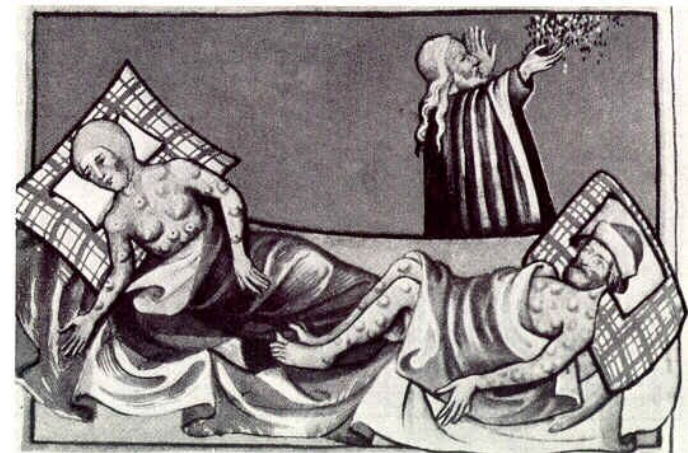


# CSI Dublin: The Hunt for the Irish Potato Killer

**Isolating a Potato Killer: Using Aseptic  
Laboratory Technique to Isolate and  
Transfer a Pathogen from a Infected  
Sample to a Healthy Sample**

# What is Disease?

- Brainstorm the idea of disease:
  - Symptoms or Suffering
  - Parasite and Host Relationship
  - Condition or Impairment of Growth or Development
  - Vector/Spread
  - **Pathogen** : What is it?
- How can we determine **one particular pathogen** that causes a particular disease when the world is full of bacteria, fungi and other microorganisms all symbiotically living together?

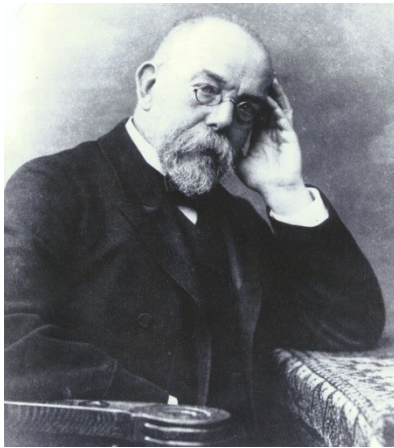


Sufferers of the Black Plague

# What Causes Disease? Koch's Postulates

- Any time one wants to identify what causes a particular disease, Koch's postulates are steps of the scientific method to determine the cause of disease.

Dr. Robert Koch (1843-1910)




Sources:

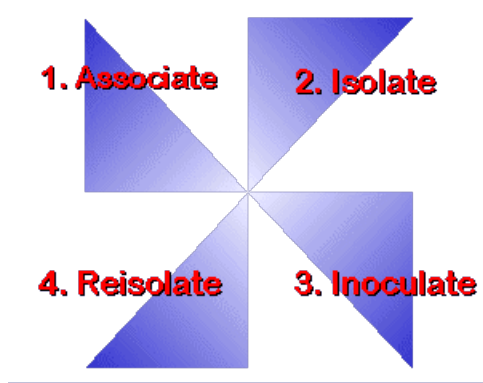
Photo Courtesy of the National Library of Medicine.  
<http://geneticsmodules.duhs.duke.edu/Design/page.asp?CourseNum=4&LessonNum=2>

### Koch's Postulates

Evidence required to establish etiologic relationship between microorganism and disease:

1. Microorganism must be observed in every case of the disease
2. It must be isolated and grown in pure culture
3. The pure culture, when inoculated in animals, must reproduce the disease
4. Microorganism must be recovered from the diseased animal

A petri dish containing a bacterial culture, showing a dense, orange-brown, granular growth on a solid medium. The dish is circular and has a dark rim.



