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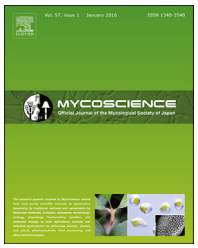
MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc



Full paper

Effects of culture conditions on conidial production of the sweet potato whitefly pathogenic fungus *Isaria javanica*

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form
10 September 2015
Accepted 15 September 2015
Available online 23 October 2015

Keywords:
Bemisia tabaci
Conidia production
Entomopathogenic fungi

ABSTRACT

Sweet potato whitefly, *Bemisia tabaci* biotype Q has been recognized as one of the most destructive insect pests worldwide because of increased resistance to some insecticide groups. To develop an alternative control agent, the effects of different culture conditions such as fermentation methods, culture temperatures, initial inoculum concentrations, substrates and medium supplements on the conidial production of the isolate *Isaria javanica* Pf04 were assessed. The results demonstrated that conidia of the isolate could be economically produced by single-phase solid state fermentation on barley substrate at 25 °C with 10^8 conidia/g of initial substrate concentrations. Using optimal conditions, the maximum conidial production obtained was 3.5×10^9 conidia/g dry substrate after 15 d of cultivation. The conidia, which were produced on barley alone and barley with 5% additives of silkworm powder and moth larvae, were virulent against second instar nymphs of whitefly, providing ≥90% mortality.

1. Introduction

The sweet potato whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) continues to be a major pest of mainly vegetables and ornamental crops worldwide (De Faria and Wraight, 2001; Nomikou et al. 2001; Cuthbertson et al. 2010). It is among the most destructive and widespread insect pests of a broad range of field and greenhouse crops (Mascarin et al. 2013). *Bemisia tabaci* can damage crops directly by feeding on phloem sap, and indirectly by the large amounts of sticky honeydew produced so lowering rate of leaf photosynthesis (Huang et al. 2010). It is also a vector of different plant viruses, especially tomato yellow leaf curl virus which seriously damage tomato (Jones 2003).

The sweet potato whitefly is a species complex containing more than 30 morphologically indistinguishable species (Boykin et al. 2007; De Barro et al., 2011; Liu et al. 2012). Among these, the B and Q biotypes are the most serious pests. Both are the main vectors for over 110 plant viruses that cause huge economic losses (Li et al. 2010; Hsieh et al. 2011). Because of its high propensity to develop resistance to insecticides and

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http://dx.doi.org/10.1016/j.myc.2015.09.002

insect growth regulators (Horowitz et al. 2003), the Q biotype of *B. tabaci* is extremely problematic in agricultural production (Han et al. 2013).

Resistance of Q biotype of sweet potato whitefly populations has resulted in research investigating alternative management options (Arno et al. 2008; Dennehy et al. 2010). Unlike other biocontrol agents, entomopathogenic fungi play important role in the natural regulation of pest populations, because they infect by penetrating the pest cuticle directly and need not be ingested to initiate disease (De Faria and Wraight, 2001). Particularly against phloem feeding pests, entomopathogenic fungi have been recognized as useful biocontrol agents (Feng et al. 2004; Dara et al. 2007).

Many species of entomopathogenic fungi have been reported to control sweet potato whitefly; isolates of *Aschersonia* spp. (Meekes et al. 2002), *Beauveria bassiana* (Santiago-Alvarez et al. 2006; Liu and Stansly 2009), *Isaria fumosorosea* (Hernandez-Torres et al. 2004; Huang et al. 2010), *I. poprawskii* (Cabanillas et al. 2013), *Lecanicillium muscarium* (Cuthbertson et al. 2005; Cuthbertson et al. 2008), and *Metarhizium anisopliae* (Potrich et al. 2011; Islam et al. 2014) and some have been commercially applied in greenhouses or fields for its control (De Faria and Wraight, 2007; Liu and Stansly 2009).

Isaria javanica is an important entomopathogenic fungus parasitic to various pests (Chen et al. 2007) and considered a good candidate for control of agricultural pests because of its ability to cause epizootics naturally (Hu et al. 2007). In our previous research, an isolate Pf04 of *I. javanica* was found to exhibit high virulence against the sweet potato whitefly (Zhu and Kim 2011).

