Primary and Secondary metabolites in fungus-plant interactions

Metabolites are involved in the growth of organisms through the process of metabolism. The metabolism is referred to as the sum of the all the biochemical reactions carried out by an organism. Depending on the origin and function, metabolites can be divided into two major categories; namely, Primary and Secondary metabolites.

Primary metabolites

Primary metabolites are essential to the growth of the cell. They are produced continuously during the growth phase and are involved in primary metabolic processes such as respiration and photosynthesis. Primary metabolites, which are identical in most organisms, include sugars, amino acids, tricarboxylic acids, the universal building blocks (carbon, nitrogen, oxygen, hydrogen, phosphorous, and sulfur), and energy sources. Other than the above compounds, proteins, nucleic acids, and polysaccharides are also considered as primary metabolites.

Secondary metabolites

Secondary metabolites are the compounds, which are derived by pathways from primary metabolic routs, and are not essential to sustain the life of cells. These compounds do not have a continuous production. Very often secondary metabolites are produced during non- growth phase of cells. Secondary metabolites are the end products of primary metabolites such as alkaloids, phenolics, steroids, essential oils, lignins, resins and tannins etc.

Phytopathogenic fungi that are basically classified as necrotrophs, hemibiotrophs and biotrophs constitute one of the main infectious agents in plants, causing alterations during developmental stages including post-harvest, gaining nutrients from the plants

they invade and, therefore, resulting in huge economic damage. Plants and fungi are rich sources of thousands of secondary metabolites (SMs), which consist of low-molecular weight compounds (the number of the described compounds exceeds 100,000) that are usually regarded as not essential for life while their role are quite versatile.

Lifestyle of phytopathogenic fungi

Necrotrophic fungi have broader host ranges than biotrophs and often enlist cell-wall-degrading enzymes and toxins, which can be small peptides or SMs. In contrast to necrotrophic and hemibiotrophic fungal pathogens, obligate biotrophs are entirely dependent on living plant tissue and characterized by a number of sophisticated infection structures including appressoria, penetration hyphae and infection hyphae allowing the invader to suppress plant defense responses and to gain excess to host nutrients. Biotrophs establish haustoria for nutrient uptake, suppress induction of host defense and reprogram metabolism. Biotrophic fungi and their metabolism has been studied on nonobligate biotrophs, such as *Cladosporium fulvum*, *Magnaporthe grisea* and *Mycosphaerella graminicola*. Much less is known about the obligate biotrophs, such as powdery mildews or rust fungi. However, it appears that biotrophy is associated with a convergent (tending toward) loss of secondary metabolic enzymes and reduction in genes encoding specific transporters of toxin secretion and extrusion of host defense compounds usual in necrotrophic fungi. Nevertheless, the infection strategy of necrotrophic fungi is less complex than that of obligate biotrophs. Appressoria formed by typical necrotrophs such as *Cercospora*, *Ramularia*, *Rhynchosporium*, *Alternaria*, *Fusarium*, *Botrytis*, *Helminthosporium*, *Sclerotinia*, or *Verticillium* species, are inconspicuous, and infection hyphae formed within the host are quite uniform.

Fungal SMs can be divided into four main chemical classes: polyketides, terpenoids, shikimic acid derived compounds, and non-ribosomal peptides. Moreover, hybrid metabolites composed of moieties (parts) from different classes are common, as in the

meroterpenoids, which are fusions between terpenes and polyketides. Analysis of available fungal genomes revealed that ascomycetes have more genes of secondary metabolism than basidiomycetes, archeo-ascomycetes, and chytridiomycetes, whereas hemi-ascomycetes and zygomycetes have none. Ascomycete genomes code for on average 16 polyketide synthases (PKS), 10 non-ribosomal protein synthases (NRPS), two tryptophan synthetases (TS), and two dimethylallyl tryptophan synthetases (DMATS) with crucial importance in SM synthesis. These types of SM genes encode signature enzymes that can be enriched in secondary metabolism gene clusters and responsible for main synthesis steps of metabolites. PKS–NRPSs have been identified only in ascomycetes, with an average of three genes per species. *Neurospora crassa* as well as human pathogens *Coccidioides* spp. and *Histoplasma capsulatum* have a lower number of PKSs (1–9 genes), NRPS (3–6 genes) and PKS-NRPSs (0–2 genes) than other ascomycetes. High number of fungal species have more than 40 genes encoding PKS, NRPS, hybrids, TS, and DMATS in their genome, including *M. grisea* (45 genes). Synthesis of siderophores, a class of SMs for iron uptake also involves a NRPS that is also very important for the virulence of several fungi (e.g., *Cochliobolus heterostrophus*, *C. miyabeanus*, *F. graminearum*, and *A. brassicicola*).

