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The photobiology of *Metarhizium acridum*: light quality, stress tolerance, and gene regulation

A fotobiologia de *Metarhizium acridum*: qualidade de luz, tolerância ao estresse e regulação gênica

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RESUMO

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Metarhizium acridum é um importante fungo entomopatogênico utilizado no controle biológico de insetos praga. O sucesso do controle biológico depende majoritariamente da habilidade do fungo em tolerar fatores ambientais geradores de estresse tais como o calor e a radiação ultravioleta. Um dos fatores ambientais geradores de estresse é a radiação solar ultravioleta-B (UV-B, 280-315 nm), que é capaz de atrasar a germinação dos conídios e até causar inativação do fungo, reduzindo assim a eficiência do controle de insetos. Foi anteriormente observado que o crescimento de *M. acridum* na presença de luz visível induz o fungo a produzir conídios com elevada tolerância à radiação UV-B. A luz visível é um importante estímulo para muitos fungos, pois ela regula diversos processos biológicos e serve ainda como sinal espacial e temporal. A resposta à luz em fungos varia de acordo com a qualidade de luz e pode ser dividida em respostas à luz azul, verde e vermelha. Na presente tese, três importantes questões são abordadas: (1) qual qualidade de luz (azul ou vermelha) é responsável pelo aumento da tolerância à radiação UV-B após exposição à luz? (2) Como a luz aumenta a tolerância à radiação UV-B? (3) Como a luz regula a expressão gênica tanto no nível transcricional como no pós-transcricional? Aqui é mostrado que a luz azul, e não a luz vermelha, aumenta a tolerância do fungo à radiação UV-B. Além disso, a luz induz a expressão de um gene que codifica uma fotoliase e foi observado que a fotorreativação, e não o reparo no escuro, é o principal mecanismo envolvido na tolerância à radiação UV-B. O uso da transcriptômica via sequenciamento de mRNA revelou que a luz regula a transcrição de aproximadamente 11% dos genes no genoma. Apesar disso, o uso de proteômica quantitativa mostrou que a luz alterou a abundância de apenas 57 proteínas, ou seja, poucas mudanças no nível de mRNA foram traduzidas em mudanças no nível proteico. A proteômica também revelou que a exposição à luz causou uma redução na abundância de proteínas envolvidas com o processo de tradução, tais como subunidades do fator de iniciação de tradução 3 e proteínas ribossomais. Essa redução na atividade traducional é consistente com um modelo em que a luz atua como sinal e como estresse para a célula. Além disso, a redução na atividade traducional é uma possível explicação para o número reduzido de proteínas reguladas pela luz. Finalmente, os resultados apresentados aqui enfatizam a importância de se medir os níveis proteicos para um entendimento completo da resposta à luz em fungos.

Palavras-chave: *Metarhizium*, fotobiologia, tolerância ao estresse, proteômica, transcriptômica.

ABSTRACT

BRANCINI, G. T. P. **The photobiology of *Metarhizium acridum*: light quality, stress tolerance, and gene regulation.** 2019. 112f. Thesis (Doctorate). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2019.

Metarhizium acridum is an important entomopathogenic fungus currently used for the biological control of insect pests. The success of biological control is heavily dependent on the fungus ability to tolerate environmental stressors such as heat and ultraviolet radiation. One of such stressor is solar ultraviolet-B radiation (UV-B, 280-315 nm), which is capable of delaying conidia germination and even inactivate the fungus, thus reducing insect control efficiency. It was previously shown that growing *Metarhizium* in the presence of visible light induces the fungus to produce conidia with increased tolerance to UV-B radiation. Visible light is an important stimulus for many fungi as it regulates a wide variety of biological processes and serves additionally as a signal for space and time. Responses to light in fungi vary according to light quality and can be divided in responses to blue, green, and red light. In the present thesis, three major questions are addressed: (1) what radiation color (blue or red) is responsible for the increased tolerance to UV-B radiation after light exposure? (2) How does light exposure increase tolerance to UV-B radiation? (3) How does light globally regulate gene expression both transcriptionally and post-transcriptionally? Here it is shown that blue light, and not red light, increases tolerance to UV-B radiation. Also, light induces the expression of a photolyase-coding gene and it was observed that photoreactivation, and not dark repair, is the major component behind UV-B radiation tolerance. Transcriptomics via mRNA-Sequencing revealed that light regulates the transcription of approximately 11% of the genome. However, quantitative proteomics showed that light changed the abundance of only 57 proteins, thus showing that few changes at the mRNA level were translated to the protein level. Proteomics also revealed that light exposure caused a reduction in the abundance of translation-related proteins such as subunits of the eukaryotic translation initiation factor 3 and ribosomal proteins. This reduction in translational activity is consistent with a model in which light is both a signal and a stress to the cell. Furthermore, decreased translational activity is a potential explanation for the reduced number of light-regulated proteins. Finally, the results presented here highlight the importance of measuring protein levels in order to fully understand light responses in fungi.

