Nevşehir Bilim ve Teknoloji Dergisi

Araştırma Makelesi (Research Article)

Makale Doi: **10.17100/nevbiltek.878877**

Geliş Tarihi:12-02-2021 Kabul Tarihi:07-05-2021

Lasiocampa trifolii (Denis & Schiffermüller, 1775)'nin Dahili Bakteriyel Çeşitliliği: Yeni Bir Olası *Okibacterium* sp.

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Öz

Böceklerin bağırsak sistemlerinde bulunan mikroorganizmaların bu böceklerin sindirim ve beslenme süreçlerinde faydalı olduğu bilinmektedir. Bu anlamda, böceklerin bağırsak sistemindeki bakterilerin tanımlanması, özellikle ekonomik olarak zararlı veya faydalı böcek türlerinin besleyici ve sindirim özelliklerinin belirlenmesi açısından özellikle önemlidir. Bu çalışmada, *Lasiocampa trifolii*'nin (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae) dahili bakteriyel florası kültür bağımlı teknikler kullanarak belirlenmiştir. Bu böceğin larvaları, çeşitli otlar ve çalılar, meşe, kavak ve süpürge çimi gibi bitki yapraklarıyla beslenmektedir. Larvalar Mayıs-Haziran (2019) aylarında Türkiye'nin Kırşehir bölgesinden toplanmış ve bakteri izolasyonu için laboratuvara getirilmiştir. Dört adet 3-4. instar larvalar bakteri izolasyonu için kullanılmıştır. İzole edilen bakteriler, 16S rRNA dizin analizi kullanılarak tanımlanmıştır. On bir bakteri izole edilmiş ve bunlar *Bacillus* sp. TT1, *Micrococcus luteus* TT2, *Arthrobacter* sp. TT3, *Corynebacterium* sp. TT4, *Arthrobacter agilis* TT5, *Micrococcus luteus* TT6, *Micrococcus luteus* TT7, *Arthrobacter* sp. TT8, *Okibacterium* sp. TT9, *Staphylococcus haemolyticus* TT10 ve *Bacillus* sp. TT11 olarak tanımlanmıştır. İzolat TT9'un, *Okibacterium* cinsi içinde yer alan yeni bir tür olarak değerlendirilebilmesi için daha ileri moleküler çalışmaların yapılması gerekmektedir. Bu bakteri bu çalışma ile şimdiye kadar herhangi bir hayvandan ilk kez izole edilmiştir. Elde edilen sonuçlar beslenme ve böcek hastalıkları açısından tartışılmıştır.

Anahtar Kelimeler: Bakteri, böcekler, simbiyoz, *Okibacterium*, 16S rRNA

Internal bacterial diversity of *Lasiocampa trifolii* (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae): A possible novel *Okibacterium* sp.

Abstract

Microorganisms in the intestinal systems of insects are known to be useful in the digestive and feeding processes of these insects. In this sense, the identification of bacteria in the intestinal systems of insects is especially important for determining the nutritional and digestive properties of economically harmful or useful insect species. In this study, the internal bacterial flora of *Lasiocampa trifolii* (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae) was determined based on culture-dependent technique. The larvae of this insect are fed with leaves of plants such as various herbs and shrubs, oak, poplar and broom grass. The larvae were collected from Kirsehir region of Turkey in May-June (2019) and brought to the laboratory for bacterial isolation. Four 3-4. Instar larvae were used for bacterial isolation. The isolated bacteria were identified using 16S rRNA sequence analysis. Eleven bacteria were isolated, and these were identified as *Bacillus* sp. TT1, *Micrococcus luteus* TT2, *Arthrobacter* sp. TT3, *Corynebacterium* sp. TT4, *Arthrobacter agilis* TT5, *Micrococcus luteus* TT6, *Micrococcus luteus* TT7, *Arthrobacter* sp. TT8, *Okibacterium* sp. TT9, *Staphylococcus haemolyticus* TT10 and *Bacillus* sp. TT11. Further molecular studies are required for isolate TT9 to be considered as a new species within the genus *Okibacterium*. This has the first time been isolated from any animal so far. The results are discussed in terms of nutrition and diseases of insects.