Stimuli in fungal SM production

A high degree of environmental interaction, particularly sources of abiotic stress for either the host or the fungus such as drought or heat stress, also affect on the interactions. Fungal genes involved in stress related responses, especially to oxidative stress, are highly represented in phytopathogenic fungi and fungal SM toxins often play a role in triggering these responses. Some fungal SMs, such as pigments, polyols and mycosporines, are associated with pathogenicity and/or fungal tolerance to several stress-inducing environmental factors, including temperature and UV light. Moreover, environmental

factors (e.g., light, temperature, pH, calcium, and nutrients) regulate SM production in a concerted (intensive) way.

Light is a requirement for deoxynivalenol (DON) toxin to exert its poisonous effect similarly to the induction of programmed cell death (PCD) during *Botrytis* infections. This might reflect the plant's need for light to produce reactive oxygen during the oxidative burst. Meanwhile, regulation of toxin production is also light-dependent through one of the most important light-regulatory protein complex, the velvet complex, comprising at least FgVe1 and FgVeB in *Fusarium* with homologous components in other fungi.

Nitrogen limitation have appeared to be an essential stimulus for the activation of virulence functions in phytopathogenic fungi. The ability to metabolize a wide variety of nitrogen sources enables fungi to colonize different environmental niches and survive nutrient limitations. Amino acids are required for SM biosynthesis, especially for the NRPS. Amino acid limitation in fungi results in the induction of a genetic network that induces genes for enzymes of multiple amino acid biosynthetic pathways as well as for aminoacyl-tRNA synthases. Inorganic N sources are also affect SM production. Ammonium activated the expression of aflatoxin genes, while nitrate served as an inhibitor of AF biosynthesis of *Aspergillus parasiticus*. The importance of global nitrogen regulators for the development of pathogenicity was shown for *M. grisea* and many other fungal plant pathogens, e.g., *Colletotrichum lindemuthianum*, *C. acutatum*, and *F. oxysporum*. In *F. graminearum*, which causes crop disease, nitrogen starvation activated the trichothecene pathway and induced the biosynthesis of the DON toxin that was identified as a virulence factor.

Fungal toxin production is also regulated by signals or even substrates from plant. The well-characterized oxylipins (a group of diverse oxygenated polyunsaturated fatty acids) such as jasmonic acid (JA) and its immediate precursor 12-oxo-phytodienoic acid are formed enzymatically in plants and accumulate in response to various stresses, in

particular wounding and pathogen infection. These compounds are also formed non-enzymatically via the action of reactive oxygen species (ROS), which also accumulate in response to pathogen infection, heavy metal uptake, or other stresses. Fungal species have been shown to harbor or secrete JA and its derivatives. Fungal oxylipins are able to mimic plant oxylipins; therefore, a reciprocal crosstalk was proposed between plant and fungus, and several examples have proven this theory. The tomato-infecting *F. oxysporum* produced JAs using a lipoxygenase enzyme related to those found in plants, suggesting that JA biosynthesis in pathogenic fungi occurs via a pathway similar to that in plants.

Effects of phytotoxins on host plant

Fungal phytotoxins are usually divided into host-selective toxins (HSTs) and non-host selective (NHSTs) toxins. Typically, HSTs are active only toward host plants, have unique modes of action and toxicity to the host; moreover, the production of the HSTs is crucial for the virulence of these fungi. Nearly all HSTs identified so far are produced by necrotrophic pathogens of the order of Pleosporales within the class of Dothideomycetes and especially in *Alternaria* and *Cochliobolus* species. These HST toxins are diverse chemically ranging from low-molecular-weight compounds to cyclic peptides.

