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ORIGINAL ARTICLE

Pathogenicity of *Simplicillium lanosoniveum* to *Coccus hesperidum*

M. Skaptsov[1], S. Smirnov[1,2], M. Kutsev[1], O. Uvarova[1], T. Sinitsyna[1], A. Shmakov[1], A. Matsyura[1]

[1]*Altai State University, Barnaul, 656049, Russia,*
E-mail: mr.skaptsov@mail.ru, m_kucev@mail.ru, uwarowa@mail.ru, t.sinitsyna@list.ru, ssbgbot@mail.ru, amatsyura@gmai.com
[2]*PlantaBio LLC, Barnaul, Russia,*
E-mail: serg_sm_@mail.ru

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Simplicillium lanosoniveum isolate SSBG2 was isolated from the diseased *C. hesper* collected from *Schefflera octophylla* in greenhouse of the South-Siberian Botanical Garden, and identified based on morphological observation and ITS region analysis. The infected plants were inoculated with conidial suspension of the isolate SSBG2 in concentrations $1.0*10^5$/mL. It was showed that after inoculation the *C. hesper* female adults were more vulnerable to infection. Larvae are affected to a lesser extent. The mycelium grows under the scale and cause the death of the insect. It was indicated that *S. lanosoniveum* had high infectivity against *C. hesper*. Infection symptoms appeared on day 7 after the inoculation, the infection reached the peak on day 20. Our study provides a new isolate that affects the *Coccus hesperidum*.
Key words: *Coccus;* entomopathogenic; fungi; PCR; *Simplicillium*

Introduction

The entomopathogenic fungi are parasitic microorganisms of various arthropods and causing acute mycoses. They can spread fast via aerial conidia and infect their host by penetration of the cuticle, grow within the internal fluids and produce toxins which kill the host. After that, the mycelium grows throughout to completing the life cycle by abundant conidial formation. Fungal species can infect a broad range of insect and several biopesticide products are developed for use in agriculture. The isolation and identification of native entomopathogenic fungi are necessary to provide a pool of biological agents for pest control. Fungal species from other areas may not be effective due to environmental differences (Lockwood, 1993). *Beauveria*, *Metarhizium*, *Lecanicillium* are among the most common bioagents. *Simplicillium lanosoniveum* is not widely used. Data on its interaction with other organisms are accumulating only now.

Materials and methods

S. lanosoniveum was isolated from greenhouse of the South-Siberian Botanical Garden. The insects were transferred to potato-dextrose agar (PDA) and incubated at 25 ºC, under diffuse light. The individual fungus colony were transferred to fresh media until a clear culture was isolated. Greenhouse experiment was carried out at 25 ºC, 95 % humidity for 10-14 days. To the Petri dish was added 1 % solution of Tween 20, then colony with aerial conidia were scraped from the agar. Conidial suspension was diluted to concentration 10^5/mL, after that the infected plants were treated. Identification was carried out morphologically and by PCR analysis. For isolate, a portion of a colony was scraped from the agar plates and transferred into a 1.5-ml Eppendorf tube. DNA was isolated by DiamondDNA kit (ABT LLc., Russia). The primers ITS1 5'- TCCGTAGGTGAACCTGCGG -3', ITS4 5'- TCCTCCGCTTATTGATATGC -3' were used for amplification (White et al., 1990). PCR(s) were carried out in 25 mkl reaction mix which included 5 ng DNA, 2.5 mkl 10x PCR buffer and 25 mM $MgCl_2$ (Sibenzyme Llc., Russia), 1 µL 5mM of mix dNTPs (Medigen Llc., Russia), 1 mkl of each 10 mM primer and 1 unit Taq DNA polymerase (Sibenzyme Llc., Russia) in the MyCycler thermal cycler (Bio-Rad, USA) using protocol: 94.0 ºC for 5 min. [94.0 ºC for 30 sec., 56.0 ºC for 30 sec., 72 ºC for 1 min.]x35, 72.0 ºC for 5 min., 4.0 ºC until the end of the process. PCR products were purified using spin columns. Sequencing by Sanger was conducted in the Syntol LLc., Russia. The sequences of ITS (MG807436) obtained in this study have been deposited in GenBank. Similarity checks were done at NCBI website. For further analysis, sequences of LSU and ITS of closely related sequences were

downloaded from NCBI. Alignment was done using Muscle, MEGA 7.0 (Edgar, 2004). Phylogenetic analysis was done using Neighbor-joining statistical method with 10000 Bootstrap replications (Saitou, Nei, 1987; Kumar et al., 2016).