Keywords: Bacteria, insects, symbiosis, *Okibacterium*, 16S rRNA

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1. Introduction

The lifestyle of many insects is based on their relationship with microorganisms. For example, microorganisms in the intestinal systems of insects have many advantages, such as termites to develop in a wood or soil diet, to feed leaf-cutting ants to fungal gardens in plant nests, and to provide plant sap eating habits of many Hemipteran beetles (aphids, white flies, etc.). More generally, all insects investigated so far have built-in microorganisms, and while some taxons are not necessarily linked to microbionts, there is also increasing evidence that these microorganisms affect many insect properties [1]. For example, symbiosis containing bacteria that cause nutrient yields have been examined especially in aphids, whiteflies and cicadas, and these bacteria have been shown to help provide essential amino acids (EAAs) and vitamin B to their hosts [2]. The capacity of using plant floem extract which has low essential amino acid content by aphids was attributed to *Buchnera aphidicola,* a symbiotic bacterium that can synthesize these nutrients [3]. In another example, the *Wolbachia* bacterium is the most common endosymbiotic bacteria that manipulates the reproduction of arthropod hosts to increase their spread throughout host populations. The *Wolbachia* infection causes parthenogenetic reproduction in many Hymenopteran insects and only female offspring provide production [4]. For Lepidopterans insects, bacteria in the intestines of these insects are known to produce digestive enzymes to help break down molecules such as cellulose, pectin and starch [5]. However, little is known about the detoxification of plant secondary metabolites by insect symbionts or their contribution to advocating of hosts against pathogens, parasitoids, or predators. In this sense, the identification of bacteria in the intestinal systems of insects is especially important for determining the nutritional and digestive properties of insect species.

At the same time, many species of entomopathogenic bacteria (especially *Bacillus thrungiensis)* are effectively used in the biological control of harmful insects. One of the most important of these bacteria is *B. thuringiensis* which is a soil bacterium with a very economical value since it is used as a biological control agent [6]. This bacterium produces proteins called as delta-endotoxins or *Cry* proteins in crystal form that show insecticidal activity against many insect pests. These bacteria are usually effective in the intestinal system of insects and eventually cause the death of the insect [7]. Therefore, the isolation of pathogenic bacteria during the identification of bacteria in the intestinal system of insects is also important. In addition, symbiotic bacteria of insects bring new approaches in the control of insect pests in the agriculture and forestry. Some studies have been shown to modify the symbiotic bacteria using genetic engineering techniques to express insect killing proteins or toxins (for example *Bt* toxins). This approach can be useful for the control of insect pests in many areas of entomological studies [8].

Therefore, it is thought that identifying symbiotic and pathogenic bacteria from different insects and determining the roles of these bacteria will be useful for future studies in increasing the production of beneficial insects and combating with insect pests. In this sense, as a first step, isolation and species determination of intestinal bacterial members should be performed correctly. For this purpose, *Lasiocampa trifolii (*Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae) was selected as the model organism in this study since it has a very widespread distribution worldwide and its larvae can feed on various plants such as *Ammodendron*, *Caragana fruticosa*, *Astragalus* spp., *Medicago* sp. (Fabaceae), *Calligonum* (Polygonaceae), *Haloxylon* (Chenopodiaceae) and *Artemisia* sp. (Asteraceae) [38]. Total bacterial isolation was performed from the larvae of this insect and the isolated bacteria were identified based on 16S rRNA sequence analysis.

2. Materials and methods

2.1. Collection of larvae

The larvae required for this study were collected from the various vegetation areas of Kırşehir (Turkey) and surrounding districts in May-June of 2019 and brought to the laboratory. The larvae collected in the field were placed in plastic boxes (25 × 25) together with the leaves of the plants where they were collected and brought to the laboratory for bacterial isolation as soon as possible. During the field studies, larvae with every growth stage were collected. After that, the larvae were kept in the laboratory for 2 days under laboratory conditions and bacteria were isolated from healthy larvae.

