BACTERIA ISOLATED FROM THE STEM BORER *SESAMIA NONAGRIOIDES* (LEPIDOPTERA: NOCTUIDAE) IN IRAN

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ABSTRACT: *Sesamia nonagrioides* (Lef.) is a major insect pest of sugarcane in southwestern Iran. It also damages maize and rice in this area. Using bacterial pathogens of insects as a biological control agent in an IPM program could be advantageous. In this study, the bacterial flora of *S. nonagrioides* were investigated in dead larvae with appearance of some bacterial disease. Samples were collected from maize and sugarcane fields of Khuzestan province as well as rice fields in Fars province. A total of 11 isolates were characterized from dead larvae of *S. nonagrioides*, according to morphology, spore formation, nutritional features, physiological and biochemical characteristics: *Acinetobacter calcoaceticus, A. baumanii, A. radioresistens* and *A. lwoffii, Enterococcus casseliflavus*, *E. gergoviae*, *Cedecea lapagei*, *Kurthia gibsonii*, *Staphylococcus auricularis*, *Listeria ivanoii* and *Erwinia herbicula*. This is the first report on the bacteria other than Bt from larvae of the stem borer *S. nonagrioides*. May be one or more of these bacteria are causative agents of death in *S. nonagrioides* and are natural suppressors of this stem borer in the fields.

KEY WORDS: *Sesamia nonagrioides***,** bacterial flora, microbial control, Iran.

The stem borer *Sesamia nonagrioides* (Lef.), is considered a major insect pest of maize in southern European countries from Spain to Turkey (Moyal et al., 2011). Larval feeding of *S. nonagrioides* causes yield losses in economically significant level. This stem borer is an important pest of sugarcane, maize and rice in Khuzestan province, southwestern Iran, and has 4-5 annual generations. It attacks several cultivated and non-cultivated graminaceous species (Daniali, 1985; Jemsi & Kamali, 1992; Esfandiari et al., 2011a,b). It also damages maize and rice in some regions of Fars province, South Iran, where it has 3-4 annual generations (Fazeli, 1992).

Apart from the hazardous effects of chemical pesticides on the health and environment, chemical control of stem borers is generally not recommended because the larvae tunnel throughout the stem from the first instar. Using bacterial pathogens of insects as a biological control agent in an IPM program may be advantageous. Studies on bacterial pathogens of *S. nonagrioides* have been focused on *Bacillus thuringiensis* (Bt) (e.g. Farinós et al., 2004; González-Cabrera et al., 2006; Andreadis et al., 2007). Nevertheless, investigating a new pathogen against a target pest is always desirable. It is also known that the success of control can be higher when native isolates are used against the target insect (Sevim et al., 2010). However, very little is known about other bacterial pathogens limiting *S. nonagrioides* populations. In this paper, we present results of a study on the isolation and identification of bacteria from *S. nonagrioides* in southern parts of Iran.

MATERIAL AND METHODS

In order to isolation and identification of the bacteria from *S. nonagrioides*, infected larvae which were soft and flaccid were collected in late summer and autumn 2007, exhibiting symptoms of some bacterial disease (e.g. lack of appetite, failure in movement, and discoloration: blackish color). Collected localities are as follows:

In Khuzestan province: from sugarcane fields of Karun (32°10´N 48°36´E) agro-industriy, as well as adjacent maize fields. In Fars province: from rice fields of Mahkuyeh village in Firuzabad (29°00´N 52°30´E). Larvae were selected and transported to the laboratory in plastic boxes.

A tissue suspension from these larvae were prepared in saline 0.9% after surface sterilization with 70 % alcohol and triturating whole larvae body in a sterile container. After filtration of this suspension through sterile, two layers cheesecloth, 100µl suspension was streaked on nutrient agar and incubated for 2 days at 25°C and 37°C. Isolates were identified based on color and morphology of colonies, gram staining, catalase, oxidase and biochemical tests.

RESULTS

In this study, a total of 11 isolates were finally selected and characterized from dead larvae of *S. nonagrioides*, according to morphology, spore formation, nutritional features, physiological and biochemical characteristics (Tables 1).