Results and discussion

Phylogenetic analysis using ITS region recovered the fungus in a good-supported (86 %) clade with species of *Simplicillium lanosoniveum* (EF513003.1). The sequence of *Beauveria bassiana* (KC253900.1) was used as outgroup (Fig. 1).

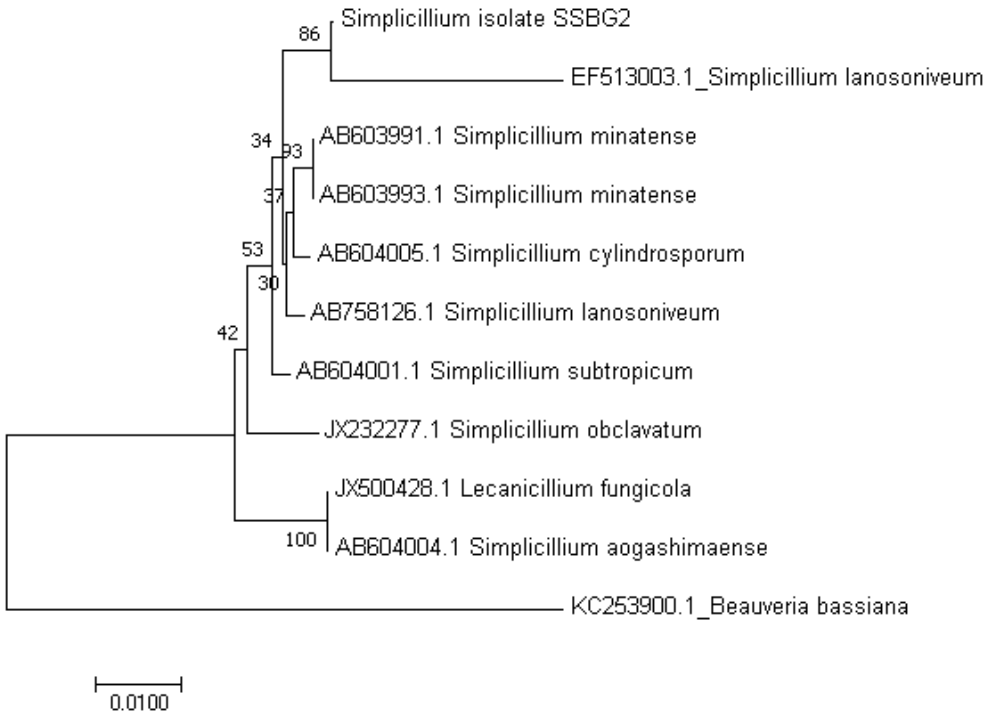

Fig. 1. Phylogenetic analysis based on ITS region of the *Simplicillium* isolate SSBG2 (MG807436).

The isolate showed a good growth rate on the PDA agar. Mature conidia were formed 3-4 days after inoculation. When *C. hesperidum* were inoculated in greenhouse with the prepared *S. lanosoniveum* spore suspension, the insects did not show signs of infection. Mycelia were observed under scale of the adult female *C. hesperidum* after 7 days of inoculation (Fig. 3). The active growth of the mycelium, the larvae damage and reduced the number of larvae were observed on 20 days after inoculation (Fig. 2).