2.2. Identification of larvae

It is often difficult to identify insect specimens based on morphological characters using the larva stage of the insect. Therefore, in order to confirm the species determination of the larvae samples collected for this purpose, a partial sequence analysis of the cytochrome oxidase (subunit I) (*COI*) gene was performed and species identification was performed by phylogenetic analysis [9]. Total genomic DNA isolation was performed from the larvae samples using DNeasy Blood & Tissue Kit from QIAGEN (Qiagen, Germantown, USA). The DNAs obtained were stored at -20 ° C until use. After genomic DNA isolation, approximately 620 bp of the cytochrome oxidase (subunit I) gene was amplified by PCR (Polymerase Chain Reaction). LCO1490-5'-GGTCAACAAATCATAAAGATATGG-3 'was used as forward primer and HCO2198-5'-TAAACTTCAGGGTGACCAAAAAATCA-3' was used as reverse primer [15]. PCR conditions were as follows: 1 µl template DNA (genomic), 5 µl 10 x PCR buffer (100 mM Tris-HCl, pH 8.3; 500 mM sterile KC1), 1.5 mM MgCl2, 1.25 U *Taq* DNA Polymerase, 0.25 mM forward primer, 0.25 mM reverse primer, 200 mM dNTPmix. The final mixture was completed to 50 µl with sterile distilled water. Reaction temperatures and times were as follows: initial denaturation at 95 °C for 5 min; 36 cycles of 94 °C for 1 min (for denaturation), 56 °C for 1 min (for primer binding) and 72 °C for 1 min (for polymerization); and final extension at 72 °C for 10 min. 5µl of the PCR products obtained from PCR reactions were run together with the marker on 1% agarose gel and were imaged under UV light after staining with ethidium bromide dye (0.5 µg / ml). The sequence of the *COI* gene replicated from genomic DNA was performed by MACROGEN (The Netherlands). The obtained 620 bp sequence of *COI* gene was compared with the sequences in GenBank, similarity ratios were revealed, and species determination of the collected larvae were determined [10,11]. In addition, the partially sequenced *COI* gene was used in phylogenetic analysis using MEGA 7.1 software [12]. The partial *COI* gene sequence was compared with the insect species (especially lepidopteran insects) used in the study of Mutanen et al. (2010) [13].

2.3. Isolation of bacteria

Bacterial isolation for aerobic bacteria was performed from 3rd and 4th instars healthy larvae. For isolation, five larvae were used, and they were first surface sterilized with 70% ethyl alcohol, then washed 3 times with sterile distilled water. Then, the larvae were homogenized in 3 ml of nutrient broth. The obtained homogenate was filtered through two layers of sterile cheesecloth to remove insect debris. After that, the homogenate was diluted from 10^{-1} to 10^{-8} using sterile serum saline and 100 µl of these dilutions were plated on nutrient agar. The petri dishes were incubated at 30 ° C for 2 days. At the end of the incubation period, the number of bacteria per larva was calculated by counting colonies. In addition, those colonies with different colors and morphologies were selected and transferred to another nutrient agar plate. Pure cultures of bacteria were stocked at -20 ° C with 20% glycerol [14].

2.4. 16S rRNA gene sequencing

Total DNA extraction from the bacterial isolates obtained from a single colony for each was performed according to the method determined by Sambrook et al. (1989) [15]. The obtained DNA pellets were dissolved in 50 µl Tris-EDTA buffer and stored at -20°C until used in PCR. 16S rRNA genes from the isolated genomic DNAs were amplified with PCR using primer pairs of 27F-(5'- AGAGTTTGATCMTGGCTCAG-3') and 1492R-(5'-TACGGYTACCTTGTTACGACTT-3') (MACROGEN). PCR ingredients were as follows: 1 µl genomic DNA, 5 ul 10 x PCR buffer (100 mM Tris-HCl, pH 8.3; 500 mM sterile KC1), 1.5 mM MgCl2, 1.25 U *Taq* DNA Polymerase, 0.25 mM forward primer, 0.25 mM reverse primer and 200 mM d NTPmix. After adding all ingredients, each mixture was completed to 50 µl with sterile distilled water. Reaction temperatures and times were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of 94 °C for 1 min (for denaturation), 56 °C for 1 min (for primer binding) and 72 °C for 1 min (for polymerization); and final extension at 72 °C for 10 min. The obtained PCR products were analyzed as described above. The obtained 16S rRNA sequences, which are about 1,400 bp long, were compared to the sequences existed in GenBank, similarities between them were revealed and species determinations of the isolated bacteria were performed [10,11]. In addition, the sequenced 16S rRNA genes were used in phylogenetic analysis (comparing the closest bacteria in GenBank) using MEGA 7.1 software [12].

Finally, the 16S rRNA sequence of *Okibacterium* sp. TT9 was used to construct phylogenetic tree to compare it with the most closely related bacterial species in GenBank.

