Trichoderma and Pseudomonas as biocontrol agent

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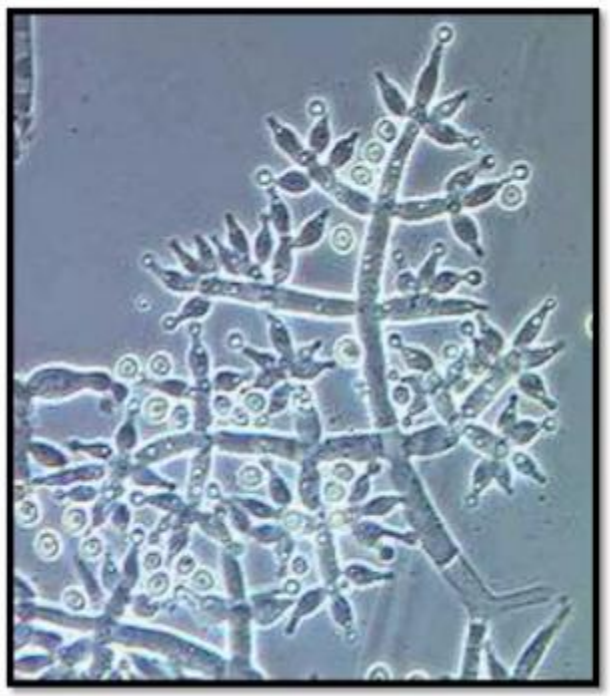
History of *Trichoderma*

- 1671 – First found in **Germany**
- 1794 – Identified by **Persoon** almost 218 years ago
- 1927 – **Gilman and Abbott** recognized four species based on colour, shape of conidia and colony appearance
- >75 years ago the potential use of *Trichoderma* by **Weindling** (1932) and first to demonstrate the parasitic activity in wilt of Pigeon pea
- Best known **mycoparasite** against many soil borne plant pathogens

- Free living fungus common in soil and root ecosystem
- Highly interactive in root, soil and foliar environment
- Suppresses the pathogen by different mechanism of biocontrol



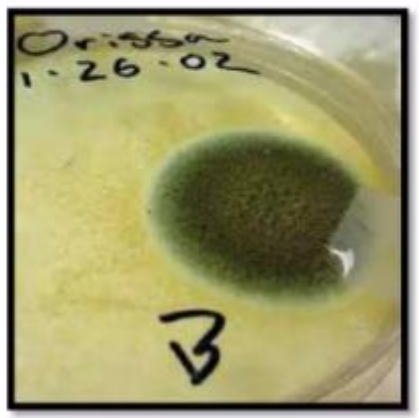
Taxonomical position of *Trichoderma*

Kulkarni and Sagar (2007) mentioned the *Trichoderma* as asexual stage and *Hypocrea* as sexual stage

Position	Asexual stage (conidia)	Sexual stage (ascospore)
Kingdom	Fungi	Fungi
Phylum	Ascomycota	Ascomycota
Sub-division	Deuteromycotina	Ascomycotina
Class	Hyphomycetes	Pyrenomycetes
Order	Monilliales	Sphariales
Family	Monilliaceae	Hypocreaceae
Genus	*Trichoderma*	*Hypocrea*

General Characters of *Trichoderma spp.*

- Cultures are fast growing at **25-30 C**
- Conidia forming within on week in compact or loose tufts in **shades of green** or **yellow or less frequently white**
- Yellow pigment may be secreted into the agar, specially on PDA
- A characteristic **sweet or 'coconut' odour** is produced by some species



