

Study on various phytotoxic compounds of *Alternaria alternata*

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Abstract:

A number of plant pathogenic fungi produce toxins that can damage plant tissues. Toxins are often classified as host selective (host specific) or nonspecific. Host-selective toxins (HSTs) are toxic only to host plants of the fungus that produces the toxin. In contrast, nonspecific toxins can affect many plants regardless of whether they are a host or non-host of the producing pathogen. Yoder (1980) classified toxins of plant pathogens as a pathogenicity factor or a virulence factor by considering the possible involvement of toxins in pathogenesis: pathogenicity is the ability to cause disease (a qualitative term), whereas virulence refers to the extent or severity of the disease caused (a quantitative term). Most HSTs are considered to be pathogenicity factors, which the fungi producing them require to invade tissue and induce disease (Yoder, 1980; Nishimura & Kohmoto, 1983; Scheffer & Livingston, 1984; Markham & Hille, 2001; Wolpert et al., 2002; Howlett, 2006). All isolates of the pathogen that produce an HST are pathogenic to the specific host; all isolates that fail to produce HSTs lose pathogenicity to the host plants. Plants that are susceptible to the pathogen are sensitive to the toxin. Such correlations between HST production and pathogenicity in the pathogens and between toxin sensitivity and disease susceptibility in plants provide persuasive evidence that HSTs can be responsible for host-selective infection and disease development. On the other hand, the exact roles of nonspecific toxins in pathogenesis are largely unknown, but some are thought to contribute to features of virulence, such as symptom development and in planta pathogen propagation.

Keywords: Phytotoxic, Physiology, Solanum, Compound

Introduction: The fungal genus *Alternaria* is a widespread and successful group growing in diverse environments worldwide, ranging from saprophytes to pathogens and even endophytes. The genus *Alternaria* was identified in the year 1816 [1]. Currently, about 300 species have been described based on phylogenetic and morphological studies, which have been further divided into 26 sections [2,3,4]. As an outstanding group of fungal pathogens, *Alternaria* species can either cause diseases in a wide range of economically important crops [1], resulting in significant economic losses, or affect human and animal health, such as through upper respiratory tract infections and asthma [4,5].

To date, over 70 toxins with different chemical structures and behaviors are known to be produced by *Alternaria* species [6]. These toxins often exhibit a variety of bioactivities, such as phytotoxic, cytotoxic, and antimicrobial properties, etc. Generally, *Alternaria* phytotoxins are divided into host-selective toxins (HSTs) and non-host-selective toxins (NHSTs) based on the susceptibility or resistance of the host. HSTs are toxic only to host plants. In contrast, NHSTs can affect many plants, regardless of whether they are a host or non-host of the pathogen producing them [7]. Most HSTs have been considered as pathogenicity factors required for fungi to invade tissues and cause disease. On the other hand, NHSTs may contribute to the development of symptoms and the proliferation of plant pathogens [8,9].

Here, we review the toxins produced by *Alternaria* spp. and summarize the classification, occurrence, mode of action, biological activity, biosynthesis, and development value of each toxin. The phytotoxins presented in the paper will be termed “toxins”, and those toxic to animals will be termed “mycotoxins”.

Literature:

Fungal kingdom is very interesting in both useful and harmful point of view, which includes more than 1.5 million species, but only 100,000 species have been described, out of them 15,000 species cause disease in plants (Maharshi and Thaker, 2012). Due to increasing plants and fungal diversity, the complexity of pathogenic mechanism also increases between them on the morphologically level by forming a highly specialized structure of infections (Hawksworth, 1991; Horbach et al., 2011). Fungi produce various secondary metabolites (SMs) which affect their host plants at different stages of pathogenesis (Berestetskiy, 2008; Friesen et al., 2008a,b; Meena et al., 2015). The fungal pathogenic SMs are regarded as

not essential for life, but their roles are quite versatile (Stergiopoulos et al., 2013; Pusztahelyi et al., 2015; Meena et al., 2016a). The genetically coded possibilities for the production of secondary metabolites, stimuli and the various phytotoxins generally predict the fungal-host plant interactions and pathogenic behavior of fungi.

The plant pathogenic fungi are divided into biotrophic, hemibiotrophic, and necrotrophic pathogens. These different pathogenic life styles require different molecular weaponry. Necrotrophic fungi infect and kill host tissue and extract nutrients from dead host cells. Biotrophic fungi colonize living host tissue and obtain nutrients from living tissue; whereas hemibiotrophic fungi display two phases during the infection process; first is an initial biotrophic phase followed by a necrotrophic stage (Lo Presti et al., 2015). Necrotrophic and hemibiotrophic fungal species basically show the contrasting mechanistic process of promoting disease, and many HSTs and proteins are the examples of effectors which fundamentally overlap (Condon et al., 2013). These life styles of plant pathogenic fungi provide general information about their interaction with the host, although the distinction between biotrophic and hemibiotrophic mode of action is still not so clear.

