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Investigation and molecular docking studies of Bassianolide from *Lecanicillium lecanii* against *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT

Entomopathogenic fungi are rich sources of bioactive secondary metabolites that possess insecticidal properties. The present study reported a novel approach for the identification of insecticidal compounds produced by *Lecanicillium lecanii* 09 and to assess their toxicity against the diamondback moth *Plutella xylostella* L. The cyclic peptides groups of toxic substances were separated from *L. lecanii* 09 through submerged liquid state fermentation. The most abundant toxic metabolite, Bassianolide was purified by high-performance liquid chromatography (HPLC) and its molecular weight and purity were determined by Liquid chromatography – mass spectroscopy (LC-MS), Fourier transformed infrared spectroscopy (FT-IR), and H^1 nuclear magnetic resonance (NMR) respectively. Subsequently, the toxicity of bassianolide was tested against third instar larvae of *P. xylostella* at three different concentrations (0.01, 0.1, 0.5 mg/ml). The results showed that higher concentration of 0.5 mg/ml had significant maximum mortality at 120 hour post inoculation. Furthermore, we investigated the ligand-target interaction of secondary metabolite binding with target insect immune receptor proteins and predicted the role of toxicity against insect host. This is the first study to report the infection process and the interaction of fungal mediated cyclicdepsipeptide compound (bassianolide) from *L. lecanii* 09 against the insect host *P. xylostella*. This novel approach provides a potential impact on biological control using natural toxic compound which acts as good inhibitor on pest insect and prevents toxicity hazards, pollution as well as ecocidal effects killing several beneficial insects.

1. Introduction

The diamondback moth *Plutella xylostella* L. (DBM) (Lepidoptera: Plutellidae) is one of the most devastating cosmopolitan pests of cruciferous crops causing huge economic loss to the farmers (Batta et al., 2011; Talekar and Shelton, 1993).This serious damage is generally caused by third and fourth instar larvae, which feed on the leaf surface thereby reducing the size and yield of the crucifers (Somvanshi and Ganguly, 2007). The increased occurrence has caused severe cruciferous vegetables losses > 70% annually, particularly in China, India, East Africa, and South America, its management of expenses have totaled about 1.2 billion US dollars annually (Godonou et al., 2009). In the recent years, conventional farming has seen a dramatic increase in the use of various synthetics pesticides such as organophosphates, carbamates, and pyrethroids to control DBM infestations, especially in the larval stages (Correa-Cuadros et al., 2016; Furlong et al., 2013). The widespread applications of synthetic insecticides also kill the beneficial antagonists of DBM, and thus have a negative impact on the natural ecosystem. Continuous applications and increased dosage of synthetic pesticides towards *P. xylostella* develop the resistance against these insecticides (Zhao et al., 2006). Alternatively, to minimize this problem, different natural biocontrol agents including entomopathogenic fungi such as *s Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria* sps. and *Lecanicillium* sps. were used against *P. xylostella* (Duarte et al., 2016; Godonou et al., 2009; Gopalakrishnan, 1989; Silva et al., 2003; Xu et al., 2011). These isolate often cause mortality within short-span and

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are considered to be safer than synthetic insecticides for both the environment and human consumption.

Entomopathogenic fungi (EPF) are considered to be one of the most promising alternative approaches for conventional use of synthetic insecticides, as they have been developed as commercial biocontrol agents against insect pests such as whitefly,aphids, psyllids, termites, mosquitoes (Dal-Bello et al., 2006; Ekesi et al., 2001; Hidalgo et al., 1998; Humber, 2008; Rice and Cogburn, 1999; Sheeba et al., 2001; Wraight et al., 2000). Generally, fungal infection process on insect pests includes three phases: firstly, the fungal conidia adhering to the cuticle of hosts via non-specific hydrophobic and electrostatic interactions (Boucias et al., 1988); secondly, the conidia germinate, forming appressorium and degrading the cuticle by secreting enzymes like proteases and chitinases that help in the process of penetration by mechanical pressure (Charnley and St. Leger, 1991; Fang and Bidochka, 2006; Wang et al., 2017) and thirdly, the fungi produce blastospores inside the host insect and evade hemocytes by secreting toxins to escape from the immune response of host, disseminating through the haemocoel to invade diverse tissues, and finally causing death of the insect pest. Previous studies on the insecticidal activities during the pathogenic process proved that most of the EPF produced several secondary metabolites with low molecular weight (Amiri et al., 1999; Kim et al., 2002; Nishanth et al., 2014; Ownley et al., 2010; Shin et al., 2013). The advantages of these bioactive compounds are their amenability for mass production, cost- effectiveness, easy storage and efficiency over an extensive range of temperature and humidity (Ravindran et al., 2016; Zimmermann, 2007).