Keywords: *Metarhizium*, photobiology, stress tolerance, proteomics, transcriptomics.

1 – INTRODUCTION

1.1 – Light responses in fungi

Visible light (400-700 nm) is perhaps the most important environmental signal for many fungi as it regulates a variety of biological processes such as the balance between sexual and asexual development, spore germination, vegetative growth, secondary metabolism, circadian time keeping, phototropism, pathogenicity, nutrient uptake, and stress tolerance (Yu and Fischer, 2019). Visible radiation is made of photons of different wavelengths and fungi possess a range of photoreceptors that sense distinct spectral colors (Fig. 1).

Fungi responses to different radiation colors will vary based on the presence or absence of the necessary photoreceptor as well as the pathways activated or inhibited after light exposure (Yu and Fischer, 2019). In the following sections, we provide an overview of light regulation in fungi focusing on blue, red, and green light responses.

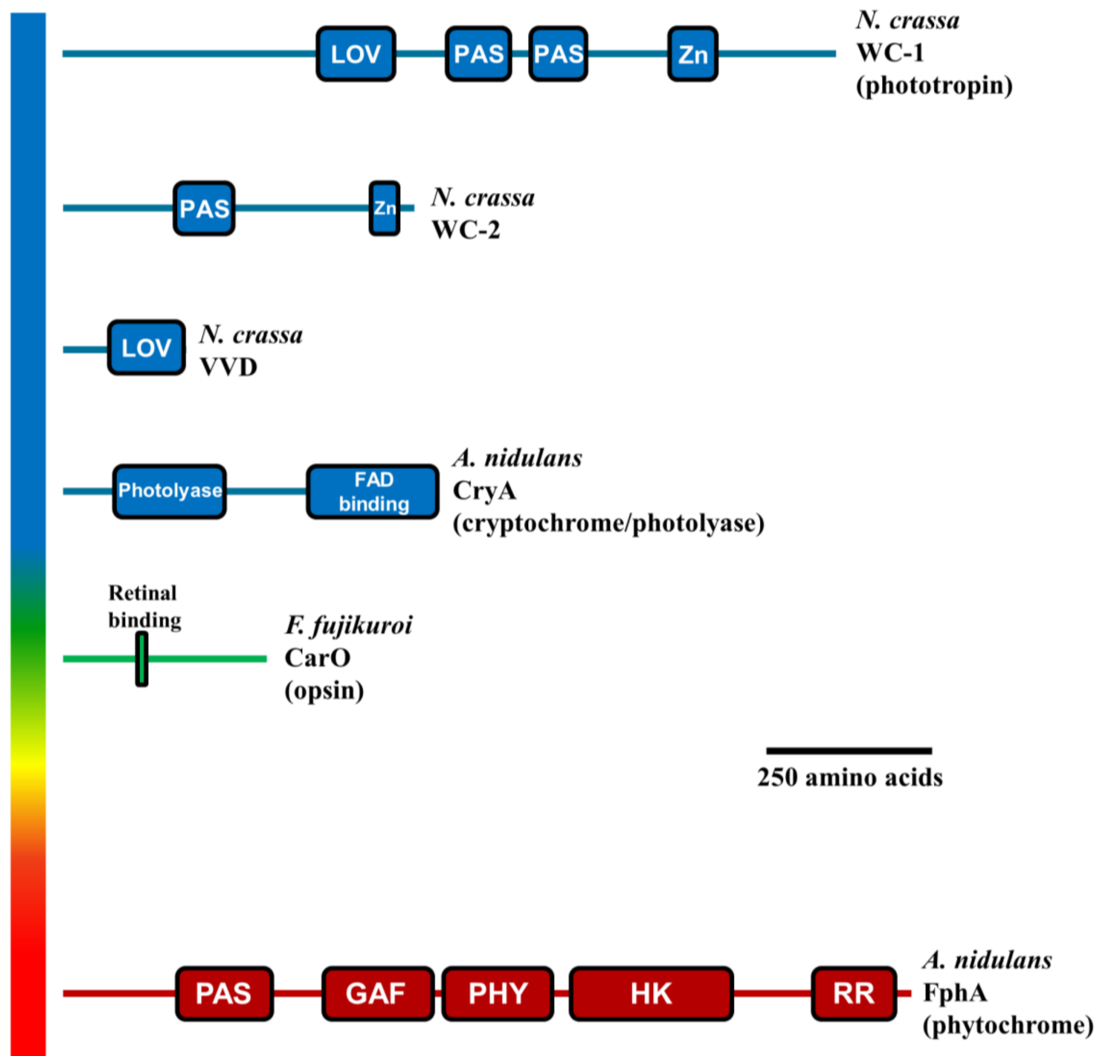

Fig. 1 – Classical fungal photoreceptors from *Neurospora crassa*, *Aspergillus nidulans*, and *Fusarium fujikuroi* responsible for sensing blue, green, and red light. LOV: *L*ight, *O*xygen, *V*oltage; PAS: *P*er-*A*rnt-*S*im; Zn: zinc finger DNA binding; GAF: c*G*MP-specific phosphodiesterase, *A*denylyl cyclase, *F*ormate hydrogen lyase; PHY: phytochrome; HK: histidine kinase; RR: response regulator. Source: adapted from Yu and Fischer (2019)

1.1.1 – Blue light responses

Responses to blue light were first characterized in the model ascomycete *Neurospora crassa*. Light regulates many aspects of *N. crassa* biology including mycelial carotenoid biosynthesis, development, conidiation, formation of protoperithecia, and the circadian clock (Linden; Rodriguez-Franco; Macino, 1997). When growing in slants in the presence of blue light (~450 nm), *N. crassa* will appear

