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Investigation of bacterial pathogens of *Chrysomela* (*Melasoma*) *populi* (Coleoptera: Chrysomelidae)

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ÖZ

***Chrysomela* (*Melasoma*) *populi* (Coleoptera: Chrysomelidae)'nin bakteriyel patojenlerinin araştırılması**

Bu çalışmada, *Chrysomela* (*Melasoma*) *populi* (Coleoptera: Chrysomelidae)'nin bakteriyel patojenleri ilk kez çalışılmıştır. İkisi spor oluşturan ve ikisi spor oluşturmayan 4 bakteri izole edilmiş ve VITEK bakteriyel tanımlama sistemleri kullanılarak tanımlanmıştır. İzole edilen bakteriler *Bacillus vallismortis*, *Bacillus thuringiensis*, *Staphylococcus haemolyticus* ve *Aerococcus viridans* olarak tanımlanmıştır. Bu bakterilerin tamamı ilk kez bu böcekten izole edilmiştir. Biyoassay denemeleri, izole edilen *B. vallismortis*, *B. thuringiensis*, *S. haemolyticus* ve *A. viridans* bakterilerinin *C. populi* erginleri üzerinde sırasıyla %65.9, %70.6, %50 ve %53.3 etki gösterdiğini ortaya koymaktadır.

Anahtar kelimeler: *Chrysomela* (*Melasoma*) *populi*, Biyolojik mücadele, Bakteriyel patojen

ABSTRACT

In this study, bacterial pathogens in the populations of *Chrysomela* (*Melasoma*) *populi* (Coleoptera: Chrysomelidae) were investigated for the first time. Four bacterial species, two spore forming and two non-spore forming were isolated and identified using VITEK bacterial identification systems. These bacteria were identified as *Bacillus vallismortis*, *Bacillus thuringiensis*, *Staphylococcus haemolyticus* and *Aerococcus viridans*. All bacteria were isolated from this insect for the first time. Insecticidal potential experiments revealed that the isolated bacteria show mortalities on the adults of *C. populi*. *Bacillus vallismortis*, *B. thuringiensis*, *S. haemolyticus* and *A. viridans* have 65.9%, 70.6%, 50% and 53.3% mortalities on the *C. populi* adults, respectively.

Keywords: *Chrysomela* (*Melasoma*) *populi*, Biological control, Bacterial pathogen

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INTRODUCTION

Poplar plays a significant role in rehabilitation of degraded forests and fragile ecosystems (FAO 2009, Gilman 1997, Konijnendijk 2005, Plotnik 2009, Ürgenç 1998). It is also a very beneficial plant for industrial use. Although its positive benefits, there are multiple biotic risk factors affecting the poplar breeding. Among the biotic risk factors, pest insects damaging poplar plantations are the most important factors hindering the efficiency of sustainable poplar breeding. One of these pest insects, *Chrysomela* (*Melasoma*) *populi* L., a member of the family Chrysomelidae, is known as the most abundant and most important species damaging on *Populus* and *Salix* and it has very extensive distribution from Europe to Asia (Urban 2006). There are several records on the harmfulness of *C. populi* particularly on poplars (Aslan and Özbek 1999, Georgiev 2000, Kasap 1988, Thakur 1999, Urban 2006, Tillesse et al. 2007, Zeki ve Toros 1996). Several control strategies have been tried to struggle with this pest. One of these strategies, chemical control, was extensively used against the pest in different countries because of that it is the most widely known suppressive method (Cavalcaselle 1972, Khan and Ahmad 1991, Tillesse et al. 2007). However, using chemical control method for poplar pests should be taken into account ecological factors (Tillesse et al. 2007). Also alternative strategies should be searched. In this perspective, natural enemies of *C. populi* have been evaluated for controlling these pests in last decades (Teodorescu 1980, Tillesse et al. 2007, Zeki and Toros 1990). Predators (Teodorescu 1980), parasitoids (Lotfalizadeh and Ahmadi 1998), ectoparasite (Tarasi et al. 2001), nematodes (Jolivet and Theodorides 1950, Rauther 1906), microsporidia (Sidor 1979, Sidor and Jodal 1986), fungi (Assaf et al. 2012, Tillesse et al. 2007) and *Bacillus thuringiensis* (Vriesen and Keller 1994) have been extensively studied. There are numerous biology, harmfulness, natural enemies and protection papers concerning *C. populi*. However, its bacterial flora and naturally occurring entomopathogenic bacteria have not been investigated yet. In the present study we aimed at; determination of the bacterial community, isolation and identification of the entomopathogenic bacteria, and testing the insecticidal potential of the identified bacteria of *C. populi* to offer ecologically alternative control agents for this pest.

