Introduction to *Fusarium* taxonomy

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Fusarium genus

Link H.F. (1809) – the generic concept of Fusarium

Defining characteristic:
Presence of canoe- or banana-shaped conidia

Other genera may have this characteristic

It is a polyphyletic form genus
Basis taxonomy of *Fusarium* genus

Wollenweber and Reinking reduced over 1000 published species of *Fusarium* prior to 1935 to 65 species.

**The Sections**

- arrange species in the sections
- are based solely on morphological characters and cultural characteristics
- are not monophyletic but are used for convenience
Taxonomic systems are based on morphological characters
The concept of a species within the genus has varied greatly in the taxonomic systems.
Teleomorphs of *Fusarium*

**Gibberella** – the majority of *Fusarium* species

**Haematonectria** – *Fusarium solani* complex

**Albonectria** – *Fusarium decemcellulare*

Teleomorph morphology usually is not sufficiently for identification purposes.

*Gibberella zeae*
Key characters for identifying *Fusarium* species

**Macroconidia**

**Size**
large versus small

**Number of septa**
range and average

**Shape**
elongate versus squat
degree of curvature
Key characters for identifying *Fusarium* species

**Macroconidia**

- **Apical cells**
- **Basal cells**

**Dorsal side**  
**Ventral side**
Apical cells shapes:

A1. Blunt  
A2. Papillate  
A3. Tapering  
A4. Hooked

Basal cells shapes:

B1. Foot-shaped  
B2. Elongated foot-shaped  
B3. Distinctly notched  
B4. Barely notched
Key characters for identifying *Fusarium* species

Shape and Size
Number of cells
False heads or/and chains

**Microconidia**

- globose
- subglobose
- oval
- apiculate
- fusiform
- truncate
- reniform
- falcate
- ampulliform
- ovoid
- obovoid
- puriform
- clavate
Key characters for identifying *Fusarium* species

**Conidiophores** are special structures of hyphae which finished in fertile cells - phialides and provide conidia with nutrients.
- unbranched, sparsely or densely branched.

**Conidiogenous cells**

**Monophialides** – having only one opening per cell

**Polyphialides** – having more than one opening per cell (but not necessarily for all conidiogenous cells)
Key characters for identifying *Fusarium* species

**Monophialides**

**Polyphialides**
Length of the Conidiogenous cell

Long (*F. solani*)

Short (*F. poae*)
Key characters for identifying *Fusarium* species

**Mesoconidia**

Spindle-shaped conidia that are typically produced in *F. sporotrichioides, F. chlamydosporum, F. semitectum, F. camptoceras* and in some isolates of *F. avenaceum* and *F. subglutinans*.

These spores are not produced in sporodochia, only in the aerial mycelium.
Key characters for identifying *Fusarium* species

**Chlamydospores**

The presence of chlamydospores is the diagnostic character. Their absence is not an informative character.

They can take a long period of time to form (up to 6 weeks). Formed singly, doubles, in clumps or in chains.

May be found in aerial mycelia or embedded in the agar.
Key characters for identifying *Fusarium* species

**Colony morphology**

Abundance and color of aerial mycelium  
Pigmentation  
Growth rates, especially at 25°C and 30°C
Beginning the identification process

The majority of isolates are not difficult to identify if you use:

• Controlled temperature and light conditions
• Standard media
• Cultures initiated from single spores
Growth Conditions
Remember, try to use the conditions outlined in the identification guide you are following!

**Temperature** – generally 25°C or fluctuating 25/20°C day/night cycle.

**Light**
Good light conditions will help to stimulate conidia formation.
Artificial daylight (cool white tubes) on a 12 hr light:12 hr dark cycle necessary to maximize production of sporodochia.
Black light (near-UF, emission ca. 310-360 nm) may be essential for teleomorph development.
Standard media for growth of *Fusarium* fungi

**Media for *Fusarium* isolation:**

Potato dextrose agar (PDA)
Czapek-Dox agar
The media are supplemented with antibiotics and fungicides after the agar is melted and the temperature is not higher than 55 degrees C.
### Antibiotic solutions