completely orange as both conidia and mycelium accumulate carotenoids. However, mutants for the *wc-1* (*w*hite *c*ollar-1) do not accumulate carotenoids in the mycelium as a response to light. This results in the formation of a white mycelial matt underneath the orange conidia resembling a white collar (Harding and Turner, 1981). Later, it was unveiled that the *wc-1* gene encodes a zinc finger transcription factor binding to GATA-like sequences and is itself light-regulated (Ballario et al., 1996). A second gene, *wc-2*, is also essential for light responses in *N. crassa*. WC-1 and WC-2 interact via their PAS (Per-Arnt-Sim) domains (Fig. 1) and form the heterodimer White Collar Complex (WCC) that then drives the expression of light-regulated genes after illumination (Talora et al., 1999; Yu and Fischer, 2019). Despite the amount of evidence at the time, confirmation that WC-1 was indeed a photoreceptor came only in 2002 after the publication of two studies. In the first, Froehlich and co-workers reported that, in the presence of light, WC-1 binds to specific regions named Light-Responsive Elements (LRE) on light-regulated genes (Froehlich et al., 2002). Simultaneously, He and coworkers reported that WC-1 possesses a specific type of PAS domain named LOV (Light, Oxygen, Voltage) that binds a FAD molecule with peak absorption at 450 nm (He et al., 2002). When taken together, these studies showed that WC-1 is both a bona fide photoreceptor and a transcription factor binding to the promoter regions of light-regulated genes.