Alternaria species have shown different life styles i.e., from saprophytes to endophytes to pathogen (Thomma, 2003; Dang et al., 2015). They are very successful pathogenic genus that causes disease in large number of economically important plants, including apple, broccoli, cauliflower, potato, tomato, citrus, pear, strawberry, tobacco, etc. (Meena et al., 2016a). *Alternaria* creates large economic losses due to their host range and their worldwide distribution. Approximately, 300 species of genus *Alternaria* have been identified worldwide which includes *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria arborescense*, *Alternaria brassicicola*, *Alternaria infectoria*, and *Alternaria solani* (Lee et al., 2015). These *Alternaria* species have been reported to cause diseases in nearly 400 plant species, in which *A. alternata* infects almost 100 plant species. It is also responsible for post-harvested diseases in various crops (Coates and Johnson, 1997; Woudenberg et al., 2015; Meena et al., 2017c; Sajad et al., 2017) causing asthma and infection of upper respiratory tract in humans (Kurup et al., 2000). The reasons behind pathogenicity are the production of diverse phytotoxins.

Alternaria mycotoxins have been frequently isolated and reported in fruits and vegetables, such as tomatoes, citrus fruits, Japanese pears, prune nectar, red currant, carrots, barley, oats, olives, mandarins, melons, peppers, apples, raspberries, cranberries, grape, sunflower seeds, oilseed rape meal, flax seed, linseed, pecans, melon, lentils, wheat, and other grains (Patriarca et al., 2007; Ostry, 2008; Logrieco et al., 2009; Andersen et al., 2015; Woudenberg et al., 2015; Meena et al., 2016a,b). More than 70 phytotoxins produced by species of *Alternaria* have been characterized, and include virulence factors that have both non-specific and specific host interactions. Several *Alternaria* SMs have been evaluated by the European Food Safety Authority (EFSA) as potentially causing risks to human health, including alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), altenuene (ALT), and tentoxin (TEN) [(EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011; Rychlik, 2013)]. *Alternaria* produces host-specific toxins as well as non-host specific toxins (nHSTs). Generally, non-host-specific toxins have relatively mild phytotoxic effects, affect a broad spectrum of plant species and are thought to be an additional factor of disease alongside, for instance, penetration mechanisms and enzymatic processes. Although, they generally act as virulence factors and intensify disease symptom severity, they are not absolutely required for establishing disease since they are also toxic to plant species outside the host range of the pathogen. In *Alternaria*, many host-specific toxins have been identified, although the precise action of only a few has been studied in detail. Structures of different toxins are given in Figure 1. Brefeldin A (dehydro-), curvularin, tenuazonic acid, tentoxin, and zinniol are examples of non-host specific toxins that are produced by several *Alternaria* species (Thomma, 2003; Meena et al., 2017a,b).

Pathogenicity of *Alternaria* Species

The genus *Alternaria*, the Hyphomycetes in the Fungi Imperfecti, includes saprophytes on organic substrates and parasites on living plants (Rotem, 1994; Thomma, 2003). The

involvement of an HST in plant disease was first suggested in the black spot disease of Japanese pear in 1933 (Tanaka, 1933). The disease appeared after the new cultivar Nijisseiki was introduced. This cultivar was discovered in 1888 as a spontaneous mutant seedling at a damp yard in Chiba Prefecture, Japan, and the economic cultivation began after 20 years of its discovery. Since then, black spot disease has become a serious threat to farmers growing Nijisseiki pears. Nijisseiki was extraordinarily susceptible to the pathogen, while older cultivars were immune. The causal organism was identified and named *Alternaria kikuchiana* (Tanaka, 1933). Tanaka (1933) reported that the fungus-free culture filtrates showed toxicity to fruits of Nijisseiki, but not to those of resistant cultivars. This work was the first confirmed example of selective toxicity by culture filtrates and suggested the presence of a host-selective fungal metabolite, which was later isolated and named AK-toxin. Studies on the toxin were resumed by several groups in Japan in the 1950s (Nishimura & Kohmoto, 1983). Beginning in the 1970s, HSTs were identified from other *Alternaria* pathogens, and there are now seven diseases caused by *Alternaria* in which HSTs are responsible for fungal pathogenicity (Table 1) (Nishimura & Kohmoto, 1983; Kohmoto & Otani, 1991; Otani et al., 1995; Markham & Hille, 2001; Thomma, 2003). The direct and indirect relationship hypothesis of pathogenicity proposed by Andrew et al. (2009), in which he expected that the genes of fungal genome database are responsible for pathogenicity and geographical distribution. The intensive study of disease pathogenesis via rDNA analysis has enabled phylogenetic classifications and investigations into the extent of DNA mutation (Lv et al., 2012). Further, there is no direct correlation observed between rDNA variant distribution and host-specific pathogenicity in toxins producing fungi (Koch et al., 1991). However, the pathogenic specialization of *A. alternata* might be controlled by some small number of genes which is helpful in HSTs toxin biosynthesis. Repeated DNA sequencing patterns of fungal supernumerary chromosomes suggest their different evolutionary history from essential chromosomes in the same genome and they may have been introduced into the fungal genome through horizontal gene transfer (HGT) from another species (Rosewich and Kistler, 2000). DNA-DNA reassociation and 16S rRNA sequence analysis are successful in bacterial taxonomy; therefore, these technologies are also applying in fungal taxonomy (Bruns et al., 2003). DNA-DNA analysis has suggested a close relationship between HSTs producing *Alternaria* fungal species and non-pathogenic *A. alternata* (Kuninaga and Yokozawa, 1987).