Several microbial phytotoxic compounds either inhibited an amino transferase or appeared to have such a mode of action, like cornexistin from *Paecilomyces variotii*, which was patented as an herbicide; or tentoxin, a cyclic tetrapeptide from *A. alternata*, which indirectly inhibited the chloroplast development. A series of structurally related fungal metabolites specifically inhibited ceramide synthase (sphinganine-N-acyltransferase) in plants, e.g., several analogs of AAL-toxin (*A. alternata*) and FB1 (*Fusarium* spp.). Fusicoccin [*Fusicoccum* (*Phomopsis*) *amygdali*] irreversibly activated the plant plasma membrane H+-ATPase. Alternariol and monomethyl alternariol are natural phytotoxins, produced by *Nimbya* and *Alternaria*, inhibited the electron transport chain. Cerulenin (*Cephalosporium cerulens*) inhibited de novo (a new) fatty acid synthesis in plastids. T-toxin (a family of C35

to C49 polyketides) from *C. heterostrophus*, which is a HST trichothecene phytotoxin, inhibited mitochondrial respiration by binding to an inner mitochondrial membrane protein in sensitive plants, resulting in pore formation, leakage of NAD+, and other ions, as well as subsequent mitochondrial swelling. Zinniol (*Alternaria* species and one *Phoma* species) bound plant protoplasts and stimulated Ca^{2+} entry into cells. The availability of fungal genome sequences, the knowledge of the biosynthesis of these toxins and gene disruption techniques, allows the development of tools for discovering the role of more and more toxins in plant cell death and disease.

Secondary metabolite production on the host's side

Based on their biosynthetic origins, plant SMs can be divided into three major groups, (i) flavonoids and allied phenolic and polyphenolic compounds, (ii) terpenoids, (iii) nitrogen-containing alkaloids and sulfur-containing compounds, while other researchers have classified plant SMs into more specific groups. Plant SMs functions as defense molecules against microbes, viruses or other competing plants or as signal molecules like hormones and attracting molecules for pollinators or seed dispersal animals. Therefore, these compounds have importance for survival and fitness.

Hormone production and plant resistance

Hormone biosynthetic pathways are typically involved in the regulation of plant resistance to pathogens and pests. Endogenous signaling molecules, e.g., ethylene (ET), salicylic acid (SA), Jasmonic acid (JA) and abscisic acid (ABA) have been associated with plant defense signaling against biotic stress. Generally, SA signaling induces defense against biotrophic pathogens, whereas JA against necrotrophic pathogens.

SA synthesis is a crucial way a plant responds to a biotic attack and involved in both local and systemic resistance. Systemic acquired resistance (SAR) is a plant immune response that is induced after a local infection and confers immunity throughout the plant to a broad spectrum of pathogens. The onset of SAR is usually associated not only with

increased levels of SA but additional small metabolites have also been involved as effectors. Some of these metabolites have been implicated in the rapid activation of defenses in SAR in response to subsequent exposure to the pathogen that called priming. The induced systemic resistance (ISR) pathway is stimulated during necrotrophic bacterial attack but was shown to protect *Arabidopsis* against the necrotrophic fungal pathogens *Alternaria brassicicola*, *Botrytis cinerea* and also *Plectosphaerella cucumerina*, where SAR was ineffective. Investigations of the regulation of ISR revealed the role of JA and ET. SAR and ISR were characterized by the coordinated activation of pathogenesis-related (PR) genes, many of which encode PR proteins with antimicrobial activity such as chitinases. Soluble chitin fragments released from fungal cell wall through the action of plant chitinases were found to serve as biotic elicitors of defense-related responses like phytoalexin synthesis in plants. The main auxin in higher plants, indole-3-acetic acid (IAA), has profound effects on plant growth and development. Only the free form of IAA and related compounds are considered to be active. The majority of produced auxin, however, is conjugated (coupled) mainly to amino acids and sugars and thereby inactivated. IAA induces e.g., the production of expansins, the proteins whose function is to loosen the cell wall. But, the loose cell wall is more vulnerable to the invasion of different types of pathogens. Similarly to bacterial pathogens, hemibiotrophic or necrotrophic fungi produced IAA, manipulated plant growth and subverted plant defense responses such as PCD to provide nutrients for their growth and colonization. *Magnaporthe oryzae* secreted IAA in its biotrophic phase especially in the area of the infection hyphae and, in turn, provoked (motivated) rice to synthesize its own IAA at the infection sites.