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Stock solution, g</th>
<th>Working concentration, ml/L of medium</th>
<th>Effective against bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptomycin</strong></td>
<td>5 / 100ml H₂O</td>
<td>20</td>
<td>G-</td>
</tr>
<tr>
<td><strong>Neomycin</strong></td>
<td>1 / 100ml H₂O</td>
<td>12</td>
<td>G+</td>
</tr>
<tr>
<td><strong>Chlotetracycline hydrochloride</strong></td>
<td>0.5 / 100ml H₂O</td>
<td>10</td>
<td>G- and G+</td>
</tr>
<tr>
<td><strong>Chloramphenicol</strong></td>
<td>0.5 / 10 ml ethanol (96%)</td>
<td>1</td>
<td>G- and G+</td>
</tr>
</tbody>
</table>

**Fungicides prevent growth of saprophytes**

**PCNB (Pentachloronitrobenzene):** 750mg/L. PCNB is usually added as 1 g of Terrachlor, which contained 75% PCNB (w/w).

**Dichloran:** 200 mg in 100 ml ethanol (96%) (1 ml is added to 1000 ml agar before autoclaving).

**Propiconazole:** 0.375 mg /1L after the agar is melted and the temperature is below 55 degrees C.

The linear growth of fungi may be suppressed by **Triton X-100**, stock solution 2% is autoclaved and used at the rate of 10 ml/L of medium.
Weak nutrient media

**Carnation Leaf-Piece Agar (CLA).** CLA is a natural substrate medium prepared by aseptically placing sterile carnation leaf pieces, 3-5 mm, into a Petry dish and adding sterile 2% water agar (5-6 leaf pieces is dishes). Water agar (WA) consist of 20 g agar in 1 L of distilled H₂O.

**Spezieiller Nährstoffarmer Agar (SNA).** In 1 L of distilled H₂O: KH₂PO₄ – 1 g; KNO₃ – 1 g; MgSO₄ x 7H₂O – 0.5 g; KCl – 0.5 g; Glucose – 0.2 g; Sucrose – 0.2 g; Agar – 20 g.
Placing 1-2 pieces of sterile filter paper (approximately 1cm²) on the agar surface after the medium has gelled can increase sporulation.

Media promotes sporulation and good conidiogeneous cell development. Media transparent, so cultures can be viewed directly with microscope (x100).

CLA- surface  CLA- reverse  SNA- surface
Criteria for identification

**PDA medium:**
Colony morphology
Pigmentation – hyphae and in agar
Growth rates

**CLA and SNA media:**
Presence/absence of conidia
Shape and formation of conidia
Nature of the conidiogenous cells
Presence/absence of chlamydospores
Single spore isolation

Can be used for macroconidia, microconidia and ascospores.

If you want to be sure in identification, use single spore cultures!

Never use cultures before single sporing in the further studies (genetics, molecular, pathogenicity, etc.)! More than one species/strain of fungi or *Fusarium* from the same substrate.
**Dilution Plate**

**Protocol:**
- Prepare sterile water in a vial
- Transfer sporodochial conidia from culture with a fine needle in a water.
- Check the concentration and dilute (5-10 conidia/drop) under a low power (10x) microscope.
- A few drops of the suspension spread over 2% dry and thin WA plates.
- Leave plates for 6-15 hr.
- Check plates for germinating conidium along under a low power (10x).
- Mark with a fine marker.
- Pick small squares of agar around a germinating conidium using a fine sterile needle and transfer to maintenance media.
* If the culture is not form conidia then pick a hyphal tip separated from others by spreading on WA plate and transfer to maintenance media.
Fusarium genus

Section Discolor

Main species:

- *F. graminearum* (*Gibberella zeae*) complex
- *F. culmorum*

*Fusarium* head blight
Section Discolor

Colony morphology
- fast growing (5 to 7 cm >growth in 7 days)
- red/pink pigments

Macroconidia
- relatively robust and thick-walled
- typically formed in sporodochia
- less often on hyphae

Microconidia
- absent or rare

Conidiophores
- monophialides

Chlamydospores
- present in conidia and hyphae
- single, chains, clumps
- inconsistently produced
Section *Discolor*

Colony appearance

**F. graminearum**

*Surface* and *reverse* images of colonies of *F. graminearum* are shown.