More recently, the WC-1-driven blue light response in *N. crassa* was found to be dependent on histone acetylation. Residue K14 of histone H3 associated with *albino-3* (*al-3*) is acetylated after illumination in a WC-1-dependent manner (Grimaldi et al., 2006). A deletion mutant for the histone H3 gene has the same blind phenotype observed for *wc-1* mutants and does not display light induction of *al-3*. Loss of photoinduction also occurs after deletion of *ngf-1* coding for an acetyltransferase

involved in histone acetylation (Grimaldi et al., 2006). Later, it was observed that WC-1 and NGF-1 interact and the resulting complex assembles in the dark on the promoters of light-regulated genes (Brenna et al., 2012). Upon light exposure, NGF-1 acetylates histone H3 and activates gene transcription (Brenna et al., 2012).

In terms of genome-wide gene regulation after illumination, transcriptomic analyses by micro-array revealed that approximately 5.6% of the genome is regulated by light in *N. crassa* (Chen et al., 2009). Light-regulated genes were divided in Early Light Response Genes (ELRG) and Late Light Response Genes (LLRG) with the former peaking within 15-45 min of light exposure and the latter after 45-90 min. The main difference between the two classes is that ELRG are regulated by the WCC itself whereas LLRG are regulated by different transcription factors. However, because LLRG are light-regulated, their transcriptional regulators must also be upregulated after light exposure and some are indeed direct targets of the WCC. Therefore, the authors unveiled that light response in *N. crassa* is hierarchical in nature (Chen et al., 2009). It was also reported that co-regulated genes normally share biological functions. For example, four genes involved in carotenoid biosynthesis present almost identical regulation. Genes involved in DNA repair were classified as ELRG while genes coding for antioxidant enzymes were placed among the LLRG (Chen et al., 2009).

In 2014, a more thorough analysis, performed with RNA-Seq, revealed that as much as 31% of all expressed genes in *N. crassa* are light-regulated (Wu et al., 2014). More importantly, the authors showed that light exposure also results in gene downregulation, which was a then-undescribed phenomenon in *N. crassa* (Wu et al., 2014). In this regard, light exposure resulted in downregulation of genes involved in ribosome biogenesis, a known cellular response to stress (Spriggs; Bushell; Willis,

2010). This is consistent with previous work showing that light exposure represents a stress to the fungal cell (Wang; Yoshida; Hasunuma, 2007; Canessa et al., 2013).

One important protein in the regulation of blue light responses in *N. crassa* is the LOV domain protein VIVID (VVD) (Fig. 1). VVD binds WC-1 and thus interferes with the formation of the WCC (Hunt et al., 2010). Because *vvd* transcription is under the control of the WCC (and is therefore light-induced) VVD plays a role in photoadaptation. Mutants for *vvd* lack the ability to photoadapt and display increased expression of light-regulated genes when grown in constant light (Shrode et al., 2001).

Cryptochromes and photolyases are also relevant blue light photoreceptors in fungi (Fig. 1). Photolyases use blue light to repair UV-damaged DNA whereas cryptochromes are defined as proteins with sequence homology to photolyases but that lack DNA repair activity, normally fulfilling regulatory roles (Sancar, 2003). In *N. crassa*, the gene *cry* encodes a cyptochrome that has been shown to bind both single- and double-stranded DNA (Froehlich et al., 2010). However, CRY does not seem to regulate gene expression for either early or late light-responsive genes (Froehlich et al., 2010). Conversely, in *Aspergillus nidulans* the cryptochrome CryA is a regulator of development. CryA represses sexual development under UV-A light (350-370 nm) and *cryA* deletion mutants undergo sexual development under inappropriate conditions (Bayram et al., 2008). Interestingly, CryA has been shown to possess DNA repair activity in repair-deficient *Escherichia coli* and also when overexpressed in UV-sensitive (*uvsB*Δ) *A. nidulans* (Bayram et al., 2008). In *Fusarium fujikuroi*, the cryptochrome CryD cooperates with the WC-1 homologue, WcoA, to modulate light-induced carotenoid biosynthesis. Furthermore, VVD homologue VvdA regulates WcoA activity and therefore also plays a role in photocarotenogenesis (Castrillo and Avalos, 2015).