Trichoderma: mechanism of action

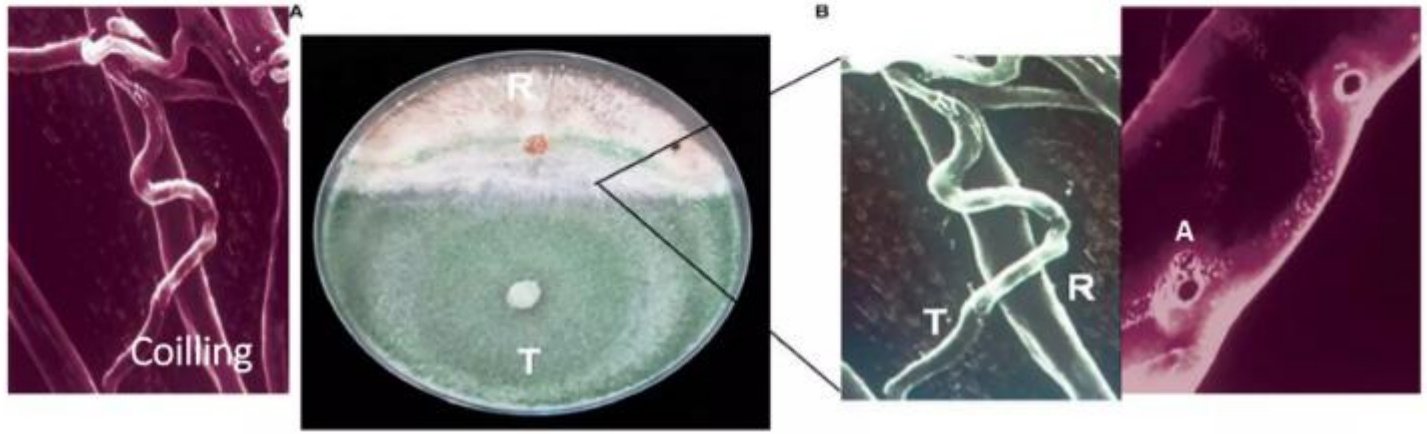
- Trichoderma spp. are present in nearly all agricultural soils.
- They are the most prevalent culturable fungi.
- Many species in this genus can be characterized as **opportunistic avirulent plant symbionts.**
- This refers to the ability of several *Trichoderma* species to form mutualistic endophytic relationships with several plant species.

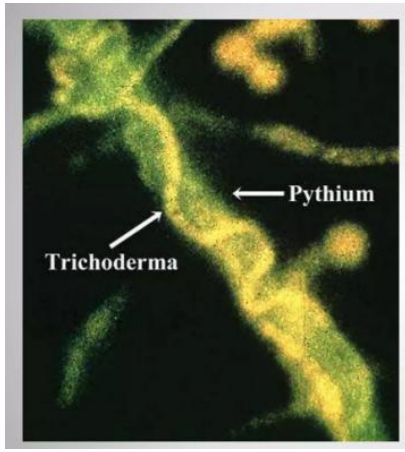
Trichoderma : biocontrol Mechanism

- Mycoparasitism
- Antibiosis
- Competition
- Induced resistance

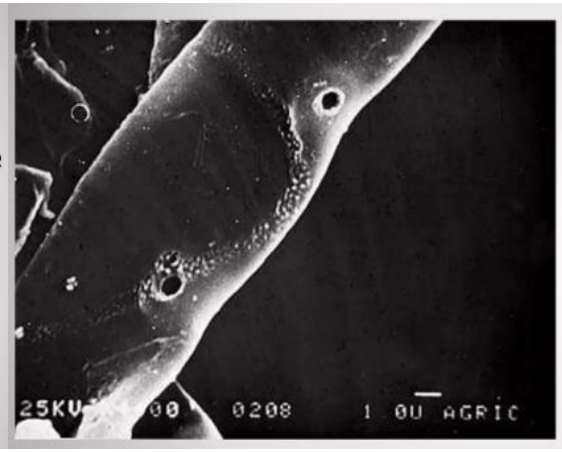
Mycoparasitism :

1. *Chemotropic growth of Trichoderma.*
2. *Recognition of the host.*
3. *coiling and appresoria formation.*
4. *secretion of hydrolytic enzymes. (glucanases, chitinases and proteases)*
5. *penetrations of the hyphae and lysis of the host.*





1. Attachment to the host hyphae by coiling
   a. Lectin-carbohydrate interaction



2. Penetrate the host cell walls by secreting lytic enzymes
   a. Chitinases
   b. Proteases
   c. Glucanases

The cellwall degrading enzymes of *Trichoderma*

1.ß-1,3-glucanases and different chitinolytic enzymes

2.Endochitinase (42- kDa),

3. chitobiosidase (40-kDa) and

4.N-acetyl-b- D-glucosaminidase (73-kDa)

5.Celluases

Antibiosis

Two different mechanisms of action.

1. The production of low molecular weight, non-polar, volatile compounds (i.e. 6PP) results in a high concentration of antibiotics in the soil environment, that have a relatively long distance range of influence on the microbial community.

2. Short distance effect may be due to the polar antibiotics and peptaibols acting in close proximity to the producing hyphae.

(i)Volatile antibiotics, i.e. 6-pentyl-a-pyrone (6PP) and most of theisocyanide derivates.

(ii)Water-soluble compounds, i.e. heptelidic acid or koningic acid.

(iii)Peptaibols, which are linear oligopeptides of 12–22 amino acids rich in a-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol (Pheol or Trpol) at the C-terminus.