2.5. Data analysis

All obtained DNA sequences were arranged by the BioEdit (Hall 1999, version 7.09) software and the percentage similarities of the sequences with other bacterial species in GenBank were determined by BLAST search [10]. Cluster analysis of DNA sequences was similarly done using the ClustalW program packed in BioEdit, and the data obtained from this was used in neighbor-joining (NJ) analysis using MEGA 7.1 [12]. Alignment gaps have been evaluated as missing data. The reliability of the generated dendrograms was tested based on 1.000 repetitions using bootstrap analysis.

2.6. GenBank Accession Numbers

16S rRNA gene sequences of the bacterial isolates (from TT1 to TT11) have been deposited in GenBank under accession numbers of MT264951, MT264952, MT264953, MT264954, MT264955, MT264956, MT264957, MT264958, MT264959, MT264960 and MT264961, respectively.

3. Results

Cytochrome oxidase (subunit I) (*COI*) gene sequencing was used to identify *Lasiocampa trifolii* (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae) larvae. Total genomic DNA was extracted from healthy larvae and the partial sequence of cytochrome oxidase (subunit I) gene was sequenced and used to determine correct identification of the collected insect. It was found that the partial *COI* gene sequences was like *Lasiocampa trifolii* voucher BC ZSM Lep 60900 at the rate of 97,09% based on Blast search. Additionally, the partial *COI* sequence was compared with other Lepidopteran insects used in the study of Mutanen et. al. (2010) using phylogenetic analysis [13]. Based on the dendogram, it was clear that the collected larvae were *L. trifolii* (Figure 1).

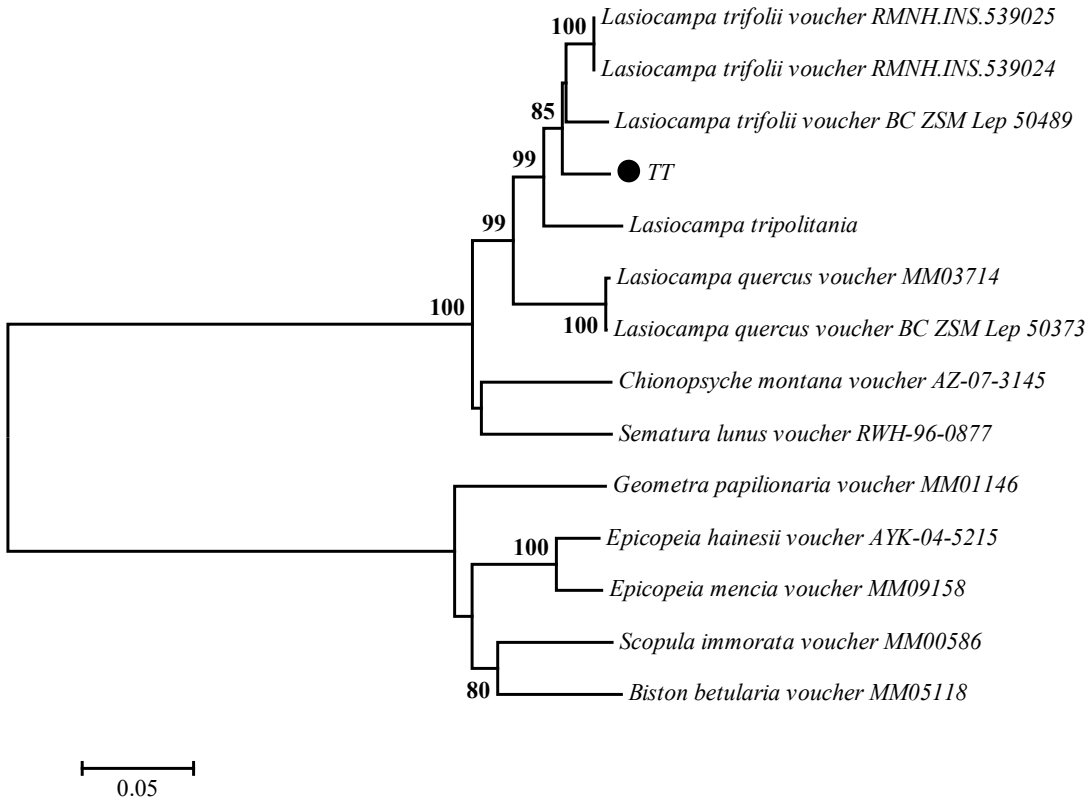

Figure 1. The N-J tree showing the taxonomic position of *Lasiocampa trifolii* based on the partial sequence of *COI* gene (620 bp long). Reference species were taken from the study of Mutanen et al. (2010) [1]. Bootstrap values were tested with 1.000 repetitions and 70% or higher were indicated. The scale below the dendogram shows the degree of dissimilarity. *L. trifolii* sample was indicated by black circle.