1.1.2 – Red light responses

Blue light responses normally dominate and fungi like *N. crassa* respond exclusively to this color. However, some fungi will also respond to red light. It is the case for *A. nidulans*. In addition to homologues for WC-1 and WC-2, named LreA and LreB (*L*ight *R*esponse), *A. nidulans* possesses a fungal phytochrome (FphA) responsible for sensing red light (650~705 nm).

Molecular studies have shown that FphA is a two-component regulatory protein made of photoreceptor and regulatory domains. The photoreceptor domain contains the PAS, GAF (c*G*MP-specific phosphodiesterase; *A*denylyl cyclase; *F*ormate hydrogen lyase), and PHY (*Phy*tochrome) domains and the regulatory domain contains a histidine kinase and a response regulator domain (Fig. 1) (Brandt et al., 2008).

The roles of red light in *A. nidulans* development were reported long ago when Mooney and Yager observed that red light (680 nm) was essential for conidiogenesis and that the fungus reproduced sexually by forming cleistothecia in the absence of light (Mooney and Yager, 1990). Later, it was shown that FphA is responsible for repressing sexual development in the presence of red light (Blumenstein et al., 2005).

In *A. nidulans*, unlike observed for *N. crassa*, both blue and red light regulate development. The blue photoreceptors LreA and LreB (homologues of WC-1 and WC-2) interact with FphA both genetically and physically (Purschwitz et al., 2008). At the genetic level, LreA and LreB are activators of sexual development and are inhibited by FphA under light, leading to asexual development and conidiogenesis. At the molecular level, there is a direct interaction between LreB and FphA (Purschwitz et al., 2008). Therefore, LreA and LreB interact to form a complex similar to the WCC and LreB also interacts with FphA which, in turn, interacts with the master regulator of development velvet A (VeA) (Purschwitz et al., 2008). Furthermore, because development and

secondary metabolism are closely linked in fungi (Calvo et al., 2002), the authors evaluated the effects of light exposure on sterigmatocystin (ST) production. In *A. nidulans*, secondary metabolite production is favored in the dark. Both white and blue light inhibited ST production. However, red light increased ST biosynthesis to levels above those of the dark control with a peak production at 700 nm (Purschwitz et al., 2008).

The phytochrome FphA was also recently found to regulate histone modification in response to light. Photoinduction of the light-regulated gene *ccgA* correlates with acetylation of lysine 9 in histone H3 (H3K9) (Hedtke et al., 2015). Because H3K9 was acetylated after illumination, the authors studied the interactions of VeA, LreA, and FphA with the acetyltransferase GcnE and the deacetylase HdaA. All three proteins interact with GcnE and HdaA. Based on the findings, the following model is proposed: in the dark, VeA and LreA are bound to the *ccgA* promoter and interact with HdaA to prevent transcription of *ccgA*. Upon illumination, LreA is released from the promoter and FphA, together with VeA, induce acetylation of H3K9 via the SAGA/AdaB/GcnE complex, thus activating *ccgA* transcription (Hedtke et al., 2015).

Histone modification is one of the functions that FphA exerts in nuclei. However, a cytoplasmic regulatory role for this photoreceptor was also unveiled. Yu and coworkers performed an *A. nidulans* screening looking for blind mutants and isolated one blind strain that presented a point mutation in the *sakA* gene involved in the high osmolarity glycerol (HOG) pathway (Yu; Armant; Fischer, 2016). Photoinduction of the conidiation-related gene *conJ* and of *ccgA* is abolished in both *fphA* and *sakA* deletion mutants, indicating that light sensing and the HOG pathway were intertwined. Indeed, *conJ* and *ccgA* expression was induced by 0.5 M NaCl. For the HOG pathway to be activated by light, illumination should result in SakA shuttling into nuclei and also

in SakA phosphorylation. A SakA-GFP protein shifted to nuclei after illumination with either white or red light in an FphA-dependent manner. Shuttling into nuclei was also observed with 0.5 M NaCl, but this was FphA-independent. Illumination with white or red light also resulted in FphA-dependent SakA phosphorylation. At the molecular level, FphA was found to directly interact with the phosphotransfer protein YpdA which is part of the two-component system involved in HOG pathway activation. Therefore, light triggers the stress-activated HOG pathway in *A. nidulans* during signaling (Yu; Armant; Fischer, 2016).