MATERIAL AND METHODS

Insect samples and bacterial isolation

Adults of *C. populi* were collected from three different localities, Akyazı, İzmit and Samsun in Turkey. The beetles were examined macroscopically to determine any disease symptom. Dead and living adults exhibiting characteristic disease symptoms were selected for bacterial isolation. They were individually placed into 70% ethanol and gently shaken for 3 min and then washed tree times for distilled water for surface sterilization (Yaman et al. 2010). After surface sterilization, a

drop of hemolymph of each beetle was taken, diluted 100 times with sterile water and spread on nutrient agar plates. The plates were incubated at 36 °C for 24-48 h. After incubation, the plates were examined and bacterial colonies were selected (Kuzina et al. 2001, Thiery and Frachon 1997). Different colony type of bacteria were selected and purified on nutrient agar plate by sub culturing. Bacterial strains were maintained for long-term storage in nutrient broth with 15% glycerol at –86 ^{o}C for further tests. The isolates were stored at Department of Biology, Faculty of Science, Karadeniz Technical University.

For identification of bacteria, all bacterial isolates were initially stained by Gram stain for Gram-positive or Gram-negative identification and tested for some biochemical reactions. Then, VITEK bacterial identification systems (bioMerieux, Prod. No; 21341 and 21342**)** were used for the identification of the isolated bacteria. Additionally *Bacillus* species were stained for the presence of crystal protein.

Bioassay with the i**solated bacteria**

The bacterial isolates were tested against *C. populi* adults. *C. populi* adults cause damage by feeding on the leaves of poplars. Therefore the adults were fed with poplar leaves sprayed with the suspended bacterial cells (Ziemnicka 2007). The control group was fed with poplar leaves sprayed with sterilized water.

A number of bioassays tests were carried out using the *C. populi* adults. Totally twenty adults were tested for each bioassay during 21 days. For the control, a set of the insects was fed with sterilized distilled water. All tested groups were kept at 24-28°C and 35-45% RH and 18:6 photoperiod in laboratory conditions (Ziemnicka 2007). Observations were carried out daily and dead adults were removed immediately. All bioassays were repeated 3 times on different days. Percent mortality data were corrected using Abbott's formula. Average mortality of isolates identified as the same species was evaluated.

RESULTS AND DISCUSSION

Chrysomela populi is the most abundant species damaging on *Populus* and *Salix* and it has very extensive distribution from Europe to Asia (Urban 2006). Although there are numerous biology, harmfulness, natural enemies and protection papers concerning *C. populi*, there is no any record on its bacterial flora. This is the first study on the bacterial flora of *C. populi.* In the present study we isolated four bacteria, two spore forming and two non-sporforming from this pest. These bacteria were identified as *B. vallismortis, B. thuringiensis, S. haemolyticus* and *A. viridans* (Table 1). All bacteria were isolated from this insect for the first time.

Two *Bacillus* species, *B. vallismortis* and *B. thuringiensis* were isolated from the population of *C. populi* in Akyazı. Members of the genus *Bacillus* can have different strains or varieties and they can be more virulent on the host insect.