**F. culmorum**

*Surface* and *reverse* images of colonies of *F. culmorum* are shown.
## Section *Discolor*

### Macroconidia

<table>
<thead>
<tr>
<th><em>Fusarium</em></th>
<th><strong>Size</strong></th>
<th><strong>Cells</strong></th>
<th><strong>Surface</strong></th>
<th><strong>Septate</strong></th>
<th><strong>Sporodo-chia</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>length</td>
<td>ratio*</td>
<td>apical</td>
<td>basal</td>
<td>ventral</td>
</tr>
<tr>
<td><em>F. graminearum</em></td>
<td>40-60</td>
<td>11</td>
<td>tapered</td>
<td>foot-shaped</td>
<td>straight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>apical</td>
<td>basal</td>
<td>ventral</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>25-35</td>
<td>6</td>
<td>blunt, rounded</td>
<td>foot-shape -notched</td>
<td>straight</td>
</tr>
</tbody>
</table>

* approximate ratio = length/ width of macroconidia

![Image of *F. graminearum* macroconidia](image1.png)  
![Image of *F. culmorum* macroconidia](image2.png)

* *F. graminearum*  
* *F. culmorum*
Section *Roseum*

Main species: *F. avenaceum* (*Gibberella avenacea*)

Cosmopolitan, especially temperate zones. Wide range of plants: seedling disease, crown & root rot.

**Colony morphology**
- highly variable
- surface: floccose, grey rose, yellow, white.
- growth rate varies from moderate to rapid

**Pigment** - grey rose to burgundy, brownish

**Conidiophores**
- branched and unbranched monophialides

**Sporodochia:** Usually bright orange

**Chlamydospores** - Absent
**F. avenaceum**

Macroconidia formed on hyphae and in sporodochia

Macroconidia in sporodochia
- long, slender, thin-walled, usually 3-5 septate
- falcate to almost straight "needle-like"

Apical cell – elongate, bent
Basal cell – notched or foot-shaped

Macroconidia & Mesoconidia in aerial mycelium
- often abundant
- fusiform, comma-shaped, 0-3 septate
Section *Sporotrichiella*
Microconidia pyriform, globular to spindle-shaped, or lemon-shaped.

Main species:

- *F. sporotrichioides*
- *F. tricinctum* *(Gibberella tricincta)*
- *F. poae*
- *F. langsethiae*
**F. sporotrichioides**

Cosmopolitan.
Occurs on a wide variety of plants & soil.

**Colony morphology**
- rapid growth, abundant floccose mycelium
- sporodochia may occur in older cultures

Surface: white, pinkish to brownish red
Pigment: rose to burgundy

**Macrococidia:** abundant
- on hyphae and sporodochia
- falcate, 3-5 septate

Apical cell: curved, pointed
Basal cell: notched or foot-shaped

**Microconidia:** abundant
- oval to pyriform (pear-shaped), spindle-shaped
- 0-1 septate, often with papilla

**Conidiophores**
- monophialides & polyphialides

**Chlamydospores** - Abundant
Cosmopolitan
More common on gramineous hosts

**Colony morphology**
- rapid growth, abundant floccose mycelium
- sporodochia rare
Surface: white, pinkish white
Pigment: rose to burgundy

**Macroconidia**: generally rare
- on hyphae and sporodochia
- falcate, 3-5 septate
Apical cell: short, curved, pointed
Basal cell: foot-shaped or notched

**Microconidia**: abundant
- globose to pyriform, often with papilla
- 0-1 septate,
- produced in clusters, "bunch of grapes"

**Conidiophores**
- unbranched & branched monophialides

**Chlamydospires** - Absent
Some isolates has fruity odor
Section *Sporotrichiella*

**F. langsethiae**

Up to now found only in Europe  
Detected in small cereals grain

**Colony morphology**  
- slow growth, nearly lack of aerial mycelium  
  Surface: white to peach, pinkish white, powder  
  Pigment: peach to violet

