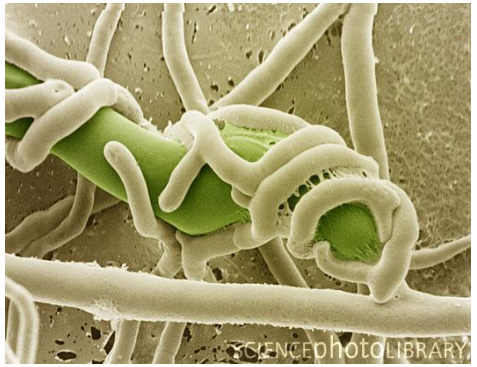
Trichoderma – interaction with plants

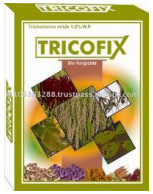


Indirect:
a. mycoparasitism,
b. competition;
Direct:
c. mycelial growth around plant rhizosphere and production of metabolites

Pathogenic fungi *Rhizoctonia sp* *Trichoderma*



Products



Trichoderma viride

Trichoderma harzianum

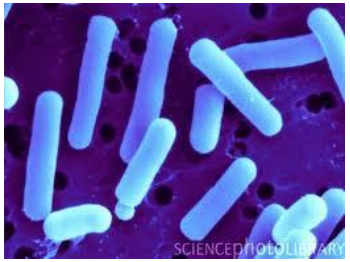
Aureobasidium pullulans

Probiotic applied in agriculture

Table 1. Currently approved microorganisms for livestock feed application[1]

Aspergillus niger	*Kluyveromyces marxianus*
Aspergillus oryzae	*Lactobacillus acidophilus*
Bacillus coagulans	*Lactobacillus brevis*
Bacillus lentus	*Lactobacillus buchneri* (cattle only)
Bacillus licheniformis	*Lactobacillus bulgaricus*
Bacillus pumilus	*Lactobacillus casei*
Bacillus subtilis	*Lactobacillus cellobiosus*
Bacteroides amylophilus	*Lactobacillus curvatus*
Bacteroides capillosus	*Lactobacillus delbrueckii*
Bacteroides ruminicola	*Lactobacillus farciminis* (swine only)
Bacteroides suis	*Lactobacillus fermentum*
Bifidobacterium adolescentis	*Lactobacillus helveticus*
Bifidobacterium animalis	*Lactobacillus lactis*
Bifidobacterium bifidum	*Lactobacillus plantarum*
Bifidobacterium infantis	*Lactobacillus reuterii*
Bifidobacterium longum	*Leuconostoc mesenteroides*
Bifidobacterium thermophilum	*Pediococcus acidilacticii*
*Enterococcus cremoris**	*Pediococcus cerevisiae (damnosus)*
*Enterococcus diacetylactis**	*Pediococcus pentosaceus*
*Enterococcus faecium**	*Propionibacterium freudenreichii*
*Enterococcus intermedius**	*Propionibacterium acidipropionici* (cattle only)
*Enterococcus lactis**	*Propionibacterium shermanii*
*Enterococcus thermophilus**	*Saccharomyces cerevisiae*

Chemical inhibition



Lactobacillus reuteri

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Competitive exclusion

Lactobacillus acidophilus against *Escherichia coli, Yersinia pseudotuberculosis, Salmonella enterica* serovar Typhimurium

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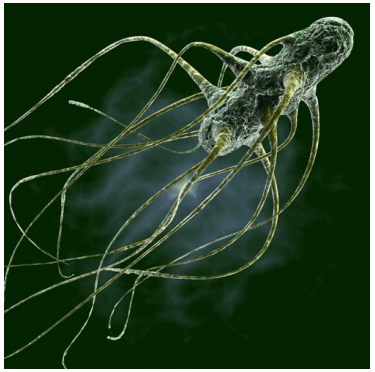
Microbially mediated immunodevelopment

Lactobacillus plantarum, L. paracasei – increase of antybodies against Salmonella, tetanus toxoid

55

Reduction of Food-Borne Pathogens – *Salmonella* sp. (*Salmonella enterica*)



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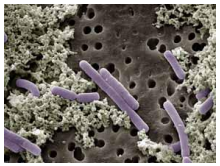
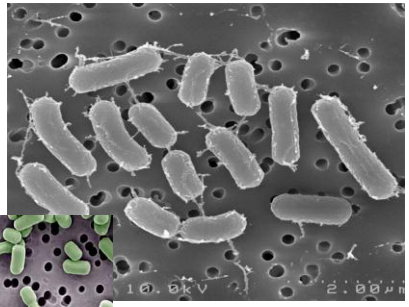
Special case : *Lactobacilli*

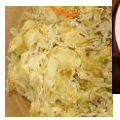




Lactobacilli as probiotics

Species	Main commercially used strain(s) (when available) with documented health benefits
Lactobacillus acidophilus	LA1; LA5
Lactobacillus johnsonii	La1; NCFM; DDS-1; SBT-2062
Lactobacillus (para)casei	F19; CRL 431; Immunitass; Shirota
Lactobacillus rhamnosus	GG; LB21; 271; GR-1; VTT E-97800
Lactobacillus plantarum	299v; Lp01
Lactobacillus delbrueckii subsp. *bulgaricus*	Lb12
Lactobacillus delbrueckii subsp. *lactis*	Lla
Lactobacillus cellobiosus	
Lactobacillus curvatus	
Lactobacillus fermentum	RC-14
Lactobacillus reuteri	MM2
Lactobacillus brevis	
Lactobacillus salivarius	UCC118
Lactobacillus helveticus	B02
Lactobacillus amylovorus	
Lactobacillus crispatus	
Lactobacillus gallinarum	
Lactobacillus gasseri	LG21

Lactobacilli - food products



fermented milk (yogurt, kefir), ice cream, cheeses, wine, beer, sourdough bread, meat (ham), fish (also as a sauce), sauerkraut, pickled cucumber, pickled barszcz, soya sauce,