Bacterial isolation from the larvae was made as mentioned above and the number of bacteria for per larva was calculated as 18.75×10^3 cfu/larva. A total of 11 bacteria was isolated and identified based on 16S rRNA gene analysis. The sequenced 16S rRNA genes were used to compare with other bacterial species which are available in GenBank using Blast search (Table 1). Also, these sequences were used in phylogenetic analysis with the most closely related bacteria (Figure 2). Based on all these data, the bacterial isolates were identified as *Bacillus* sp. TT1, *Micrococcus luteus* TT2, *Arthrobacter* sp. TT3, *Corynebacterium* sp. TT4, *Arthrobacter agilis* TT5, *Micrococcus luteus* TT6, *Micrococcus luteus* TT7, *Arthrobacter* sp. TT8, *Okibacterium* sp. TT9, *Staphylococcus haemolyticus* TT10 and *Bacillus* sp. TT11.

Table 1. Percentage similarities of the bacterial isolates with the most closely related bacteria in GenBank based on 16S rRNA gene sequences.

Strain	Species	GenBank ID	Query coverage (%)	Similarity (%)
TT1	*Bacillus tropicus* strain AOA-CPS1	CP049019	%100	%99,85
	Bacillus wiedmannii PL1	AP022643	%100	%99,85
	Bacillus albus strain DLOU-Yingkou	CP040344	%100	%99,85
	Bacillus thuringiensis strain BDzB	MN203613	%100	%99,85
TT2	*Micrococcus luteus* strain NCCP	CP043842	%100	%99,54
	Micrococcus luteus strain MS1-14	MN238740	%100	%99,54
	Micrococcus luteus strain SA211	CP033200	%100	%99,54
TT3	*Arthrobacter* sp. MARAP2	KU882738	%99	%99,78
	Arthrobacter sp. AFG19.2	KT314143	%99	%99,86
	Arthrobacter sp. AFG20.2	KT314137	%99	%99,86
TT4	*Corynebacterium aurimucosum* ATCC 700975	CP001601	%100	%97,45
	Corynebacterium nigricans	AY227208	%100	%97,45
	Corynebacterium nigricans isolate 92-0360	AF537608	%100	%97,45
TT5	*Arthrobacter agilis* strain UMCV2	CP024915	%100	%99,19
	Arthrobacter agilis strain F1	MG279727	%100	%99,19
	Arthrobacter agilis strain IHBB	KR085842	%100	%99,19
TT6	*Micrococcus luteus* strain SA211	CP033200	%100	%99,93
	Micrococcus luteus strain SA211	CP033200	%100	%99,93
	Micrococcus sp. A7	AM235879	%100	%99,93
TT7	*Micrococcus* sp. strain CLC-F33	MH518215	%100	%99,42
	Micrococcus luteus strain SA211	CP033200	%100	%99,35
	Micrococcus luteus strain FC1737	MH665979	%100	%99,35
TT8	*Arthrobacter* sp. AFG19.2	KT314143	%100	%98,68
	Arthrobacter sp. AFG20.2	KT314137	%100	%98,68
	Arthrobacter sp. AFG15.3	KT314123	%100	%98,68
TT9	*Okibacterium* sp. strain A3P100	MN989104	%99	%100
	Okibacterium sp. strain D4K016	MN989090	%99	%100
	Microbacterium sp. strain I-S-L2-4	MK398014	%99	%100
TT10	*Staphylococcus haemolyticus* strain EGI317	MN704523	%100	%99,57
	Staphylococcus haemolyticus strain XAAS.x148	MN187274	%100	%99,57
	Staphylococcus haemolyticus strain IADCACS10	MH973470	%100	%99,57
TT11	*Bacillus wiedmannii* strain BB32-3	MN314860	%99	%97,80
	Bacillus cereus strain ck-01	MK592620	%99	%97,80
	Bacillus thuringiensis serotype H57	DQ286352	%99	%97,80