The EPF *L. lecanii* play a crucial role in the control agent of DBM insect populations in nature and has been fast killing efficiency, apparent high virulence than the other species entomopathogenic fungi (Cuthbertson et al., 2005; Liu et al., 2009). It grows naturally in soil and has broad geographical distribution (Goettel et al., 2008). The host immune response factor involved to inhibit or prevent fungal proliferation triggers the EPF to produce immunosuppressive toxins (Kershaw et al., 1999). *Lecanicillium* sp. produces a family of cyclodepsipeptide substances that target broad range of hosts by exhibiting cytotoxic and immunosuppressive effect (Hung et al., 1993). EPF produce a wide array of cyclodepsipeptides such as *Metarhizium anisopliae* (destruxins), *I. fumosoroseus* and *Beauveria bassiana* (beauvericin, beauverolides, and bassianolide), and *L. lecanii* (aphidicolin, helvolic acid, dipicolinic acid, and bassianolide) possessing insecticidal activities against a variety of insect pests have been reported (Bernardini et al., 1975; Vilcinskas et al., 1999; Vega et al., 2008). These findings confirmed that the EPF are rich source of bio-insecticide compounds that can revolutionize the agriculture industry.

However, to our knowledge, only few studies were focused on the compound bassianolide, produced during the infection of *L. lecanii* (Claydon and Groves, 1982; Challa et al., 2014; Kanaoka et al., 1978). These studies were evaluated the pathogenicity of insect pest and limited information of bassianolide from EPF such as *Isaria fumosoroseus* and *Beauveria bassiana*. They also lack the information on dynamic role of secondary metabolites during the pathogenesis and fungal development in in-vivo insect host. For example, Kanaoka et al. (1978) extracted the toxic compound bassianolide from *L. lecanii* and tested the insecticidal activity against silkworm larvae, *Bombyx mori* L. Hence, our study is the first to report the characterization of bassianolide and its immunosuppressive activity against insect host *P. xylostella*. The study aimed to investigate i) synthesis and characterization of toxic compounds from isolate *L. lecanii* 09 and to assess the larvicidal activity against *P. xylostella* in-vitro ii) quantification, purification and identification of toxic compounds through high performance liquid chromatography (HPLC), Liquid chromatography – electro spray ionization - mass spectroscopy (LC-ESI-MS), Fourier transformed infrared spectroscopy(FT-IR) and H^1 nuclear magnetic resonance (NMR) analysis and iii) prediction of ligand-receptor interaction through molecular docking study.

2. Materials and methods

2.1. Fungal culture and maintenance

The virulent strain *Lecanicillium lecanii* 09 was obtained from College of Plant Protection, Fujian Agriculture and Forestry University, China and it was maintained in Sabouraud Dextrose Agar (SDA) plate. For long-term storage, the conidial suspension was prepared in 20–30% glycerol solution and stored at −80 °C as a stock culture and sub-cultured for further analysis (Zimmermann, 2007). The white mycelial mat of *L. lecanii* 09 was harvested from 14-day-old sporulating culture by scraping the surface with a spatula and suspended in the sterile 0.05% aqueous (w/v) Triton X-100. The isolate was further cultivated in 250 ml Erlen-meyer flasks containing 100 ml of Czapek Dox (CD) agar medium with 0.5% (w/v) bacto peptone, pH 7.0. The flasks were inoculated with 1 ml of conidial suspension containing 10^7 conidia/ml and incubated at 25 ± 1 °C on an orbital shaker at 150 rpm for 12–15 days.

2.2. Insect rearing

P. xylostella was reared at 21 ± 1 °C and 14:10 (light:dark) photoperiod in the insect rearing laboratory. The larvae were reared on cabbage leaves kept in cages (60 × 60 × 60 cm) and maintained until the required larval developmental stages (III^{rd} and IV^{th} instar). The adults were separated from the cages and transferred to separate cages for mating and oviposition. Adults were fed on an artificial diet consisting of sucrose (50 g), Methyl *p*-hydroxy benzoate (1 g), vitamin stock solution (5 ml), and water (500 ml). Cotton balls soaked in artificial diet solution were placed in the cages. The eggs were removed daily twice and the larval stage was maintained (Freed et al., 2012).

2.3. Extraction of crude secondary metabolites

Mycelium from the 14-day-old culture of *L. lecanii* 09 isolate was blended and separated by centrifugation. The bioactive compounds were recovered from the isolate after the incubation period of 14 days. Extracellular metabolites were separated from the cells using methanol: chloroform (1:2, v/v). Further, impurities of fatty acids were removed by centrifugation and the compound was repeatedly extracted with these solvent. The organic layer was collected and concentrated in a vacuum rotary evaporator. By this way, partially purified insecticidal toxic compounds were obtained. The bioactive compounds were analyzed and its structures were determined using analytical methods.

2.4. Preparative HPLC analysis

The crude extract was dissolved in methanol and acetonitrile-soluble mobile phase was flashed in chromate graph on a Merck (Darmstadt, Germany) K-60 silica gel column (230–400 mesh, 240 × 325 mm) and employed in a 50-ml stepwise methylene: dichloride-methanol (95:5, at the beginning) solvent gradient to give desired polarities. Further purification was performed on a preparative HPLC column (Merck LiChrosorb, 7 mm, and 250 × 310 mm). The sample was filtered, diluted with MeOH and analyzed using reverse phase HPLC column and the peaks were detected by UV absorption at 240 nm. A typical running gradient was used as follows: 0 min (0% acetonitrile), 30 min (40% acetonitrile), 40 min (50% acetonitrile), 60 min (50% acetonitrile) to collect the two fractions at 3.2 min and 7.2 min. Purity of the compound was calculated based on the total HPLC chromatogram peak area determined at 240 nm where the response coefficient for all the presented components was assumed to be equal. Fractionated samples were collected and tested for further biological activity (Ravindran et al., 2014).