So far we have seen that whereas blue-light responses dominate in *N. crassa*, red light is more important to *A. nidulans*. Some fungi display more complex interplay between blue- and red-light responses. It is the case of the plant pathogen *Alternaria alternata*. This fungus possesses blue, red, and green light photoreceptors that were named exactly the same as their *A. nidulans* orthologues. Light induction of the conidiation-related gene *ccgA* is reduced in a Δ*fphA* background and lost in Δ*lreA* strains (Igbalajobi; Yu; Fischer, 2019). This is markedly different from *N. crassa* and *A. nidulans*. In the former, *ccg-1* induction is strictly dependent on WC-1 whereas in the latter *ccgA* photoinduction depends on FphA. There is also interplay between light and HOG signaling pathways in *A. alternata*. Both blue and red light cause HogA phosphorylation and this response is lost in both Δ*fphA* and Δ*lreA* strains together with *ccgA* photoinduction (Igbalajobi; Yu; Fischer, 2019). Interestingly, Δ*fphA* and Δ*lreA* strains display higher tolerance to oxidative stress (H_2O_2 and menadione) and increased expression of genes coding for catalases and superoxide dismutases (Igbalajobi; Yu; Fischer, 2019). This is in contrast to *Botrytis cinerea* in which deletion of WC-1 led to reduced tolerance to oxidative stress (Canessa et al., 2013) and highlights the variability between fungi in terms of light response and photoreceptor roles.

1.1.3 – Green light responses

Green light sensing in fungi is achieved via opsins. The *N. crassa* NOP-1 is a G protein coupled receptor with the classical seven helix transmembrane domain (Bieszke et al., 1999a). A conserved Lys residue in the seventh helix binds all-*trans*-retinal via a protonated Schiff base, thus forming a bacterial rhodopsin-like protein with peak absorption at 534 nm (Bieszke et al., 1999a). The mechanism through which NOP-1 operates is not known, but light exposure causes isomerization of retinal followed by deprotonation of the Schiff base. These are followed by protein conformational changes that likely trigger signal transduction (Bieszke et al., 1999a).

nop-1 mRNA levels increase during *N. crassa* asexual development and after light exposure, indicating it could perform a role in conidiation and/or light response, despite the fact that Δ*nop-1* mutants present no overt conidiation or photoresponse phenotype (Bieszke et al., 1999b). Interestingly, *nop-1* levels were higher in a Δ*wc-2* background, indicating WC-2 can negatively regulate *nop-1* expression (Bieszke; Li; Borkovich, 2007). Furthermore, deleting *nop-1* resulted in increased expression of conidiation-related genes *con-10* and *con-13* irrespective of light exposure (Bieszke; Li; Borkovich, 2007). More recently, photoinduction of *con-6* and *con-10* was found to be higher in a Δ*nop-1* background, indicating that NOP-1 can negatively regulate WCC activity at certain loci, presumably by regulating a WCC repressor (Olmedo et al., 2010).

The plant pathogen ascomycete *F. fujikuroi* has two genes coding for opsins, namely *opsA* and *carO*, and both have been shown to be light regulated (Estrada and Avalos 2009). Strains lacking the *N. crassa wc-1* homologue *wcoA* have increased expression of *opsA* and *carO*, indicating that WcoA is a negative regulator of their expression. However, photoinduction of *opsA* and *carO* is lost in the *wcoA* mutant

(Estrada and Avalos, 2009). *opsA* is homologous to *N. crassa nop-1* and its deletion in *F. fujikuroi* leads to no overt phenotype be it growth, development, virulence or secondary metabolite production. However, there is reduced expression of some structural genes involved in carotenoid biosynthesis, such as *carB* and *carRA* (Estrada and Avalos, 2009).