*Sporodochia* absent  
Micro morphology similar to *F. poae*

**Macroconidia**: absent  
**Microconidia**: abundant  
- globose to pyriform, often with papilla  
- 0-1 septate,  
- produced in clusters, "bunch of grapes"

**Conidiophores**  
- unbranched & branched monophialides

**Chlamydospores** - Absent  
No odor
**F. tricinctum**

Cosmopolitan, more common in temperate areas

**Colony morphology**
- moderate rapid growth,
- mycelium as compact cushion
- sporodochia may occur in older cultures
  - Surface: red to vinaceous or purple
  - Pigment: red to vinaceous or purple

**Macroconidia:**
- usually form in sporodochia
- falcate to crescent-shaped, slender
- 3-5 septate
Apical cell: curved, pointed
Basal cell: notched or foot-shaped, narrow and pointed

**Microconidia:** abundant
- lemon-shaped to pyriform, ellipsoidal
- 0-1 septate

**Conidiophores:**
monophialides, richly branched, slender

**Chlamydospores:** formed singly or in chains
Section Gibbosum

Main species:
*F. equiseti* *(Gibberella intricans)*  
*F. acuminatum* *(Gibberella acuminata)*

Weak pathogens of wide range of plants, in soil.

**Macroconidia**
Usually strongly curved on both ventral and dorsal sides of conidia  
striking foot-shaped basal cell  
Often quite large  
**No microconidia**  
Chlamydospores present  
**Pigment:** red or brown
Section *Gibbosum*

**F. equiseti**

**Culture morphology:**
- fast growing
- abundant mycelium
  Surface: white to beige or brown
  Pigment: pale to dark brown with brown flecks

**Macroconidia**
- various sized - occasionally very long
- 5-septate, with strong curvature
  Apical cell: tapering cell
  Basal cell: foot shaped

**Chlamydospores** abundant in hyphae, brown
Section *Gibbosum*

**F. acuminatum**

**Culture morphology:**
- slow-middle growing
- floccose mycelium
Surface: peach-beige
Pigment: carmine red
Sporodochia blight orange

**Macroconidia**
- slender, sickle-shaped
- 3-5-septate
Apical cell: tapering cell
Basal cell: pedicellate shaped

**Chlamydospores** in hyphae
**F. proliferatum**

Worldwide distributed on a wide variety of plants

**Colony morphology**
- rapid growth, abundant floccose mycelium
- sporodochia may occur
Surface: white, becoming purple-violet with age
Pigment: varies in violet intensity

The tester strains of *G. fujikuroi* complex

Fertile perithecia *G. fujikuroi* after making crosses with the tester strain *F. proliferatum*
Section *Liseola*
*Gibberella fujikuroi* species complex

**F. proliferatum**

**Macroconidia:** usually abundant
- on hyphae and sporodochia
- slender, thin-walled, relatively straight, 3-5 septate
Apical cell: curved
Basal cell: poorly developed

**Microconidia:** abundant
- oval with flattened base, pyriform also may occur
- 0 septate
- in short chains and in false heads

**Conidiophores**
- monophialides & polyphialides

**Chlamydospores** - absent
**F. oxysporum species complex**

Worldwide distributed on a wide variety of plants. An important vascular wilt and root rot pathogens. Many isolates are host specific and differentiated as *forma speciales* (f.sp.) – infraspecific subdivision.

**Colony morphology** varies widely!!!
- may be floccose, sparse, abundant
- sporodochia may occur, pale orange

Surface: white, becoming purple-violet with age
Pigment: unpigmented to dark violet

**Macroconidia**: usually abundant
- on hyphae and sporodochia
- short to medium length
- straight, to slightly curved
- thin walled, usually 3- septate

Apical cell: tapered and curved
Basal cell: foot shaped to pointed

**Microconidia**: abundant
- oval, elliptical
- 0 septate
- in false heads

**Conidiophores**
- Short monophialides

**Chlamydospores**
- usually formed abundantly and quickly
- singly or in pairs

Section *Elegans*