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MiniReview

# Photoactivated perylenequinone toxins in fungal pathogenesis of plants

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## Abstract

Several genera of plant pathogenic fungi produce photoactivated perylenequinone toxins involved in pathogenesis of their hosts. These toxins are photosensitizers, absorbing light energy and generating reactive oxygen species that damage the membranes of the host cells. Studies with toxin-deficient mutants and on the involvement of light in symptom development have documented the importance of these toxins in successful pathogenesis of plants. This review focuses on the well studied perylenequinone toxin, cercosporin, produced by species in the genus *Cercospora*. Significant progress has been made recently on the biosynthetic pathway of cercosporin, with the characterization of genes encoding a polyketide synthase and a major facilitator superfamily transporter, representing the first and last steps of the biosynthetic pathway, as well as important regulatory genes. In addition, the resistance of *Cercospora* fungi to cercosporin and to the singlet oxygen that it generates has led to the use of these fungi as models for understanding cellular resistance to photosensitizers and singlet oxygen. These studies have shown that resistance is complex, and have documented a role for transporters, transient reductive detoxification, and quenchers in cercosporin resistance.

*Keywords:* Plant disease; Non-specific toxin; Active oxygen; Pyridoxine; Zinc cluster transcription factor

## 1. Introduction

Plant pathogenic fungi utilize multiple strategies for infection of host plants. Among them is the production of toxins. Toxins produced by plant pathogenic fungi differ in structure as well as in their role in disease and mode of action [1]. Toxins play diverse roles in disease, from impacting symptom expression and disease progress to being absolutely required for pathogenesis. Some toxins are directly toxic, killing cells and allowing for infection of dead cells. Others interfere with induction of defense responses or induce programmed cell death-mediated defense responses in order to generate necrosis required for pathogenesis [2].

One very interesting group of toxins are the photoactivated perylenequinones. These toxins act by absorbing light energy and generating reactive oxygen species which damage host cells. Studies with toxin-deficient mutants as well as observations on the importance of light in symptom expression have documented the importance of these toxins in pathogenesis, and have led to investigations of toxin resistance as a means of generating resistance to disease. The production of highly reactive oxygen species and the autoresistance

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by the producing fungus have also led to the use of these fungi as models for understanding cellular resistance to singlet oxygen and photosensitizing compounds. This review focuses on our state of knowledge of these compounds, with an emphasis on cercosporin, the toxin produced by fungi in the genus *Cercospora*.

## 2. Production and mode of action of fungal perylenequinone toxins

To date, all of the identified perylenequinone toxins are produced by members of the Ascomycota, the largest phylum within the fungal kingdom. Most producers are classified within the Loculoascomycetes; however, recent reports have documented production by a Pyrenomycete (*Hypomyces*) as well as a Discomycete lichen (*Graphis*) (Table 1). All of the perylenequinones identified to date share a basic 3,10-dihydroxy-4,9-perylenequinone chromophore and differ mainly in side chains (Fig. 1). These fungal compounds show close structural similarity and mode of action to the extended quinones produced by protozoans and plants, for example, hypericin (Fig. 1), the active compound produced by the medicinal herb St. John's wort [3].

The similarity of the fungal perylenequinone structure to hypericin led to investigations of the fungal compounds as photosensitizers [17,18]. Photosensitizers are structurally diverse compounds classified together based on their common ability to be activated by visible and UV-A wavelengths of light and to generate active oxygen species [19]. Many biological molecules are photosensitizers. These include compounds important in human biology such as riboflavin and porphyrins, plant-derived compounds such as chlorophyll, coumarins, and acetylenes, and common dyes such as acridine orange, rose bengal, and methylene blue. In nature, photosensitizers play diverse roles as defense compounds in plants, pathogenesis determinants in fungi, and as molecules responsible for photomovement of protozoans [20].

Photosensitizers absorb light and are converted to an electronically active triplet state. Triplet state photosensitizers react in one of two ways. They may react by electron transfer (radical) reactions via a reducing substrate (type I reaction), leading to the production of a reduced sensitizer molecule. This molecule may react directly with cellular molecules or with oxygen, leading to the production of lipid free radicals and active oxygen species such as superoxide ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^\bullet$) [21]. Alternatively, the triplet sensitizer may react directly with oxygen by an energy transfer mechanism (type II reaction) [19] leading to the production of the non-radical, but highly toxic singlet oxygen ($^1O_2$). Almost all macromolecules in cells are susceptible to oxidative damage caused by photosensitizers. Most commonly, photosensitizers damage lipids, proteins, and DNA, with the type of damage being determined by where the photosensitizer molecule localizes in cells such as in the membranes, cytoplasm or nucleus [22].

Table 1
Production of perylenequinones by fungi

| Fungal species | Ecological niche | Compound(s) | References |
|---|---|---|---|
| *Alternaria* species<br>*A. alternata*<br>*A. eichorniae* | Decay saprophytes on fruits and vegetables; pathogens of water hyacinth and spotted knapweed | Alteichin<br>Alterlosins<br>Altertoxins<br>Stemphyltoxin III | [4–6] |
| *Cercospora* species | Plant pathogens (multiple host species) | Cercosporin<br>Isocercosporin | [7] |
| *Cladosporium cucumerinum*<br>*C. cladosporioides*<br>*C. herbarum*<br>*C. phlei* | Saprophyte; pathogens of cucumber, sugar beet, and timothy | Calphostin C<br>Cladochrome<br>Ent-isophleichrome<br>Phleichrome | [8–11] |
| *Elsinoe* species | Pathogens of citrus, other plant species | Elsinochromes | [11] |
| *Graphis hematites* | Lichen | Isohypocrellin | [12] |
| *Hypocrella bambusae* | Pathogen of bamboo | Hypocrellins | [11,13] |
| *Hypomyces* species | Pathogen of mushrooms, shelf fungi | Hypomycin A | [14] |
| *Scolecotrichum graminis* | Pathogen of orchardgrasss | Cercosporin<br>Isocercosporin<br>Acetylisocercosporin | [15] |
| *Shiraia bambusicola* | Pathogen of bamboo | Shiraiachromes | [16] |
| *Stemphylium botryosum* | Saprophyte; plant pathogen (multiple host species) | Stemphyltoxins<br>Altertoxins | [4] |