

PATHOGENESIS – ROLE OF ENZYMES, TOXINS, GROWTH REGULATORS AND POLYSACCHARIDES

The term pathogenesis means step by step development of a disease and the chain of events leading to that disease due to a series of changes in the structure and /or function of a cell/tissue/organ being caused by a microbial, chemical or physical agent. The **pathogenesis** of a disease is the mechanism by which an etiological factor causes the disease. The term can also be used to describe the development of the disease, such as acute, chronic and recurrent. The word comes from the Greek *pathos*, "disease", and *genesis*, "creation".

There are several chemical weapons secreted by pathogens that are utilized as they carry out their activities. These weapons include enzymes, toxins, growth regulators and polysaccharides.

Enzymes

Cutinases, cellulases, pectinases and lignases are often secreted by the pathogenic organism. Fungi, nematodes and bacteria are all known to produce one or more of the above enzymes in specific pathogen-host combinations. Viruses and viroids are generally not considered to secrete enzymes, although some viruses may encapsidate an enzyme in their particle.

Pathogenic organisms either continually secrete enzymes or upon contact with the host plant. As we mentioned in class on Tuesday, the first surface an organism comes into contact with is cuticle and the cell wall of the plant. As we also mentioned, the cuticle is comprised of a complex wax, cutin, which impregnates the cellulose wall. The cell wall is comprised of cellulose, which makes up the structural framework of the wall, along with the matrix molecules hemicellulose, glycoproteins, pectin and lignin. Thus, penetration into living parenchymatous tissues and degradation of middle lamella is due to the action of one or more enzymes which degrade these chemical substances. Cutinases degrade the cutin on the cuticle layer presoftening the tissue for mechanical penetration or as a first step in tissue degradation. Studies have shown that several fungi and at least one bacterial species produce cutinases. Further, evidence indicates that cutinases are continually produced, albeit in low concentrations, with degradation products often inducing even higher levels of cutinase secretion. Studies have shown that in some organisms, cutinase production may be linked to virulence. Pectic substances comprise the

middle lamella and also form an amorphous gel between the cellulose microfibrils in the primary cell wall. Pectin degrading substances often termed pectinases or pectolytic enzymes include pectin methyl esterases (PME), polygalacturonases (PG) and pectin lyases or transeliminases. Pectin methyl esterases remove small groups such as methyl groups (CH₃) often altering solubility and thus affecting the rate of chain splitting by polygalacturonase and pectin lyase. Polygalacturonases split chains by adding a molecule of water, while pectin lyases split chains by removing a water molecule from the linkage. Pectin degrading enzymes are involved in a wide range of plant diseases particularly in the soft rot diseases (Examples: bacterial soft rot due to *Erwinia carotovora*, leaf drop of lettuce and water soft rot of crucifers (*Sclerotinia sclerotiorum*) and damping off in various seedlings due to *Rhizoctonia solani*). Organisms such as these, as well as, elaborate pectic enzymes lead to tissue maceration. In fact, these enzymes are sometimes referred to as macerating enzymes.

Cellulases

Cellulose is the major framework molecule of the plant cell wall existing as microfibrils with matrix molecules (glycoproteins, hemicelluloses, pectins, lignins) filling the spaces between the microfibrils and cellulose chains. Cellulases have been shown to be produced by many pathogenic fungi, bacteria and nematodes. Cellulolytic enzymes play a role in softening and disintegration of cell walls. No doubt cellulolytic enzymes are involved in the invasion and spread of the pathogen, but also are instrumental in the collapse of cells and tissues. As pointed out in Agrios text, indirectly cellulolytic enzymes participate indirectly in disease development by releasing soluble sugars that may be used as nutrients by pathogens and also may be involved in the release of materials in the vascular system interfering with transport or translocation of water.

Hemicellulases

Hemicelluloses are complex polysaccharide polymers that link the ends of pectic compounds to cellulose microfibrils. Since hemicelluloses are such a diverse group of polymers such as xyloglucans, glucomannans, galactomannans, arabinoglucans, etc., several hemicellulases have been identified in many plant pathogenic fungi. The mechanism by which they participate in cell wall breakdown is not clear, nor is it known how they contribute to pathogenesis.

Ligninases

Lignin is a phenylpropanoid which is found in the middle lamella and secondary cell wall of plants. More than anything else, lignin confers the tough, woody nature to woody tissues. According to Agrios text, only about 500 species of fungi are capable of decomposing wood. Most lignin degradation is by basidiomycetes known as white- rot fungi. These fungi produce ligninases that enable the fungi to utilize lignin.

