

Isolation and identification of bacteria from four important poplar pests

Aislamiento e identificación de bacterias de algunas plagas de álamo

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Abstract: In this study, the bacterial flora of important poplar pests was studied. This included *Cryptorhynchus lapathi* (Coleoptera: Curculionidae), *Sciapteron tabaniformis* (Lepidoptera: Sesiidae), *Nycteola asiatica* (Lepidoptera: Nolidae) and *Gypsonoma dealbana* (Lepidoptera: Tortricidae). The final goal was to propose alternative ecological control agents for poplar pests and decrease the undesirable effects caused by chemical pesticides in urban areas and urban forests. Forty-three bacteria were isolated from the larvae and adults exhibiting characteristic disease symptoms of these pests in five different localities for the first time. All bacterial isolates were cultured and identified using VITEK bacterial identification systems (VITEK® 2 GN ID card prod. no; 21341 and VITEK® 2 GP ID card prod. no; 21342, bioMérieux, Marcy l'Etoile). The members of the genera from Bacillaceae and Enterobacteriaceae families were most commonly isolated from both pest insects.

Key words: Entomopathogenic bacteria. Biological control. Turkey.

Resumen: Se registra el estudio de la flora bacteriana de cuatro importantes plagas de álamo, *Cryptorhynchus lapathi* (Coleoptera: Curculionidae), *Sciapteron tabaniformis* Rott (Lepidoptera: Sesiidae), *Nycteola asiatica* (Lepidoptera: Nolidae) y *Gypsonoma dealbana* (Lepidoptera: Tortricidae) en la búsqueda de agentes de control ecológicamente alternativos contra las plagas del álamo y disminuir los efectos indeseables causados por los plaguicidas químicos en el área urbana y los bosques urbanos. Se aislaron e identificaron cuarenta y tres bacterias de las larvas y adultos de estas plagas a partir de cinco localidades diferentes. Todos los aislados bacteriales fueron cultivados e identificados usando los sistemas VITEK (VITEK® 2 GN ID card prod. no; 21341 and VITEK® 2 GP ID card prod. no; 21342, bioMérieux, Marcy l'Etoile). Los miembros de los géneros de las familias Bacillaceae y Enterobacteriaceae fueron aislados con más frecuencia de ambos insectos de la plaga.

Palabras clave: Bacterias entomopatógenas. Control biológico. Turquía.

Introduction

Poplars play a significant role in afforestation and reforestation, rehabilitation of degraded forests and fragile ecosystems; enhance the contribution of forests and trees in mitigating the effects of climate change; reverse the loss of natural forests and ensure the restoration of landscapes (FAO 2009). The adults and larvae of several poplar pests feed on roots, trunks and leaves. Authorities have been trying to control these pests using chemical insecticides. Chemical control is the most widely known suppressive method, but has many undesirable effects on the natural and beneficial animals, plants and other elements of the environment. Therefore, this method should be discontinued in urban forests (de Tillesse *et al.* 2007). On the other hand, entomopathogenic organisms are safe, sustainable and environmental-friendly control agents and they are highly host-specific; thus, other biotic and abiotic elements of the environment are unharmed (Yaman 2003; Contarini *et al.* 2013; Ruiu *et al.* 2013; Sabino *et al.* 2015). There are only a very few studies (Sidor and Jodal 1986; Vriesen and Keller 1994) that have explored the entomopathogenic organisms which can be used as control agents against poplar pests. Furthermore, there isn't any study on the entomopathogenic bacteria of some important poplar pests such as *Sciapteron tabaniformis* (Rottemburg, 1775) (Lepidoptera: Sesiidae), *Nycteola asiatica* (Krulikovsky, 1904) (Lepidoptera: Nolidae) and *Cryptorhynchus lapathi* Lin-

naeus, 1758 (Coleoptera: Curculionidae). In this study, we aimed at investigating the entomopathogenic bacteria of four important poplar pests: *Cryptorhynchus lapathi* L. (Coleoptera: Curculionidae), *S. tabaniformis* Rott. (Lepidoptera: Sesiidae), *N. asiatica* (Lepidoptera: Nolidae) and *Gypsonoma dealbana* (Frölich, 1828) (Lepidoptera: Tortricidae).