Entomopathogenic fungi do not sporulate readily in liquid culture (Feng et al. 2000). Therefore, the common method used to produce fungal conidia is solid-state or two-phase fermentation. Compared with solid-state fermentation (single-phase cultivation), two-phase fermentation fungi grow in liquid medium for mycelium growth and sporulate on solid substrate for conidia production. To optimize production efficiency of *I. javanica* Pf04 and improve its virulence for *B. tabaci*, conidia productions in single- and two-phase fermentation were compared. In addition, we looked into the optimal temperature, inoculum concentration, cultivation substrate and additives in solid-state fermentation for conidia production. In order to understand how the different substrates and additives impact on conidial virulence of *I. javanica*, bioassays were also conducted on eggplant leaf infested with second instar nymphs of sweet potato whitefly.

2. Materials and methods

2.1. Microorganism and inoculum

Isaria javanica Pf04 was originally isolated from infected Aleyrodidae and used throughout this study. In our previous study, this isolate showed high pathogenicity against *B. tabaci* (Zhu and Kim 2011). The fungus was grown on potato dextrose agar (PDA, Difco, Sparks, MD, USA) at 25 ± 1 °C for 15 d. The conidia were harvested from the medium surface by pouring sterilized aqueous Tween 80 (0.05%) and scrubbing with glass bar. The concentration of conidial suspension was counted using a hemocytometer. Germination rates of the conidia which used for next tests, on PDA medium was over 90% after incubation for 24 h at 25 ± 1 °C.

2.2. Comparison of conidia production in single- and two-phase fermentation

Brown rice as solid substrate was soaked in tap water for 30 min, and then strained for 1 h; a 100 g of the rice was placed in each polyethylene bag, followed by autoclaving at 121 °C for 60 min. In the single-phase fermentation experiment, a 15 ml of 1×10^7 conidia/ml conidial suspension was inoculated into the 100 g rice and cultured at 25 °C for 15 d. For two-phase fermentation, two different concentrations (10^5 and 10^7 conidia/ml) were cultured in 100 ml potato dextrose broth (PDB) (Difco, Detroit) in baffled flasks which were held on a rotary shaker (180 rpm) at 25 °C for 3 d. Then, a 15 ml of the culture solution was inoculated into solid media (pre-soaked and autoclaved brown rice) and incubated at 25 °C for 12 d.

After fermentation, 3 g of solid substrate were randomly taken from each polyethylene bag and placed in 27 ml aqueous Tween 80 (0.05%). The suspensions were mixed with vortex mixer for 1 min before filtering through 4 layers of sterilized cheese cloth. Concentration of the conidial suspension was enumerated using a hemocytometer. Conidial viability was determined by inoculating one drop of conidial suspension (10^6 conidia/ml) which were produced from single-phase and two-phase fermentation media, on 1.5% water agar and observing germination after 24 h incubation at 25 ± 1 °C.

2.3. Effects of different temperatures and inoculum concentrations on conidia production

To investigate the effects of temperature on conidia production, 15 ml conidial suspension (10^7 conidia/ml) was inoculated into pre-soaked and autoclaved brown rice and cultured at temperatures of either 20, 22.5, 25, 27.5 or 30 °C for 15 d. To test the effects of inoculum concentration on conidia production, the sterilized rice in plastic bags was inoculated with different conidial concentrations in order to achieve initial substrate concentrations of 10^5, 10^6, 10^7 and 10^8 conidia/g wet rice, followed by incubation of inoculated rice at 25 °C for 15 d.

2.4. Effects of different substrates and additives on conidial production and virulence of I. javanica

To investigate the effects of different substrates and additives on conidial production and virulence of *I. javanica* Pf04, conidial suspension (10^7 conidia/ml) was inoculated into different solid substrates (white rice, brown rice and barley) with 5% of either silkworm powder or ground *Spodoptera exigua* caterpillars as additives and cultured at 25 ± 1 °C for 15 d. The conidial suspensions (10^7 conidia/ml) from barley substrates with different additives were sprayed onto eggplant leaves infested with 2nd instar sweet potato whitefly nymphs as previously described by Zhu and Kim (2011). Whitefly mortality was recorded for 6 d at 25 ± 1 °C. Each bioassay was composed of three replicated leaf discs infested with 82–112 nymphs.