Toxins as Chemical Weapons of Pathogens

Toxins have been implicated in plant disease as far back as deBary who advanced a theory of plant disease often termed the “toxin theory”. A primary tenant of the toxin theory is that a toxin elaborated by a pathogen may produce all of the symptoms of the disease. As more information was developed the theory was largely discarded. As we will see a little later in this discussion, the discovery of the toxin victorin, a host specific toxin, revived interest in the toxin theory of plant disease. Toxins may act directly on living host cells, damaging or even killing the host. Some toxins are active on a wide range of plant species (non-host-specific) or in some cases, as with the toxin victorin (host-specific).

Non-Host Specific Toxins

Tabtoxin---*Pseudomonas syringae* p.v. *tabaci*

Phaseolotoxin---*Pseudomonas syringae* p.v. *phaseolicola*

Tentoxin---*Alternaria alternata*

Tabtoxin

This is the toxin involved in the “wildfire disease of tobacco”. In this disease, leaves exhibit necrotic spots surrounded by a yellow halo. Identical symptoms of the disease may be induced by culture filtrates of the organism or purified toxins with symptoms identical to that of wildfire of tobacco. Similar effects may also be observed on a relatively wide range of hosts, thus making the toxin non-host specific. Tabtoxin chemically is a dipeptide composed of the amino acids threonine and tabtoxine. In the cell tabtoxin is cleaved releasing the tabtoxine moiety which is the active toxin. The inhibition of the enzyme glutamine synthetase is the primary mode of action of the toxin.

Phaseolotoxin

This is the toxin involved in one of the bacterial bean blights called "halo blight". Symptoms of the disease incited by the bacterium can be produced by the toxin alone.

Chemically the toxin is a tripeptide of ornithine-alanine-arginine with a phosphosulfinyl group. Within cells the toxin is enzymatically cleaved releasing phosphosulfinylornithine which is the toxic moiety. Cellular affects are a result of the inactivation of the enzyme ornithine carbamoyl-transferase.

Tentoxin

This is the toxin produced by *Alternaria alternata*. The disease induced by this organism is primarily a seedling disease in a wide range of plant species. Seedling death results when greater than one-third of the leaf area become chlorotic, and reduce vigor with less than that amount of leaf chlorosis. The toxin is a cyclic tripeptide that binds to and inactivates a chloroplast-coupling factor protein involved in energy transfer and also the inhibition of light dependent phosphorylation of ADP to form ATP.

Host-Specific Toxins

The idea that toxins play causal roles in plant diseases is attractive and dates back to the time of deBary. A very good reference on the role of toxins in plant disease is the following: "Microbial Toxins in Plant Disease" by Harry Wheeler and H. H. Luke published in Annual Review of Microbiology, Vol 17, 1963. This review gives an excellent account of the discovery of host-specific toxins. When one reduces the toxin theory to its most elementary form, it can be stated that all of the symptoms of a given disease result from the direct action of a toxic product of the pathogen in that disease. Tenents of this theory can be tested by the following criteria:

- a) the toxin, applied at concentrations which could be reasonably expected in or around the diseased plant, produces in a susceptible host all the symptoms characteristic of the disease;
- b) the pathogen and the toxin exhibit similar suscept (host) specificity
- c) the ability of the pathogen to produce the toxin varies directly with its ability to cause disease
- d) a single toxin is involved.

The first toxin isolated that fulfilled the above criteria was that produced by *Cochliobolus* (*Helminthosporium*) *victoriae*. "Helminthosporium leaf blight of oats appeared in 1945 with the introduction and widespread use of the oat variety Victoria' and its derivatives which contained the Vb gene for resistance to the crown rust disease. In the victoria lines, the infects the basal portions of plants, and as it infects, the fungus elaborates a toxin that is carried to the leaves, causing a severe leaf blight, and death of the plant. All other oat varieties and other plant species are either immune or sensitivity to the toxin was proportional to their susceptibility to the

fungus. In addition to the toxin producing external symptoms identical to that induced by the pathogen, internal biochemical and histochemical induced by the toxin are identical to that induced by the pathogen.