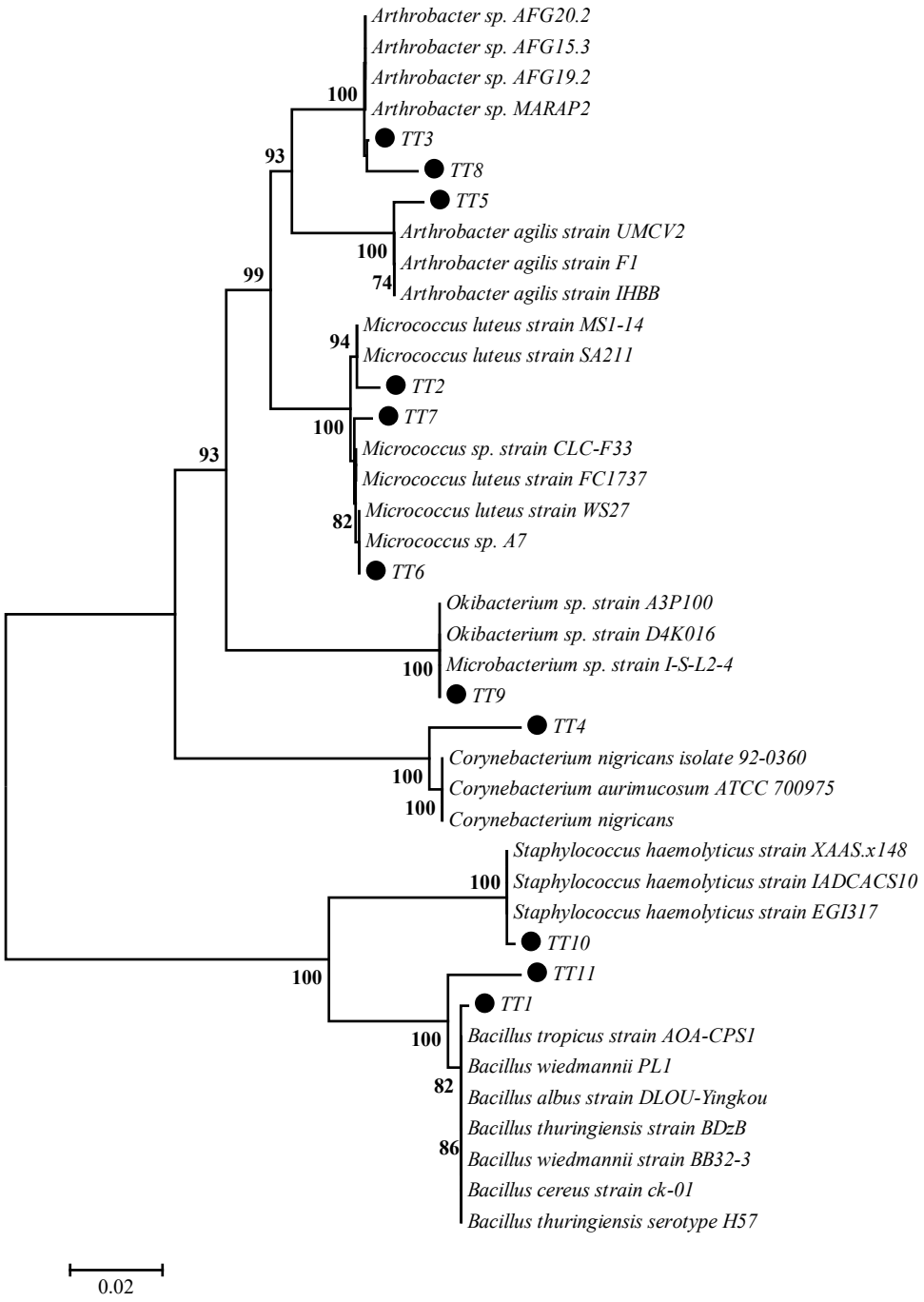

Figure 2. The N-J tree showing the taxonomic position of the bacterial isolates from *Lasiocampa trifolii* based on the 16S rRNA sequence (approximately 1.400 bp long). Reference species were taken from GenBank based on Blast search. Bootstrap values were tested with 1.000 repetitions and 70% or higher were indicated. The scale below the dendogram shows the degree of dissimilarity. *L. trifolii* isolates were indicated by black circle.

Based on the phylogenetic tree constructed using 16S rRNA gene sequence of TT9 and it is the most closely related bacteria species in GenBank, it is possible to say that this isolate seems to be a novel species (Figure 3).