Unlike *opsA*, *carO* encodes an opsin displaying green light-dependent proton pump activity and it mainly localizes to conidia plasma membrane (Garcia-Martinez et al., 2015). Conidia from $CarO^{-}$ strains germinate faster than those from $CarO^{+}$ strains under illumination, indicating that CarO slows down germination under light (Garcia-Martinez et al., 2015). It is speculated that this is achieved by regulating a local proton gradient as ambient pH can affect conidia germination. Furthermore, CarO could also be playing a role in nutrient uptake by maintaining a proton gradient across the membrane. This could prove energetically advantageous to the fungus as maintaining proton gradients are normally a function of H^{+} ATPases (Garcia-Martinez et al., 2015). Finally, green light sensing opsins are much more prevalent in phytopathogenic and endophytic fungi, presumably because green light is abundant in the phyllosphere as a result of chlorophyll-filtered sunlight (Garcia-Martinez et al., 2015).

1.2 – *Metarhizium*

1.2.1 – *Metarhizium* spp. as biological control agents

Metarhizium (Ascomycota; Sordariomycetes) is a genus of filamentous entomopathogenic fungi that can be found in both cultivated and undisturbed soil worldwide. Owing to their ability of infecting and killing insects, *Metarhizium* spp. have been used for the biological control of insect pests and disease vectors in an attempt to replace – either totally or partially – the use of chemical insecticides (Lacey

et al., 2015). Biological control is usually performed by applying formulations containing fungal conidia in the field. Upon contact with the host cuticle, conidia germinate and invade the insect, eventually causing its death by a combination of hyphal growth, nutrient consumption, and production of toxic metabolites (Kershaw et al., 1999; Scholte et al., 2004).

In 2007, the number of commercial products containing *Metarhizium* spp. worldwide was 47 (Faria and Wraight, 2007). In Brazil, the annual production of *Metarhizium* by ten sugarcane mills and private companies in the states of Alagoas and São Paulo is estimated at 1,882 tons (Li et al., 2010). In the states of Alagoas, Pernambuco, and Sergipe, *Metarhizium anisopliae* is used control the sugarcane spittlebug *Mahanarva posticata* and the root spittlebug *Mahanarva fimbriolata* with an estimated 300,000 ha of sugarcane being treated each year (Li et al., 2010). In China, *Metarhizium acridum* is used to control grasshoppers in grasslands in the northern part of the country. The fungus is also used to control grasshopper populations on rice and bamboo plantations in southern China (Li et al., 2010).

The fungus *M. acridum* is considered a specialist entomopathogen, capable of infecting exclusively orthopteran insects such as locusts and grasshoppers (Hu et al., 2014). Compared to other *Metarhizium* species, *M. acridum* has an increased number of genes related to genomic stability and the fungus is indeed the most tolerant to abiotic stresses such as UV radiation and heat, which is advantageous to biological control (Hu et al., 2014; Braga et al., 2015).

The success of biological control employing mycoinsecticides depends on maintaining fungal viability under stressful field conditions. Environmental factors such as UV radiation and high temperatures, both resulting from solar exposure, can delay conidia germination and even inactivate the fungus (Braga et al., 2001; Rangel et al.,

2005b). Ultraviolet-B radiation (UV-B, 280-315 nm) is known to interact directly with and damage DNA by inducing the formation of cyclobutane pyridimide dimers (CPD) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PP) which hinders germination and growth until the damages are repaired (Nascimento et al., 2010). *Metarhizium* inactivation by heat and high temperatures is also a concern (Keyser et al., 2014) and locusts infected with *M. acridum* bask in the sun to increase their body temperature in a phenomenon termed behavioral fever (Clancy et al., 2018).