The question arises that how *Alternaria* does actually causes disease, and which symptoms result? The fungus on the attack of host plants secretes some hydrolytic enzyme during penetration process to gain entry into plant tissue and at that time plants also secrete some chemical compounds. In this way, there is a generation of cell fragmentation of plants and fungi. These oligosaccharides compounds can elicit broad host range defense responses that slow pathogen ingress. Thus, the rapid elicitation of plant's defense responses mandates the successful pathogenic fungi must have developed strategies to suppress the response or avoid the host's potential responses (Jackson and Taylor, 1996; Knogge, 1996). The effect of these fungi is mainly on genetics, mode of action, and biosynthesis. The low weight SMs have their target on the biochemical pathway, and their action that can have pleiotropic effects on host plant metabolism.

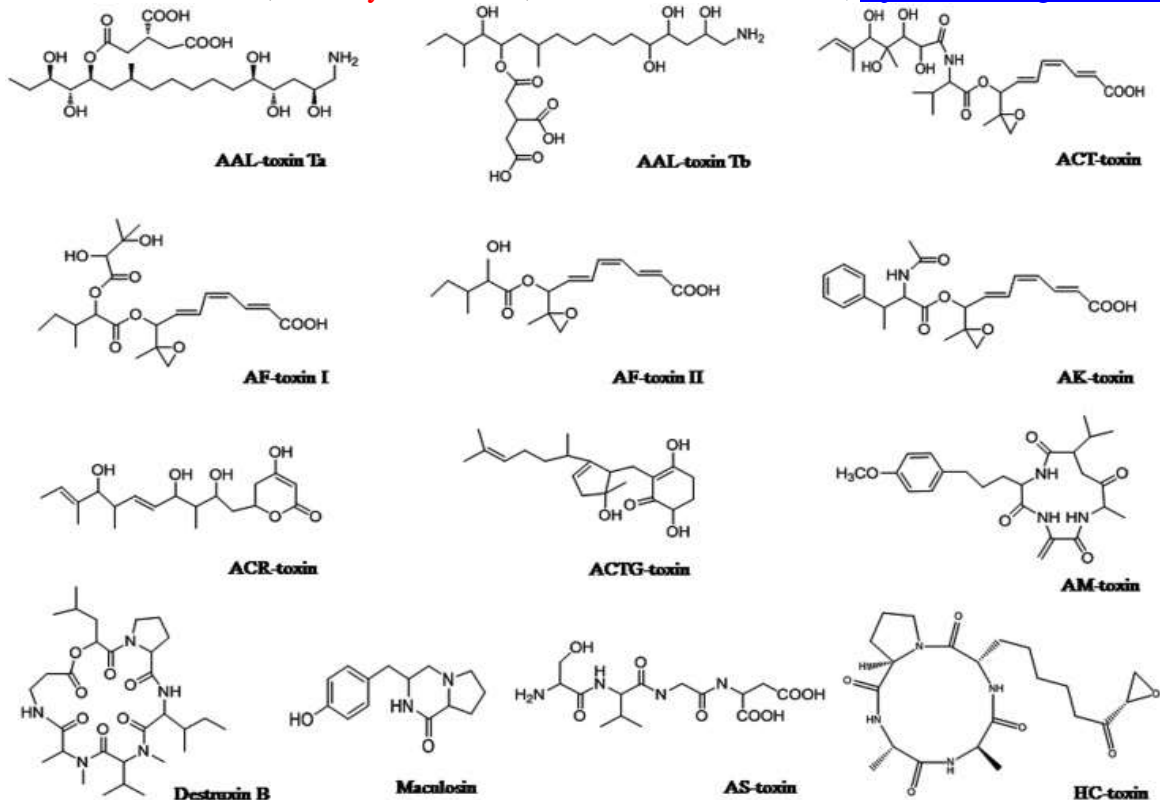


Figure 1: Chemical structures of host-specific toxins produced by various species of *Alternaria*

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