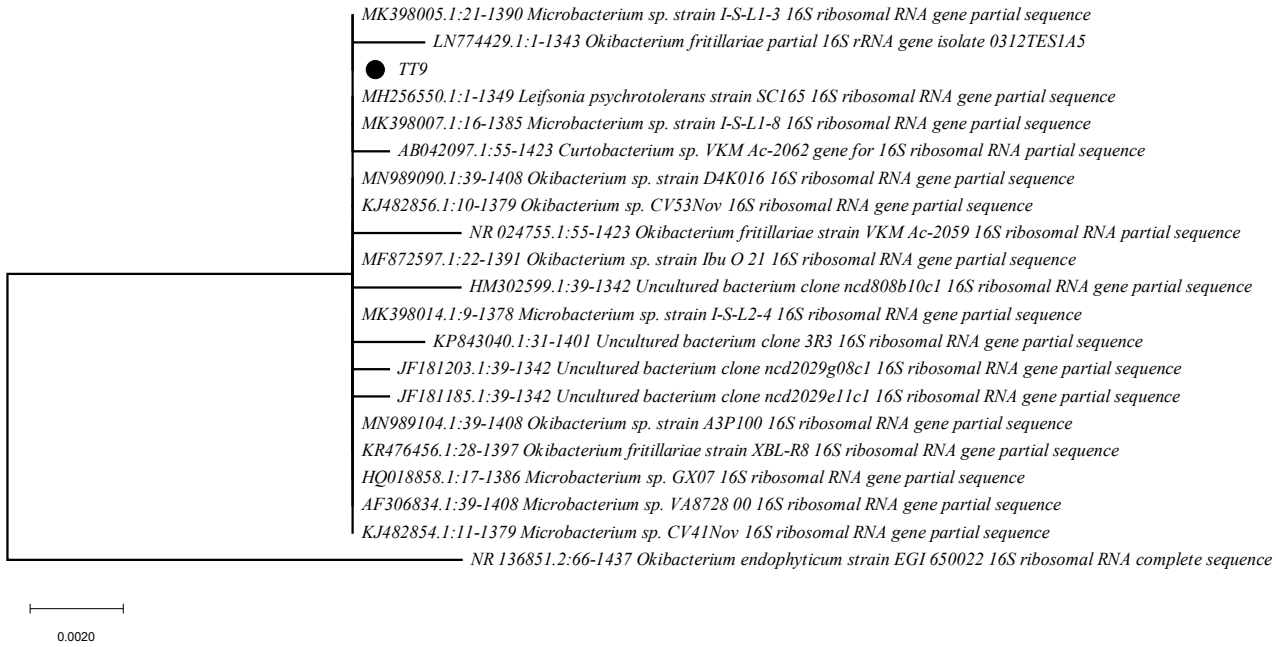

Figure 3. The N-J tree showing the taxonomic position of *Okibacterium* TT9 based on the 16S rRNA sequence (approximately 1.400 bp long). Reference species were taken from GenBank based on Blast search (first twenty closely related bacteria were selected). Bootstrap values were tested with 1.000 repetitions and 70% or higher were indicated. The scale below the dendogram shows the degree of dissimilarity. TT9 was indicated by black circle.

4. Discussion and Conclusion

Insects are experimentally tractable and cost-effective model systems to investigate animal-bacterial interactions. So far, microorganisms living in symbiotic relationship with insects have been shown that they play an important role in nutrition, development, and evolution of their hosts. Some of these microorganisms help their hosts use weak digestible foods, gain resistance to biotic and abiotic stress conditions, and to regulate different metamorphosis events. Based on the some studies, some of the endosymbionts contained in insects have been shown that they were left from ancient infections [16]. In addition to symbionts associated with insects, there are many types of pathogenic bacteria that cause the death of insects by damaging insects' intestinal system [17]. Some of these bacteria (especially *Bacillus thuringiensis*) are used as environmentally friendly challenges to insects that cause harm in agriculture and forestry. For this purpose, *L. trifolii* which feeds on various oaks, beech, poplar, and single-year herbaceous plants, was selected as a model organism. Both endosymbiont and pathogenic bacteria in the intestinal system of this insect were isolated and identified.