Many studies have focused on increasing *Metarhizium* tolerance to environmental stressors, some of which by the genetic engineering of strains (Ortiz-Urquiza and Keyhani, 2015). Tseng and coworkers cloned an *A. alternata* gene for melanin biosynthesis and inserted it in the *M. anisopliae* genome. Because melanin can serve a photoprotecting pigment, there was increased tolerance to UV-B radiation in the mutant strain. Also, increased mortality of *Plutella xylostella* larvae due to a reduction in UV-induced germination delay was also observed (Tseng; Chung; Tzean, 2011). In a similar way, inserting a photolyase coding gene from *Halobacterium salinarum* into the fungus genome resulted in increased survival of the mutant strain after UV-B exposure and increased virulence against *Anopheles gambiae* (a malaria vector) when compared to the wild type (Fang and St Leger, 2012).

While the success of genetic engineering is evident, there are other ways of increasing *Metarhizium* tolerance to stressors without having to resort to transgenic organisms. Both chemical and physical environmental factors are known to regulate *Metarhizium* tolerance to a variety of stresses. For instance, cultivating *M. acridum* on rich medium results in conidia with higher tolerance to UV-B radiation when compared to conidia obtained by infecting the grasshopper *Melanoplus sanguinipes* (Rangel et al., 2005a). Similarly, *Metarhizium robertsii* conidia produced in a medium with

supplemented salicylic acid are more thermotolerant when compared to conidia obtained from non-supplemented medium (Rangel et al., 2012). One environmental stimulus that can regulate *Metarhizium* tolerance to stress is light.

1.2.2 – The photobiology of *M. acridum*

As previously shown, light is an essential environmental signal that regulates many biological processes in fungi. Exposure to visible light also modulates stress tolerance in *Metarhizium*. It was previously shown that growing *M. robertsii* under a 12:12h light:dark photoperiod induces the fungus to produce conidia with increased tolerance to UV-B radiation when compared to conidia obtained from cultures that grew in the dark (Rangel et al., 2011). The genome of *M. acridum* bears genes encoding for all classical photoreceptors except green light-sensing opsins (Table 1).

Table 1 – The *Metarhizium acridum* genome bears genes coding for the classical photoreceptors with the exception of opsins

	Metarhizium acridum	
Photoreceptor	**Name**	**Gene ID**
WC-1	White collar 1	MAC_01685
WC-2	Cutinase gene palindrome-binding protein	MAC_09544
VVD	Cellulose signaling associated protein ENVOY	MAC_03457
Cryptochromes/Photolyases	Putative cryptochrome DASH	MAC_07571
	Cryptochrome-2	MAC_03703
	Photolyase	MAC_05491
Opsins	-	-
Phytochromes	Putative phytochrome-like histidine kinase	MAC_04734

Knowing that light regulates UV (particularly UV-B) radiation tolerance and that *M. acridum* possesses genes encoding for different photoreceptors, three immediate questions rise to mind: (1) what are the effects of different light colors on the acquisition of UV-B tolerance? (2) What is the molecular mechanism behind such

increase in tolerance? (3) How does light globally regulate gene expression in *M. acridum*? These questions are addressed in the following three chapters. In Chapter 1, we investigate the effects of blue and red light on the acquisition of UV-B tolerance and also examine the kinetics of tolerance acquisition after light exposure. In Chapter 2, we study the photoinduction of photolyase- and UV endonuclease-coding genes and the roles of photoreactivation in determining UV-B tolerance. Finally, in Chapter 3 we combine RNA-sequencing and mass spectrometry-based high-throughput quantitative proteomics to study how light regulates gene expression at both the mRNA and the protein levels.

4 – CONCLUSIONS

Light induces tolerance to UV-B radiation in the mycelium of *M. acridum*. This induction in dependent on light quality and only blue light achieves the increase. Light exposure also results in increased photoreactivation ability which is crucial to UV-B radiation tolerance. The molecular mechanism behind this phenomenon is possibly the photoinduction of a photolyase-coding gene with subsequent protein accumulation. Finally, light also affects the transcription of as much as 11% of the genome. However, these changes at the mRNA level are frequently not translated to the protein level. This is potentially due to light also acting as a stress and ultimately causing a reduction in translational activity.

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