Therefore, different isolates of these bacteria have been tested against pest insects to find more effective ones (Ertürk et al. 2008, Yaman and Demirbağ 2000). For example, a new strain of *B. circulans* was isolated from the larvae of *Culex quinquefasciatus* and *Epargyreus clarus* (Cramer) (Lepidoptera: Hesperidae) (Darriet and Hougard 2002, Brooks et al. 1988). *S. haemolyticus* and *A. viridans* are other bacteria isolated from *C. populi*. The genus *Staphylococcus* includes some entomopathogenic species isolated from insects (Katı and Katı 2013, Manimegalai and Shanmugam 2013, Nagaraju et al. 2012, Yaman et al. 2002). *S. aureus* was isolated from termites and *Bombyx mori* (Lepidoptera: Bombycidae) (Nagaraju et al. 2012, Manimegalai and Shanmugam 2013). *S. sciuri* was isolated from *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae) (Katı and Katı 2013). This bacterium was found the most common isolate associated with adults of the Asian longhorned beetle (Coleoptera: Cerambycidae) (Podgwaite et al. 2013).

Insecticidal potential experiments revealed that the isolated bacteria show mortalities on the adults of *C. populi* (Figure 1). *B. vallismortis*, *B. thuringiensis*, *S. haemolyticus* and *A. viridans* have 65.9%, 70.6%, 50% and 53.3% mortality on the *C. populi* adults, respectively.

Shakoori et al. (1999) isolated *B. thuringiensis* from soil and found 82% mortalities against housefly, *Musca domestica.* Sturz and Kimpinski (2004) found that this bacterium possessed activity against root-lesion nematodes around the root zone of potatoes in soils.

Table 1. Isolated bacteria from *Chrysomela* (*Melasoma*) *populi* (Coleoptera; Chrysomelidae)

Isolate No	**Isolated bacterium**	**Locality**
52, 53, 54	*Bacillus vallismortis*	Akyazı
55	*Bacillus thuringiensis*	Akyazı
56, 77, 81	*Staphylococcus haemolyticus*	İzmit/Samsun
82	*Aerococcus viridans*	İzmit

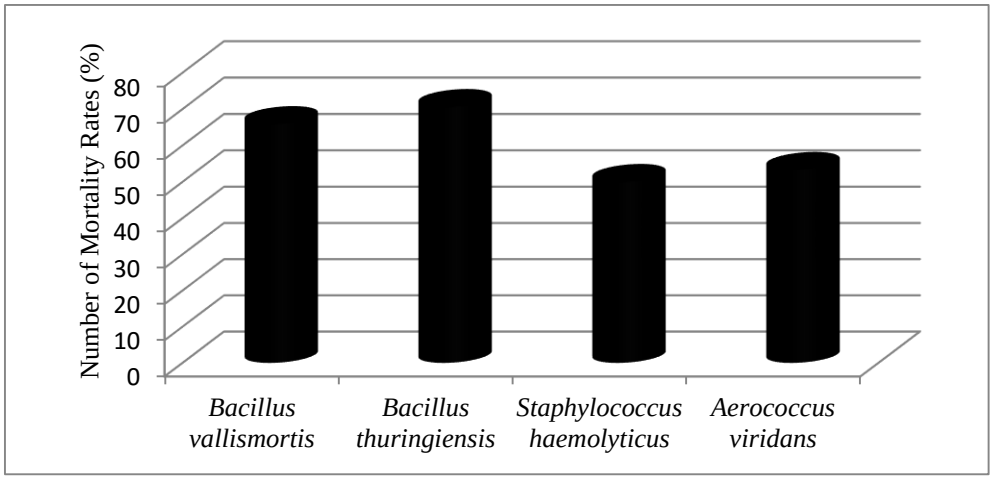

Figure 1. Insecticidal potential of the isolated bacteria on *Chrysomela populi.*

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