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Review

Cellular signaling in cross protection: An alternative to improve mycopesticides

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HIGHLIGHTS

- New technologies based on gene manipulation are substitutes to chemical pesticides.
- Restrictions in the use of GMO limit the application of those alternatives.
- Cellular signaling control can improve quality of conidia from mycopesticides.
- Cross protection to environmental stresses relays on cell signaling pathways.
- Moderate oxidative stress in some entomopathogenic fungi improves conidial quality.

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ABSTRACT

The use of chemical pesticides have damaged the environment and human health over the past 50 years. Biotechnological alternatives include genetically modified plants such as Bt cultures and recently those based on RNAi and CRISPR-Cas9, although resistance may appear in some insects, and also there are restrictions due to legal regulations. Microbial biopesticides based on conidia from entomopathogenic fungi are an excellent choice to face the present situation. In addition to high conidial yields, the quality of conidia should be assured in production processes. Sublethal stress during cultures may lead to cross protection, by which conidia acquire tolerance to other sort of stresses like those found in open fields. These stress response mechanisms involve G proteins and MAPK pathways. These current challenges are discussed here.

Contents

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1. Introduction

Chemical pesticides have controlled agricultural pests during the last 50 years. Nevertheless, the extensive use of these chemicals affects human health (Bassil et al., 2007; Kamel, 2013), and the environment (Jennings and Li, 2014), due to toxicity against non-target organisms (Hallmann et al., 2014). Paradoxically, these compounds may kill other insects, even natural predators of insect pests (Douglas et al., 2014). In addition, pests of up to 500 arthropoda species have developed resistance to several pesticides (Hajek, 2004). Furthermore, tons of expired pesticides are disposed of without any precaution (FAO, 2016). Nonetheless, agriculture requires the use of chemical pesticides in order to maintain production and avoid aggressive price variations, although the current agricultural policy is unsustainable due to the negative impacts mentioned above.

Besides keeping (or increasing) production in agriculture, a rational use of the resources, i.e. the sustainability of cropping, is a critical goal (Bulgari et al., 2015). The demand for environmentally friendly agricultural practices has increased (Duc et al., 2015); this would warrant environmental health as a strategy to support sustainability in agriculture, leading to even higher production and the suitable prevention of damages by pests since ecosystems are not altered (FAO, 2014).

Integrated Pest Management (IMP) comprises the development of healthy crops, minimizing the damage in agroecosystem; IMP is mainly based on natural methods of pest control such as biopesticides (FAO, 2014), which are obtained from natural materials (EPA, 2016), with great prospect when used properly (Seiber et al., 2014). In recent years, biotechnological mechanisms have been developed for the control of pests, which are used in the present and are projected to be used in the near future.

Nowadays, the main biotechnological mechanism for pest control are crops expressing the bacterial endotoxin from *Bacillus thuringiensis* (Bt). The first genetically modified plant (corn) which expressed the *B. thuringiensis* endotoxin was registered in Environmental Protection Agency (EPA) in the United States in 1995. Even though the use of this technology has increased worldwide, some limitations still remain; one of the most important is the inability to control certain insect plague species (Bortolotto et al., 2014), as well as cases of resistance acquired by some insects to this control technique (Tabashnik et al., 2013). Besides, many restrictions for the implementation of GM crops still exist, although sowed areas have increased up to 94-fold comparing to that reported in 1996 (Raven, 2014). In Europe, for instance, the only GM field occupies 114,000 ha (Bt Corn MON810), which represents less than 1% of the total corn fields in this continent (Lotz et al., 2014). Besides, the paper claiming the appearance of tumors and other diseases in rats fed with transgenic corn is still debated, after hard criticism from scientific community causing the subsequent removal. Nevertheless, attending such criticism, this paper was recently republished (Seralini et al., 2014). Finally, according to a World Bank report (World Bank, 2008), transgenic technology is still controversial, due to the perception of potential risks towards the environment and human health.