The purpose of this study is to reduce the economic burden caused by poplar pests on the poplar breeding industry and also propose alternative ecological control agents against important poplar pests by decreasing the undesirable effects caused by chemical pesticides in urban areas and urban forests.

Material and methods

Insect samples. In this study, the larvae and adults of four important poplar pests, which were *C. lapathi* L. (Coleoptera: Curculionidae), *S. tabaniformis* Rott. (Lepidoptera: Sesiidae), *N. asiatica* (Lepidoptera: Nolidae) and *G. dealbana* (Lepidoptera: Tortricidae), were collected from five different localities in Turkey from April to September 2013.

Isolation and identification of bacteria from insect pests.

After macroscopic observations, dead and living larvae and adults exhibiting characteristic disease symptoms such as no feeding, slow mowing and colour changes on the body were selected for bacterial isolation. The larvae and adults were

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individually placed into 70 % ethanol and gently shaken for 3 min and then washed three times with distilled water for surface sterilization (Kuzina *et al.* 2001; Yaman *et al.* 2002). After surface sterilization, the insects were dissected in sterilized distilled water depending on their body size to avoid any damage in their guts. A drop of hemolymph was taken from the dissected insect, diluted 100 times with sterile water and spread on nutrient agar plates. The plates were incubated at 25–36 °C for 24–48 h. After incubation, the plates were examined and bacterial colonies were selected (Thiery and Frachon 1997). The selected colonies were purified by subculturing on plates. Different colony types of bacteria were selected and purified on nutrient agar plates by subculturing. Individual colonies were subcultured twice to ensure purity (Kuzina *et al.* 2001). Bacterial strains were preserved for long-term storage in nutrient broth with 15 % glycerol at –86 °C for further tests. The isolates were stored at the Department of Biology, Faculty of Science, Karadeniz Technical University.

All bacterial isolates were initially stained by Gram's dye for the identification of Gram-positive or Gram-negative bacteria and tested for some biochemical reactions. Then, VITEK bacterial identification systems (bioMérieux, Prod. No; 21341 and 21342) were used for the identification of the isolated bacteria. Additionally, *Bacillus* species were stained to reveal the presence of crystal protein (Sharif and Alaeddinoglu 1988).

Results and discussion

In the present study we isolated forty-three bacteria from the larvae and adults of four important poplar pests, which were *C. lapathi* (Coleoptera: Curculionidae), *S. tabaniformis* (Lepidoptera: Sesiidae), *Nycteola asiatica* (Lepidoptera: Nolidae) and *G. dealbana* (Lepidoptera: Tortricidae), in five different localities (Tables 1, 2, 3 and 4). The members of the genera from the Bacillaceae and Enterobacteriaceae families

Table 1. Bacteria isolated from *Cryptorhynchus lapathi* L. (Coleoptera: Curculionidae).

Isolate No	Isolated bacterium	Host Stage	Locality
1	<i>Bacillus vallismortis</i>	Adult	Kocaeli
2	<i>Serratia liquefaciens</i> group	Adult	Kocaeli
3	<i>Enterobacter amnigenus</i>	Adult	Kocaeli
5	<i>Bacillus vallismortis</i>	Adult	Kocaeli
6	<i>Bacillus thuringiensis</i>	Adult	Kocaeli
16	<i>Enterobacter amnigenus</i>	Adult	Samsun
18	<i>Enterobacter cloacae</i> complex	Adult	Samsun
19	<i>Staphylococcus lentus</i>	Adult	Samsun
20	<i>Enterococcus faecalis</i>	Adult	Samsun
21	<i>Enterococcus casseliflavus</i>	Adult	Samsun
22	<i>Enterobacter cloacae</i> complex	Adult	Samsun
23	<i>Bacillus thuringiensis</i>	Adult	Samsun
24	<i>Staphylococcus haemolyticus</i>	Adult	Samsun
25	<i>Bacillus thuringiensis</i>	Adult	Samsun
26	<i>Enterococcus casseliflavus</i>	Adult	Samsun
43	<i>Staphylococcus sciuri</i>	Adult	Samsun

Table 2. Bacteria isolated from *Sciapteron tabaniformis* Rott. (Lepidoptera: Sesiidae).