The *Bacillus* genus contains phenotypically large, Gram-positive (some species Gram-variable), endospore-forming, aerobic or facultative anaerobic and rod-shaped bacteria. Advances in molecular biology have shown a high degree of phylogenetic heterogeneity in this genus. Members of this genus are widely found in many environments and often stand out as contaminants in a laboratory environment. The most important pathogenic types of this genus, which contains approximately 200 species, are *B. anthracis, B. cereus, B. subtilis* and *B. thuringiensis* [18]. Among these species, B. *thuringiensis* are especially important for biological control of insect pests [19]. In this study, a total of two *Bacillus* sp. (TT1 and TT11) was isolated, and these two isolates were found to be similar with *B. thuringiensis* with 99% based on 16S rRNA gene sequence. However, more detailed tests are needed to distinguish the species within the *Bacillus* genus. In addition, it is necessary to determine whether these two isolates contain insecticidal crystal proteins or not. Therefore, more detailed tests are needed to determine the potential of TT1 and TT11 isolates in terms of biological control.

The *micrococcus* genus contains Gram-positive, non-motile, non-spore forming bacteria that form sphere-shaped tetrads or irregular communities. Many species produce carotenoid pigments [20]. Members of this genus are found in many habitats, from soil to water. They are often also found in the skin flora of other animals and humans [21]. There are many studies showing that some species of this genus were isolated from different insects [22,23]. In this study, three *Micrococcus* (*Micrococcus luteus* TT2, *Micrococcus luteus* TT6 and *Micrococcus luteus* TT7) were isolated. These studies show that some members of this gene are closely related to insects. However, further studies based on host-microorganism relationship are needed to determine the role of these bacteria in insects.

The genus of *Arthrobacter* is a very wide genus and contains very common species in nature. Due to the versatility of their diet, they can often be easily isolated from soil, sewerage, food and some other environments [24]. There are also some studies showing the isolation of some members of this genus from various insects [25]. Some members of this group are also known to break up agricultural pesticides [26]. Although there have been many studies on this genus, studies based on their roles in insects are very limited.

Corynebacterium genus is often composed of rod-shaped, Gram-positive, non-motile, non-spore forming, aerobic and catalase positive members. Members of this group are located in the normal skin flora of many hosts and mucous membranes, but can also be found in other ecological environments such as soil and water [27]. The members involved in this genus are classified as pathogenic, opportunistic, and saprophytic. Some members (such as *C. diphteriae* and *C. pseudotuberculosis)* cause disease in humans and animals [28]. In this study, one *Corynebacterium* sp. (TT4) was isolated from *T. trifolii*. Although there are some studies showing isolation of some members of this genus from insects, there is a limited information about their roles in insects [29].

Okibacterium which was placed in Microbacteriaceae is composed of Gram-positive and non-spore-forming bacteria. This genus was first defined in 2002 by Evtushenko et al. (2002) and includes only two species identified so far (*O. fritillariae* and *O. endophyticum*) [30,31]. These two species were isolated from plant samples (roots and seeds) and these limited studies may indicate that these bacteria are endophytically associated with plants. In this study, one *Okibacterium* sp. (TT9) was isolated, and this is the first study showing the isolation of this genus from any animals or insects. According to the phylogenetic tree, it seems that the genus of *Okibacterium* may include some undefined species and it may need a complete molecular systemic revision. With advanced molecular identification techniques, it is necessary to determine whether this isolate is a new species or not.

Staphylococcus haemolyticus which is a member of coagulase negative staphyloccocci and is found in human's normal skin flora. This bacterium can also be colonized in primates, pets and is known as a well-known opportunistic pathogen [32-35]. In some studies, performed so far, various types of *Staphylococcus* have been isolated from many insect samples [26,36,37]. Based on these studies, it should be considered that members of the *Staphylococcus* genus are closely related to insects and are involved in normal intestinal flora of insects. When evaluated in terms of animal micro-ecology, it can be said that the nature, quantity, and conditions of natural intestinal flora in insects are closely related to the feeding of the insect and digestive physiology. In this study, one *Staphylococcus haemolyticus* (TT10) was isolated and is a bacterium which is important for human health. Although it is unusual for such bacteria to take part in the intestinal flora of insects, it should be kept in mind that healthy insect microbiota may contain pathogens when insects are considered as vectors of certain human diseases. However, further studies strictly are needed to prove this.

In this study, a total of 11 bacterial strain was isolated from *L. trifolii* and they were characterized based on 16S rRNA sequence analysis. The obtained and defined bacterial strains were discussed in terms of nutrition, physiology, and diseases of insects. Studies are required to identify some bacterial strains obtained from this study at the species level using advanced molecular techniques (especially O*kibacterium* sp. TT9) and to determine the role of these bacteria in insects.

5. Acknowledgements

We would like to thank Kırşehir Ahi Evran University (BAP unit) for supporting this study (Project number: ZRT.A4.19.023).

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