RNA interference (RNAi) is a method projected to have an impact on the control of insect pests in the near future. In eukaryotic cells, this is a natural mechanism of post-transcriptional regulation in gene expression, where the aims of such regulation are messenger RNAs or mRNA's (Zhu, 2013). The first related study was with plants, at the end of the 1980s (Napoli et al., 1990). Further studies with animals were performed, specifically with the *Caenorhabditis elegans* worm (Fire et al., 1998). The basis of RNA interference is essentially that, following the artificial introduction of a double-stranded RNA (dsRNA), silencing of the target gene will occur, i.e., the gene has a similar mRNA sequence to the artificially-introduced fragment (Belles, 2010). Currently, the use of this genetic regulation pathway was suggested for insect plague control, based on the genetic modification of plants of agricultural interest. For example, Raza et al. (2016) reported a mortality of more than 70% was achieved in the insect pest *Bemisia tabaci* using transgenic plants of *Nicotiana tabacum*. In addition, there are publications that summarize the perspectives of this genetic technique for the control of pests such as *Helicoverpa armigera* (Lim et al., 2016), aphids (Yu et al., 2016) and coleopterans (Baum et al., 2007). Finally, San Miguel and Scott (2016) studied the use of dsRNA actin for the control of the Colorado potato beetle (*Leptinotarsa decemlineata*); the novelty of this research is that the dsRNA was used to protect potato plants by foliar application, avoiding the genetic modification of the plant. Some expectations in the control of insect pests using RNAi are addressed more extensively by Huvenne and Smagghe (2010).

Concerning RNAi, one of the crucial aspects to consider in this control method are the selection of genes to be silenced, as well as the uptake dose required for insect plague control. Nevertheless, just as with Bt crops, there are many use limitations; a couple of the most important are the fact that several insect pests are naturally resistant (Baum and Roberts, 2014; Gu and Knipple, 2013), and resistance has developed in insects, perhaps due to polymorphisms in the sequence of the target gene (Gordon and Waterhouse, 2007).

A third mechanism for the control of agricultural pests, postulated to be relevant in the future, is the Clustered Regularly Interespaced Short Palindromic Repeats (CRISPR). This technique is based on an ancient mechanism in which bacteria acquire protection against viral infections. In this technique, short sequences of RNA (guide RNA's o gRNA's) bind to a specific nuclease (like Cas9) to silence or modify specific genes which have homologies with the gRNA. Even when it is recent the discovery and develop of this genetic technique, the use has been studied for the control of insect pests as *Spodoptera litura* (Bi et al., 2016) and the insect model *Tribolium castaneum* (Gilles et al., 2015). However, before the general use of this technique in the control of pests, the methods must be improved to achieve the specificity in the modification of the target gene found in the pest of interest. In addition, it is also important to consider the possible alternate mutations, off-target mutations, caused in the target pest if the gRNA is poorly designed (Webber et al., 2015).

According to the Environmental Protection Agency (EPA), Bt crops and RNAi are considered as biopesticides, which are specifically named plant-incorporated protectants (PIPs); however, according to legislations in other countries, such technologies should not be considered biopesticides (Seiber et al., 2014).

Thus, alternative methods of plague control are still required considering the current regulations, prohibition of the use of chemical pesticides and the problems found in certain biopesticides now used; emphasis is focused on those showing high efficacy and low risk. In this context, mycopesticides based on conidia from entomopathogenic fungi, are alternatives meeting those requirements with full potential as a biocontrol agent.

2. Entomopathogenic fungi

As part of Integrated Pest Management (IPM) program, biological control uses the ability of organisms such as bacteria, viruses, nematodes and fungi, to infect and kill insect pests. Regarding the entomopathogenic fungi, an interesting hypothesis is that pathogenicity to insects is an evolutionary step, a feature that these organisms developed from an original habitat as endosymbionts of plants. As endosymbionts fungi can provide nitrogen to