Chemically, the toxin is a complex chlorinated cyclic pentapeptide. Ultrastructural studies demonstrate the primary target of the toxin is the plasma membrane. Here, it apparently binds to proteins and in some yet to be determined mechanism alters the metabolism of susceptible cells

T-Toxin

This is another good example of a host-specific toxin. The toxin is produced in common corn disease "Southern Corn Leaf Blight" incited by the fungus *Cochliobolus heterostrophus* formerly known as *Helminthosporium maidis*. T-toxin is produced by race T of the fungus that first appeared in the United States in 1968. By 1970, severe losses occurred throughout the corn-belt in corn that carried the Texas male-sterile (Tms) cytoplasm. Corn varieties with normal cytoplasm were found to be resistant to the fungus and also to the toxin. The toxin, chemically, is a mixture of linear, long polyketols with 35 to 45 carbon atoms. The toxin specifically affects mitochondria of susceptible cells where ATP synthesis is inhibited.

Hc-Toxin

One other example of a host-specific toxin is the HC-Toxin produced by the fungus *Cochliobolus (Helminthosporium) carbonum* which incites a leaf spot disease in corn. The toxin is specific to only certain maize (corn) lines. The mechanism of action of the toxin is poorly understood at the present time, although the molecular and biochemical basis for resistance is well understood

Growth Regulators as Weapons

As you know, plant growth is the result of three factors: cell divisions, cell elongation and cell differentiation. Plant growth is controlled by a group of naturally occurring compounds with hormonal action that are often referred to as plant growth regulators, plant growth hormones, etc. The major groups of plant growth regulators are the auxins, gibberellins, kinins and ethylene. Plant growth regulators are not unlike any hormone, in that, they act in very small concentrations, with slight changes in the normal level resulting in profound changes in the growth pattern of the plant. Pathogens often cause an imbalance in the hormonal system by causing the infected plant to produce more or less hormone, or in some cases the pathogen itself elaborates hormone thus changing the hormone level. Some of the commonly observed

symptoms related to effects on plant growth regulation are: stunting, overgrowths, galling, root branching, adventitious root formation, defoliation, rosetting, leaf epinasty, etc.

Auxins

Auxins are the major growth regulator in plants and are defined as growth regulators whose major mode of action is cell elongation; they resemble indole-3-acetic acid in their activity. The naturally occurring auxin is indole acetic acid (IAA). Increased levels of auxin occur in plants infected with fungi, bacteria, viruses, mollicutes and nematodes. Following are a few important diseases directly related to altered auxin levels:

Corn smut (*Ustilago maydis*), clubroot of crucifers (*Plasmodiophora brassicae*), southern bacterial wilt (*Rolstonia solanacearum* formerly *Pseudomonas solanacearum*, crown gall (*Agrobacterium tumefaciens*) good examples of diseases where altered auxin levels have been implicated in disease.

Gibberellins

A century ago, rice farmer in Asia noticed some exceptionally tall seedlings growing in their paddies. Before these rice seedlings could mature and flower, they grew so tall and spindly that they toppled over. In Japan, this aberration in growth pattern became known as *bakanae* ('foolish seedling disease') disease of rice. In 1926, Kurosawa, a Japanese scientist discovered that the disease was caused by a fungal pathogen, *Gibberella fujikuroi*. By the 1930s, Japanese scientists had determined that fungus produced hyper-elongation of rice stems by secreting a chemical, which was given the name gibberellin. Gibberellins are normal constituents of green plants and also produced by several other microorganisms. The best known gibberellin is gibberellic acid. In the past years, scientists have identified more than 80 different gibberellins, many of them occurring naturally in plants. Spraying of diseased plants with gibberellin overcomes some of the symptoms (stunting) caused by several virus- or mollicute-pathogens indicating that gibberellin involves in disease development.

Ethylene

Ethylene production in infected tissues can be dramatically induced. This induction is largely dependent on activation of the ethylene biosynthetic pathway in plant tissues. Genes encoding several key enzymes involved in the ethylene biosynthesis are highly activated at the transcriptional level. It has not been shown that ethylene is produced directly by plant pathogenic fungi and bacteria.

Ethylene has been considered as a signal in plant for wounding and senescence responses. Recent studies show that ethylene together with another signal component jasmonic acid may play an essential role in plant defense responses of several pathosystems.

- Induced ethylene production in infected tissues.
- Ethylene induces expression of certain types of PR-genes and some signal components involved in defense signaling.
- Ethylene insensitive mutants block several PR-gene-expression.
- Ethylene insensitive mutants can be beneficial against some pathogens but deleterious to resistance against other pathogens in a specific gene-for-gene manner.

Other chemical weapons include polysaccharides, plant defense suppressors, transporters etc.

Plant viruses and viroids are not known to produce any substances themselves, but they adapt, induce and manipulate the host metabolism to replicate themselves



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