DISCUSSION

All of the isolates in our study were determined for the first time from the bacterial flora of *S. nonagrioides*. Although several of the species of *Acinetobacter* spp., *Staphylococcus* spp. and *Enterococcus casseliflavus* have been reported as pathogens for humans and/or other mammals, nevertheless, they have been previously reported from insects. For example; such species have been reported from *Hylesia metabus* Crammer (Lep.: Saturniidae), (Osborn et al., 2002), as well as the cotton bollworm *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) (Xiang et al., 2006). *Acinetobacter lowffii* has been found in *Lutzomyia longipalpis* (Dip. Psychodidae) (Oliveira et al., 2000) and the oil fly *Helaeomyia petrolei* (Coquillett) (Dip.: Ephydridae) (Kadavy et al., 1999). *Acinetobacter baumanii* was also reported from *Bemisia argentifolii* Bellows & Perrin (Hom.: Aleyrodidae) (=*B. tabaci* B biotype) (Davidson et al., 2000).

Enterococcus casseliflavus is one of the most common enterococci in a large variety of insects, including beetles, flies, bees and termites (Willems, 2010). These bacteria are not regarded as primary pathogens, but they are generally recognized as nosocomial pathogens worldwide (Linden & Miller, 1999). *Enterococcus casseliflavus* has been found previously among locusts gut microbiota (Dillon et al., 2010). Channaiah et al. (2010) also showed that *E. casseliflavus* was the most common enterococcal isolate among 95 stored-product insects.

The bacterium *Cedecea lapagei* (Enterobacteriaceae) has found to be pathogenic to the tick *Boophilus microplus*; this bacterium infects ticks via the genital opening and under laboratory conditions can produce up to 100% mortality (Samish et al., 2001). *E. herbicola* bacteria have been found in different insect species. In their studies, Takahashi et al. (1995) and Watanabe et al. (1998) showed that this bacterial species is present in brown planthoppers, mulberry

pyralids, and silkworms, and originate from their host plants. *E. herbicola* was isolated from the majority of populations and biotypes of *T. tabaci* examined by De Vries et al. (2008). We isolated *Enterobacter gergoviae* as well. Several species of *Enterobacter* have been isolated from several insect species and used for biological control purposes (Sandra & Douglas, 2004; Bahar & Demirbag, 2007).

In conclusion, we determined members of the bacterial flora of *S. nonagrioides* larvae. We didn't test the pathogenicity of these bacteria on *S. nonagrioides*, but since all of the tested larvae were showing bacterial disease symptoms in different crop fields with distances up to 500 kms, maybe one or more are causative agents of death in *S. nonagrioides* and are natural suppressors of *S. nonagrioides* populations and could be a promising biocontrol agent for use against this pest. Laboratory experiments are necessary to determine insecticidal activities and pathogenicity of these bacteria and their potential as a biocontrol agent against *S. nona*grioides.

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Table 1. Results of identification of bacteria isolates from *S. nonagrioides* larvae collected in agricultural fields of SW Iran.

Identified species	Sampling locality	Field crop
Acinetobacter calcoaceticus	Karun Agro-industry	Maize + Sugarcane
Acinetobacter baumanii	Karun Agro-industry	Maize
Acinetobacter radioresistens	Karun Agro-industry	Maize
Acinetobacter lwoffii	Karun Agro-industry	Maize
Enterococcus casseliflavus	Mahkuyeh + Karun Agro-industry	Rice + Sugarcane
Enterobacter gergoviae	Karun Agro-industry	Sugarcane
Cedecea lapagei	Mahkuyeh + Karun Agro-industry	Rice + Sugarcane
Kurthia gibsonii	Mahkuyeh	Rice
Staphylococcus auricularis	Karun Agro-industry	Sugarcane
Listeria ivanoii	Mahkuyeh	Rice
Erwinia herbicula	Mahkuyeh + Karun Agro-industry	Rice + Sugarcane