# Koch's Postulates in Plant Pathology

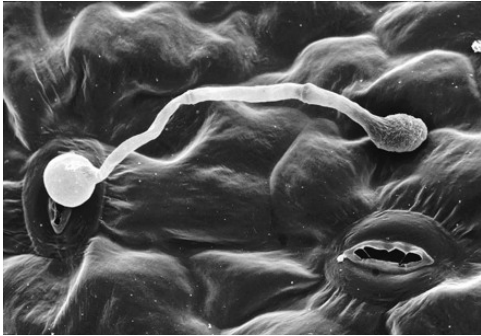
- **Pathogen must ALWAYS be associated with disease in ALL diseased plants.** There are no exceptions allowed.
- **Pathogen must be isolated and established in PURE culture.** This may be difficult with obligate parasites, but methodologies have been developed to fulfill this requirement even with obligate parasites.
- **Inoculation of a healthy plant of the same variety must reproduce EXACTLY the same symptom(s).** Inoculation must be of a healthy plant of the same species and cultivar. This may be difficult if one isolates from a plant of unknown cultivar. The symptoms must be reproduced essentially identical to the initial diseased plant, taking into account differences between the initial plants environment and the health inoculate plant.
- **Pathogen must be re-isolated from inoculated plant and its identity confirmed as the same as the original isolate.** The organism recovered must be the identical to the original isolate. There are no exceptions.

# Day 1: The Isolate “Sandwich”

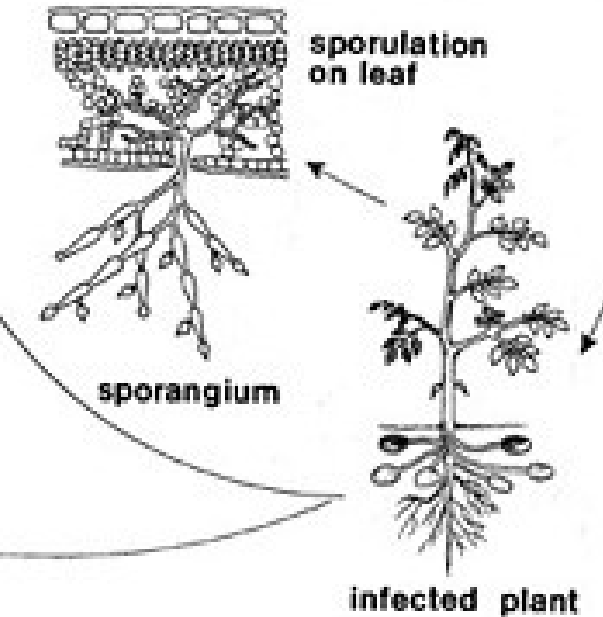


- In today’s lab, we are going to create an Isolate “sandwich” by taking infected potato tissue and inoculating healthy potato tissue to isolate the pathogen *P. infestans* in culture.
- In a research lab, the process is done several times to ensure a pure sample.

# Why the Isolate Sandwich?



- We use the sandwich method to separate out *P. infestans* from other pathogenic microorganisms by taking advantage of how they infect and sicken their host.
- The pathogen grows from the **underside** of a leaf and **through** to the top of a healthy tuber.
- The pathogen spreads between tubers via contact through whitish, threadlike mycelium between cells and **haustoria** within cells.
- That way, we know we have *P. infestans*!



Sources:

<http://www.apsnet.org/online/feature/lateblit/chapter1/1-10.jpg>

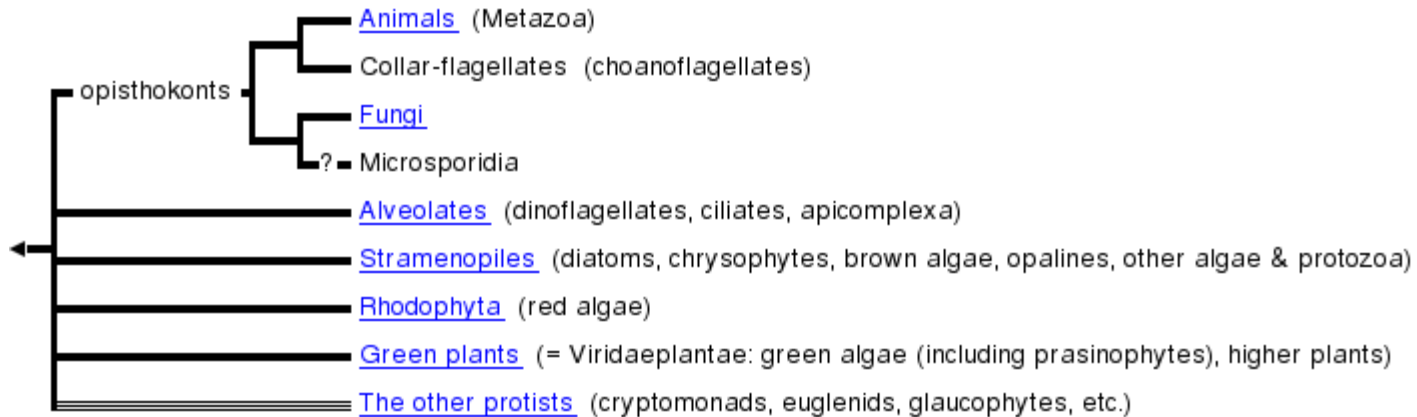
<http://www.plantcell.org/content/vol20/issue3/cover.dtl>

# Day 7: Viewing *P. infestans*

- Today you are going to remove isolate samples and view them under the microscope.
- Make sure you use proper laboratory technique with the microscope as well as safety precautions.
- Wipe up your lab station with rubbing alcohol often, wear safety glasses and dispose of any infected potato tissue (sandwich or microscope slide) in the autoclave or teacher provided bleach solution.

# New Kingdom: Stramenopila

Stramenopiles contain diatoms, brown algae and other protozoa.