Antibiotics produced

- Trichodermin
- Dermadin
- Trichoviridin
- Sesquiterpenes
- Trichozianines
- Isonitriles
- Pentyl analogues
- Gliotoxin
- Gliovirin

Competition

1. Competition for substrates is the most important factor for fungi as is competition for light in the case of evolution of plants (Garrett, 1956).

2. Root exudates and rhizosphere are rich source of nutrients such as sugar, amino acids, iron, vitamins, organic acids etc.

3. The proficient mobilization of immobile nutrients and their utilization makes it more efficient and competitive.

4. Production of organic acids, such as ***gluconic, citric*** and ***fumaric acids***, which decrease soil pH and allow the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium. (Vinale *et al.*, 2008).

"In the aerobic environment (with oxygen and neutral pH) iron exists mainly as Fe3+ and tends to form insoluble ferric oxide, making it unavailable for root absorption and microbial growth. (Miethke, 2013).

Iron act as cofactor of numerous enzymes

Trichoderma secrete ***siderophore***, an iron chelating compounds which bind with insoluble iron **(Fe^{+3})** and converted to soluble form **(Fe^{+2})** .

Induced-resistance

Interaction of *Trichoderma* with the plant, different classes of metabolites may act as elicitors or resistance inducers.

These molecules include:

(i) proteins with enzymatic activity, such as xylanase

(ii) avirulence-like gene products able to induce defence reactions in plants

(iii) low-molecular-weight compounds released from fungal or plant cell walls by the activity of *Trichoderma* enzymes.

Pseudomonas fluorescens

- *Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium.
- It belongs to the *Pseudomonas* genus.
- *P. fluorescens* has multiple flagella.
- It has an extremely versatile metabolism, and can be found in the soil and in water.
- It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration.

P. fluorescens as biocontrol agent

The bacteria *P. fluorescens* possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to-

- Grow rapidly *in vitro* and to be mass produced.
- Rapidly utilize seed and root exudates.
- Colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plants.

Mode of action

- Antibiotic Production
- Siderophores Production
- Induced Systemic Resistance
- Competition
- Hydrogen Cyanide Production
- Plant Growth Promotion Antibiotic Production

Antibiotic production

- The *P. fluorescens* is very effective antibiotic producer.
- Many secondary metabolites of *P. fluorescens* acts as antibiotics against plant pathogens.
- The *P. fluorescens* produces antifungal compounds which are fungistatic, inhibiting spore germination and lysis of fungal mycelia.

Antibiotics produced

- Phenazine-1-Carboxylic Acid (PCA)
- 2, 4 – Diacetylphloroglucinol (DAPG)
- Pyocinine
- Pyrrolnitrin
- Pyoluteorin
- Oomycin-A

Siderophore production

- Siderophores are extra cellular, low-molecular weight compounds with very high affinity for ferric iron.
- As siderophore sequester the limited supply of iron in the rhizosphere, they limits it's availability to pathogens and ultimately suppress their growth.

Siderophores

- Ferribactin
- Ferrichrome
- Ferroxamine B
- Pseudobactin
- Pyochelin
- Pyoverdine(soluble fluorescent pigment)

Induced systemic resistance

- The *P. fluorescens* induce systemic resistance in plants that is phenotypically similar to Systemic Acquired Resistance (SAR). Induction of resistance by *P. fluorescens* is mainly through the:
  - **Production of phytoalexins.**
  - **Increased lignifications.**
  - **Production of PR-protein in the induced plants.**

Phytoalexins

- Phytoalexins are antimicrobial and often antioxidative substances synthesized *de novo*(Ln. from the new or by scratch) by plants that accumulate rapidly at areas of pathogen infection.
- They are broad spectrum inhibitors and are chemically diverse with different types characteristic of particular plant species.

PR– proteins

- **Pathogenesis-related (PR) proteins** are proteins produced in plants in the event of a pathogen attack.
- They are induced as part of systemic acquired resistance.
- Infections activate genes that produce PR proteins. Some of these proteins are antimicrobial, attacking molecules in the cell wall of a bacterium or fungus.
- Others may function as signals that spread "news" of the infection to nearby cells. Infections also stimulate the cross-linking of molecules in the cell wall and the deposition of lignin, responses that set up a local barricade that slows spread of the pathogen to other parts of the plant

Competition

- The *P. fluorescens* pre-empt the establishment of other rhizosphere microorganisms through competition for favuored sites on the roots and in the rhizosphere.

Hydrogen cyanide production

- Hydrogen cyanide (HCN) is representative of class of volatile inhibitors.
- The *P. fluorescens* produces HCN which can check growth of phytopathogens.

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