Isolate No	Isolated bacterium	Host stage	Locality
7	<i>Citrobacter braakii</i>	Adult	Kocaeli
8	<i>Bacillus thuringiensis</i>	Adult	Kocaeli
9	<i>Bacillus vallismortis</i>	Adult	Kocaeli
11	<i>Bacillus vallismortis</i>	Adult	Kocaeli
12	<i>Bacillus vallismortis</i>	Adult	Kocaeli
13	<i>Bacillus thuringiensis</i>	Adult	Kocaeli
14	<i>Bacillus vallismortis</i>	Adult	Kocaeli
15	<i>Bacillus thuringiensis</i>	Adult	Kocaeli
27	<i>Bacillus vallismortis</i>	Larva	Samsun
28	<i>Bacillus licheniformis</i>	Larva	Samsun
29	<i>Enterobacter cloacae</i> complex	Larva	Samsun

were isolated most commonly from both pest insects. It is known that these families include entomopathogenic bacterial species while several species isolated from the insects were found to be pathogenic and tested as biological control agents against insects (Kuzina *et al.* 2001; Yaman 2003; Yaman *et al.* 2002, 2010; Aslan *et al.* 2005; Ertürk *et al.* 2008).

There isn't any study conducted on bacterial pathogens for biological control of *C. lapathi*. In total, sixteen bacteria were isolated from *C. lapathi* L. (Coleoptera: Curculionidae). These bacteria were classified in five different genera: *Bacillus*, *Enterobacter*, *Serratia*, *Enterococcus* and *Staphylococcus*. The isolated bacteria were identified as the members of ten different species, and the species from two different localities that were far from one another had similarities (Table 1). *Bacillus thuringiensis*, *Bacillus vallismortis*, *Enterobacter amnigenus* and *Enterobacter cloacae* were observed to be the most common species of *C. lapathi*. *Serratia liquefaciens* was isolated once. *B. thuringiensis* and *E. amnigenus* were isolated from both populations (Kocaeli and Samsun) of *C. lapathi*, which were far from one another. Most of the *Bacillus* species are insect pathogens and have different insecticidal effects (Yaman and Demirbağ 2000; Ertürk *et al.* 2008). Yaman *et al.* (2010) isolated *Enterobacter amnigenus* from the bark beetle *Dendroctonus micans* and found it to be pathogenic on this pest with a rate of 72.2 % on the larvae and 37.5 % on the adults. One of our isolates from *C. lapathi* was *Serratia liquefaciens*. Some insect pathogenic species of the genus *Serratia* have been isolated from different insects (Yaman *et al.* 2010) and would possibly be the bio-control agents against some insects (Sezen *et al.* 2001). *S. marcescens* is one of the best-known and pathogenic species (Thiery and Frachon 1997). *C. lapathi* is one of the most destructive poplar pests. In the literature, there isn't any study on the bacterial flora of *C. lapathi*. In this study, all bacteria were isolated and identified from *C. lapathi* for the first time.

The second poplar pest investigated in this study was *S. tabaniformis* (Lepidoptera: Sesiidae). Eleven bacteria were isolated from the larvae and adults of this pest. These bacteria were classified in three different genera: *Bacillus*, *Enterobacter* and *Citrobacter*. The isolated bacteria were identified as the members of five different species, and one species from two different localities was the same in both

Table 3. Bacteria isolated from *Nycteola asiatica* (Lepidoptera: Nolidae).

Isolate No	Isolated bacterium	Host stage	Locality
60	<i>Kocuria kristinae</i>	Adult	Mustafa Kemal aşı
61	<i>Pantoea</i> spp.	Adult	Mustafa Kemal aşı
63	<i>Sphingomonas paucimobilis</i>	Adult	Mustafa Kemal aşı
64	<i>Bacillus licheniformis</i>	Adult	Mustafa Kemal aşı
66	<i>Sphingomonas paucimobilis</i>	Adult	Lüleburgaz
67	<i>Kocuria kristinae</i>	Adult	Lüleburgaz
68	<i>Bacillus circulans</i>	Larva	Lüleburgaz
70	<i>Sphingomonas paucimobilis</i>	Larva	Lüleburgaz
71	<i>Staphylococcus aureus</i>	Larva	Lüleburgaz
72	<i>Sphingomonas paucimobilis</i>	Larva	Lüleburgaz
73	<i>Bacillus circulans</i>	Larva	Lüleburgaz
74	<i>Streptococcus alactolyticus</i>	Larva	Lüleburgaz
75	<i>Pantoea</i> spp.	Larva	Lüleburgaz
76	<i>Pantoea</i> spp.	Larva	Lüleburgaz