Plant gibberellins are important phytohormones promoting plant growth and fungi also synthesize gibberellins among other several important terpenes. Gibberellic acids produced as SMs in the rice-infecting *F. fujikuroi* were good examples of phytohormone mimics.

Plant secondary metabolites - antifungal compounds

Most of the SMs like phytocassanes have been reported to have antifungal properties at least in vitro. The flavonoids and allied phenolics, e.g., coumarins, lignans, and polyphenolic compounds, including tannins and derived polyphenols form one major group of phytochemicals. These compounds or their precursors are present in high concentrations in leaves and the skin of fruits and are involved in important defense processes such as UV resistance, pigmentation, disease resistance, stimulation of nitrogen-fixing nodules. Phenolic compounds are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These are known to alter microbial cell permeability and to interact with membrane proteins, which cause deformation in the structure and functionality of these proteins. These disadvantageous changes may lead to dysfunction and subsequent disruption of the membranes including the following events: (i) dissipation (degeneracy) of the pH gradient and electrical potential components of the proton motive force, (ii) interference with the energy (ATP) generating and conservation system of the cell; (iii) inhibition of membrane-bound enzymes, and (iv) prevention of substrate utilization for energy production.

Antimicrobial compounds such as the steroidal glycoalkaloid saponins, e.g., avenacin and α-tomatine, restrict the growth of pathogens in the apoplast. Saponins have strong antifungal activity; the tomato saponin α-tomatine activates phosphotyrosine kinase and monomeric G-protein signaling pathways leading to Ca^{2+} elevation and ROS burst by binding to cell membranes followed by leakage of cell components in *F. oxysporum* cells. Different plant species produce different types of saponins, which are effective against a wide range of pathogenic fungi. Plant antifungal metabolites are preformed inhibitors that constitutively produced in healthy plants (phytoanticipins), or they may be synthesized de novo in response to pathogen attack or various non-biological stress factors such as short-wavelength UV light, treatment with heavy metal ions (e.g., copper

or mercury salts). The letter pathogen and environmental stress elicited compounds are called phytoalexins. These groups cannot be separated strictly as the same compound may be a preformed antifungal substance in one species and can be phytoalexin in another. Plant SMs usually accumulate in smaller quantities than the primary metabolites; however, they can accumulate in particular tissues at a higher concentration. This accumulation is regulated in a highly sophisticated manner in appropriate compartments because some plant SMs are even toxic to the plants themselves if they are mislocalized.

Phyllosphere and rhizosphere microorganisms can live in a close mutualistic association with plants or even colonize plant tissues (endophytes). Plant growth-promoting non-pathogenic microorganisms like rhizobacteria and fungi are soil-borne microbes with beneficial effects on plant performance in the rhizosphere. They can stimulate plant growth by increasing tolerance to abiotic stress or by suppressing plant diseases. Plants may actively shape microbial communities either inhabiting their outer surface or colonizing their interior. The growing plant secretes a wide range of chemicals, e.g., in root exudates, to communicate with rhizosphere microbes such as arbuscular mycorrhiza. Altered exudation patterns, putative direct arbuscular mycorrhiza effects, different root size and architecture, altered physiology may contribute to quantitative and qualitative microbial community changes in the mycorrhizosphere caused by arbuscular mycorrhiza fungi. Organic acids, amino acids and phenolic compounds present in root exudates play an active role in root-microbe communications.

Manipulation of programmed cell death

Different fungal strategies mediate killing of the plant host cells such as secretion of low molecular weight or peptide toxins or eliciting PCD in the host by secretion of reactive oxygen species (ROS). From the host's side, chloroplasts have a critical role in plant defense as these organelles are not only sites for the biosynthesis of the plant signaling

compounds: SA, JA and nitric oxide but for ROS production as well. Therefore, chloroplasts are regarded as important players in the induction and regulation of PCD in response to both abiotic stresses and pathogen attack. Moreover, toxin effectors from necrotrophic fungi can target one of the host's central signaling/regulatory pathway to trigger resistance (R) gene-mediated resistance or to down-regulate defense enzymes, and, as a consequence, to increase thereby host susceptibility to fungal attack. *Aspergillus* mycotoxin, ochratoxin A, induced necrotic lesions in detached leaves through oxidative burst induction with increased ROS level and concomitant down-regulation of plant antioxidant defense enzymes.

References of this lecture:

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