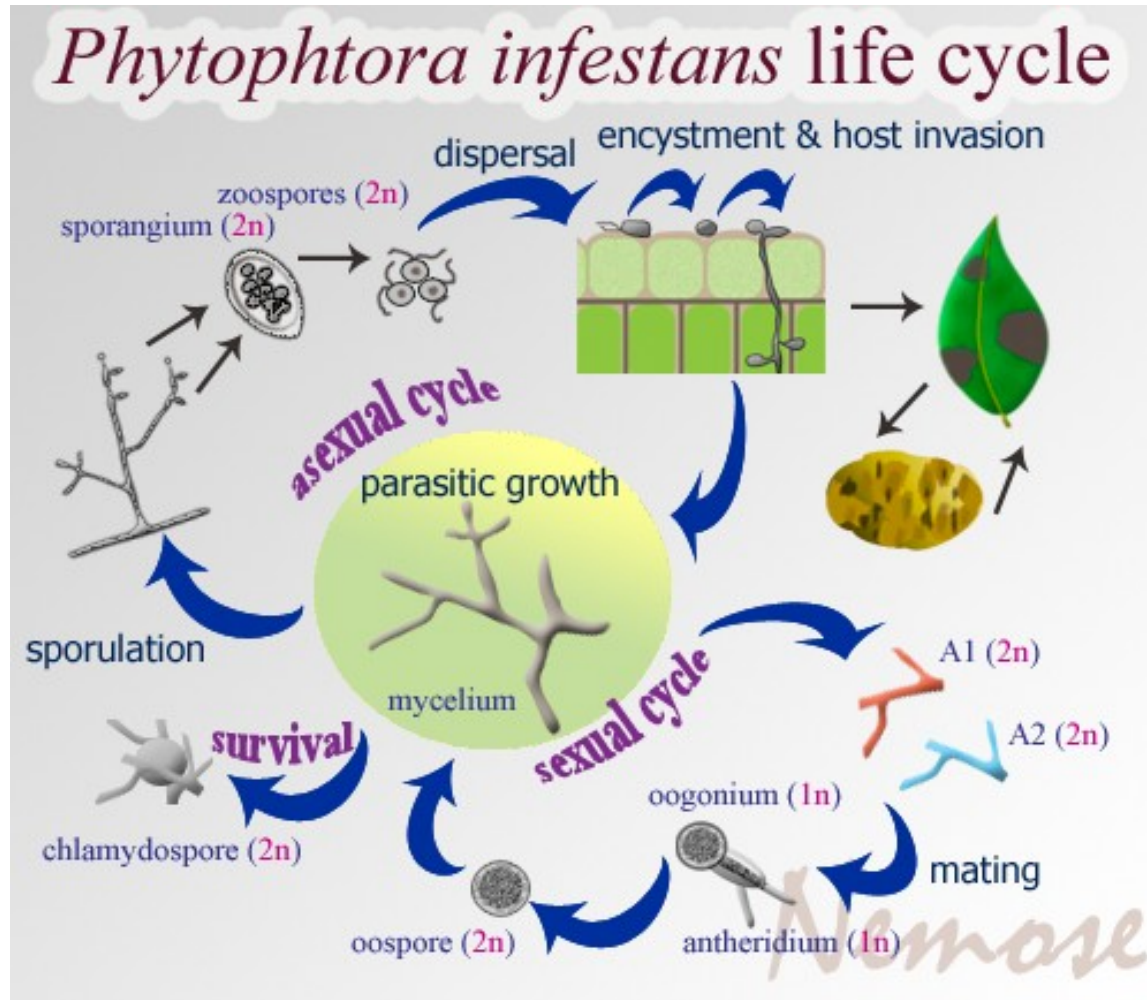


# The Evolution of Oomycetes

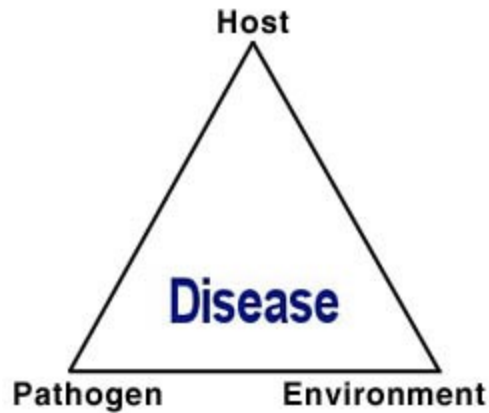
## “Lower Fungi”

- **Oomycetes** (pronounced o-o-my-seats) are often commonly referred to as “water molds.”
  - Means “Egg Fungus” because of the phyla's fungal qualities and oogamous production.
- Called *Lower fungi* since they resemble fungi in growth and life style, but are not fungi!
  - They do not have cell walls made of chitin (mixture of cellulose), cannot see cell walls in mycelium.
  - Have lemon shaped structures called sporangia that protect and release contents when conditions are optimum.
  - Contain biflagellate zoospores that require **water and wind** to spread asexual cells for **reproduction**.

# Life Cycle



Source: [http://www.metapathogen.com/IMG/Pin\\_lc.png](http://www.metapathogen.com/IMG/Pin_lc.png)



Unique for plants:

Immobility does not permit plants to escape poor environments.

Lacks the sophisticated immune systems of mammals.

Because of the type of agents that attack plants, they are environmentally dependent.

# The Disease Triangle

- The existence of a disease caused by a living agent absolutely requires the interaction of...
  - a susceptible **host**,
  - a virulent **pathogen**,
  - an **environment** favorable for disease development.
- Conversely, plant disease is prevented upon elimination of any *one of these three* causal components.
- Other parameters may include **human activity, vectors and time**.

Source:

<http://www.apsnet.org/education/InstructorCommunica>

# Sources

## (Pictures have internal links)

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- Stevens, R.B. 1960. Pages 357-429 in: *Plant Pathology, an Advanced Treatise*, Vol. 3. J.G. Horsfall and A.E. Dimond, eds. Academic Press, NY.
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