larvae and adult (Table 2). The members of the genus *Bacillus* were most commonly seen in *S. tabaniformis* populations. Most of the *Bacillus* species are insect pathogens and have different insecticidal effects (Thiery and Frachon 1997; Ertürk *et al.* 2008; Rui *et al.* 2013). Twenty-two *Bacillus* species that are most frequently found in nature are well-identified and recognized worldwide (Thiery and Frachon 1997).

Another poplar pest investigated in this study was *Nycteola asiatica* (Lepidoptera: Nolidae). Fourteen bacteria, six from the adults and eight from the larvae, were isolated from *N. asiatica*. These bacteria were identified as the members of seven different species from six different genera: *Bacillus*, *Kocuria*, *Pantoea*, *Streptococcus* and *Staphylococcus*. Two species from two different localities was the same in both larvae and adult (Table 3). The results showed that bacterial flora of *N. asiatica* consisted of the species from several different genera. *Pantoea* spp., *Bacillus licheniformis* and *B. circulans*, identified from this pest are known to be insect pathogens (Thiery and Frachon 1997; Yaman *et al.* 2010).

Another lepidopteran poplar pest examined in this study was *Gypsonoma dealbana* (Lepidoptera: Tortricidae). Two bacteria were isolated from the larvae from *G. dealbana* collected from two different localities (Table 4). Both isolates belonged to the genus *Pantoea*; *Pantoea* spp. and *Pantoea agglomerans*. Yaman *et al.* (2010) found this bacterium to be pathogenic for *D. micans* with a rate of 86 % on the larvae and 44.4 % on the adults.

In this extensive study, a total of forty-three bacteria were isolated from the larvae and adults of four important poplar

pests, which were *Cryptorhynchus lapathi* L. (Coleoptera: Curculionidae), *Sciapteron tabaniformis* Rott. (Lepidoptera: Sesiidae), *Nycteola asiatica* (Lepidoptera: Nolidae) and *Gypsonoma dealbana* (Lepidoptera: Tortricidae), from six different localities in Turkey. All bacteria were isolated and identified from these pests for the first time, and this study is the first bacteriological study on the biological control of three important poplar pests, that are *C. lapathi*, *S. tabaniformis* and *N. asiatica*. Nineteen bacterial isolates out of 43 were different species. As presented in Tables 1, 2, 3 and 4; the bacterial flora of the important poplar pests has very rich species diversity. Fortunately, some other plant pests (Yaman and Demirbağ 2000) such as *Bacillus* spp. have also been frequently isolated from poplar pests; furthermore, *Bacillus thuringiensis* was the most common bacterium among the isolated bacteria in the present study. Several species of *Bacillus* are insect pathogens and they have different insecticidal effects on insect pests (Yaman and Demirbağ 2000; Yaman *et al.* 2002; Ertürk *et al.* 2008; Rui *et al.* 2013). As shown in tables 1, 2 and 3; this study confirms that poplar pests have a rich diversity in *Bacillus*. On the other hand, *Entorebacter amnigenus*, *Serratia liquefaciens* and *Pantoea agglomerans* isolated and identified from poplar pests in this study also have a potential insecticidal effect on insect pests. These bacteria have a very high pathogenicity against plant pests. Moreover, some bacteria produce chitinase enzyme that degrades chitin, a structural polysaccharide in insects (Zhang *et al.* 2002). The chitinase activity among bacteria has been recorded maximally in the members of the genera *Streptomyces*, *Serratia*, *Vibrio* and *Bacillus*, while this enzyme is of great importance for the suppression of pest insect populations (Reguera and Leschine 2001). The members of the genera *Serratia* and *Bacillus* were the most commonly found bacteria in poplar pests in the present study.

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Table 4. Bacteria isolated from *Gypsonoma dealbana* (Lepidoptera: Tortricidae).

Isolate No	Isolated bacterium	Host stage	Locality
83	<i>Pantoea</i> spp.	Larva	Akyazı
84	<i>Pantoea agglomerans</i>	Larva	Koza

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