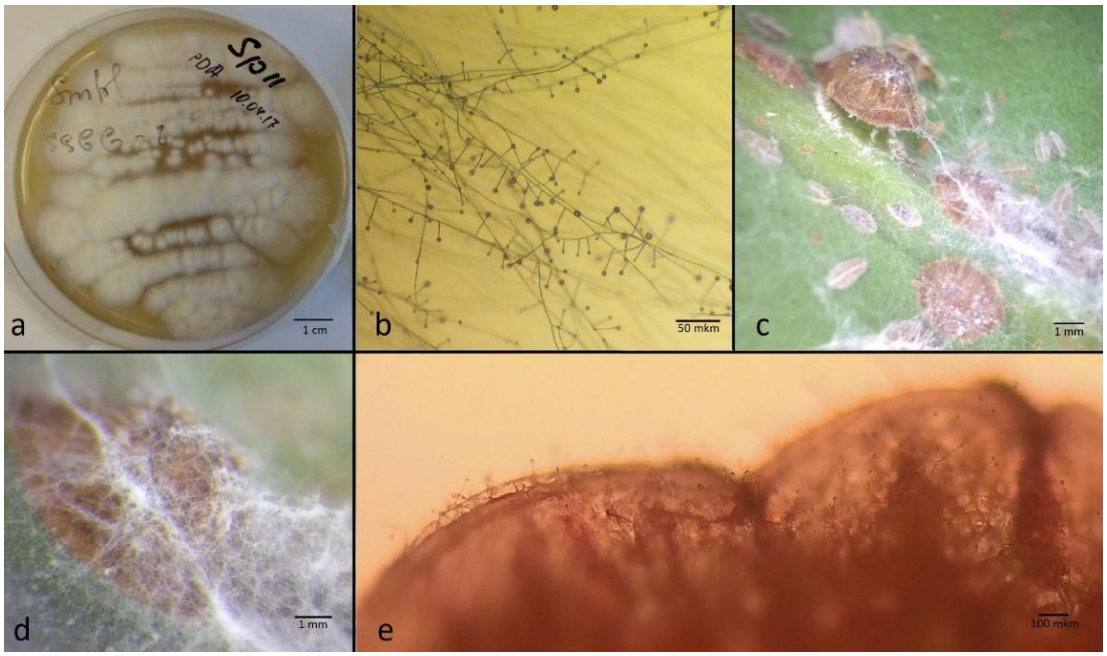

Fig. 2. *Simplicillium lanosoniveum*. a. Agar plate; b. Light microscopic analysis of mycelia and conidia; c-d. Entomopathogenicity assay in *Coccus hesperidum* (greenhouse experiment); *S. lanosoniveum* mycelia under scale of *C. hesperidum.*

Most studies have been devoted to its impact on rust fungi. *S. lanosoniveum* hyper parasitism was observed on *Aecidium elaeagni-latifoliae* isolated in India (Baiswar et al., 2014).

Colonization by the *S. lanosoniveum* was found on a rust fungus *Phakopsora pachyrhizi* (Ward, 2011). *Simplicillium* was associated with *Hemileia vastatrix* and *Uromyces pencanus* (Chen et al., 2017). *In vitro* tests showed virulence of the *S. lanosoniveum* to the silkworm. *S. lanosoniveum* activity is noted to be comparable with *Beauveria bassiana* (Lim et al., 2014). As our work, but on the other scale insects, entomopathogenicity was noted for *Pseudaulacaspis pentagona* (Wang, 2016). *S. lanosoniveum* isolate Cs0701 had high virulence against the aphids, *Aphis gossypii* and *Ceratovacuna lanigera* (Chen et al., 2017). Some works reported that *S. lanosoniveum* can be pathogen of plant parasitic nematodes (Liu, Cai, 2012; Zhao et al., 2013). However, according to some sources, the *S. lanosoniveum* can be a cause of plant diseases. Symptoms of the disease included many irregular, dark brown spots on both upper and lower leaf surfaces were found on the *Salvinia auriculata* and *S. molesta* in Taiwan. Of these, isolates of fungi were obtained and identified morphologically as *S. lanosoniveum* (Chen et al., 2008). In some works, a symbiotic role of *S. lanosoniveum* was identified. For example, for the blue-green alga *Chroococcus* sp. the phenomenon of mutualism with *S. lanosoniveum* was noted. In this case, phototrophic *Cyanobacterium* provides carbon and energy source for *S. lanosoniveum*; as in the aquatic systems, carbon source is relatively deficient and usually the key factor limiting the growth of heterotrophic fungus (Dong et al., 2013). Therefore, a broad assessment of pathogenicity of *S. lanosoniveum* on other organisms needs to be further studied. Our work presents the first data on the pathogenicity of *S. lanosoniveum* in relation to *C. hesperidum*.

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