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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Bacterial diversity associated with the Hatay yellow strain silkworm (*Bombyx mori* L.): Isolation, identification and characterization

Hatay sarısı ırkı ipekböceği (Bombyx mori L.) ile ilişkili bakteri çeşitliliği: İzolasyon, teşhis ve karakterizasyon

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ABSTRACT

The Hatay yellow strain silkworm (*Bombyx mori* L.), which is in danger of extinction, is one of the most important local cultural heritages of Türkiye. Bacterial pathogens of silkworm are highly destructive and cause mostly acute diseases. The aim of this study was to determine the bacterial diversity and potential pathogenic bacterial species in infected and dead larvae of Hatay yellow race. A total of 16 bacterial isolates from Hatay yellow race were identified according to their morphological, biochemical and molecular characteristics. The bacterial isolates isolated from infected and dead larvae of Hatay yellow race were *Staphylococcus* sp. (BM-1), *Staphylococcus xylosus* (BM-5), *Staphylococcus succinus* (BM-7), *Bacillus thuringiensis* (BM-8), *Bacillus subtilis* (BM-9), *Bacillus* sp. (BM-10), *Staphylococcus saprophyticus* (BM-16, BM-19), *Klebsiella* sp. (BM-17), *Staphylococcus arlettae* (BM-18), *Pseudomonas aeruginosa* (BM-20), *Enterococcus mundtii* (BM-21), *Pantoea agglomerans* (BM-22), *Kluyvera intermedia* (BM-23), *Serratia* sp. (BM-24), *Mammaliicoccus sciuri* (BM-25). The high bacterial density and number of species indicate that Hatay yellow race is highly susceptible to bacterial diseases. Insecticidal activity studies revealed that species belonging to *Bacillus* and *Staphylococcus* genera are important pathogens of hybrid silkworm culture and Hatay yellow race.

ÖZET

Nesli tükenme tehlikesiyle karşı karşıya olan Hatay sarı ırkı ipekböceği (*Bombyx mori* L.), Türkiye'nin en önemli yerel kültür miraslarından biridir. İpekböceğinin bakteriyel patojenleri son derece yıkıcı olup, çoğunlukla akut hastalıklara neden olurlar. Bu çalışmanın amacı, Hatay sarı ırkının enfekteli ve ölü larvalarında bakteri çeşitliliğini ve potansiyel patojenik bakteri türlerini belirlemektir. Hatay sarı ırkından izole edilen toplam 16 bakteri izolatı morfolojik, biyokimyasal ve moleküler özelliklerine göre tanılanmıştır. Hatay sarı ırkının enfekteli ve ölü larvalarından izole edilen bakteri izolatları *Staphylococcus* sp. (BM-1), *Staphylococcus xylosus* (BM-5), *Staphylococcus succinus* (BM-7), *Bacillus thuringiensis* (BM-8), *Bacillus subtilis* (BM-9), *Bacillus* sp. (BM-10), *Staphylococcus saprophyticus* (BM-16, BM-19), *Klebsiella* sp. (BM-17), *Staphylococcus arlettae* (BM-18), *Pseudomonas aeruginosa* (BM-20), *Enterococcus mundtii* (BM-21), *Pantoea agglomerans* (BM-22), *Kluyvera intermedia* (BM-23), *Serratia* sp. (BM-24), *Mammaliicoccus sciuri* (BM-25) olarak belirlenmiştir. Bakteri yoğunluğu ve tür sayısının fazla olması Hatay sarı ırkının bakteriyel hastalıklara karşı son derece duyarlı olduğunu göstermektedir. İnsektisidal etkinlik çalışmaları, *Bacillus* ve *Staphylococcus* cinslerine ait türlerin hibrit ipekböceği kültürü ve Hatay sarı ırkında önemli patojenleri olduğunu ortaya koymuştur.

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INTRODUCTION

The mulberry silkworm, *Bombyx mori* is an economically very important insect domesticated for silk production. The domestication history of the silkworm began about 5000 years ago and *B. mori* breeds spread to other countries from China about 1500 years ago (Li et al., 2005). More than 4000 strains are maintained in the germplasm of *B. mori* (Bindroo & Moorthy, 2014). The wild silkworm *B. mandarina* (*Theophila mandarinai*) is usually considered to be the ancestor of the native silkworm *B. mori* (Jiang, 1982). The wild silkworm is widespread all parts of Asia and shows great diversity.

With its cocoon production with an extraordinary yellow color scale, Hatay yellow strain of silkworm is one of the most important cultural heritage values, endemics and endangered in Türkiye. "Hatay Yellow" is the 3rd local breed identified after "Bursa White Pied" and "Bursa White" in Turkey. Hatay yellow strain was domesticated about 5000 years ago and it's one of the important privileges for Turkey to have it. However, Hatay yellow strain hasn not been bred for nearly 50 years (İleri, 2019). Turkey's domestic silkworm breed Hatay yellow, which is facing the danger of extinction, attracts the attention with its different colored cocoons from cream to orange.

Hatay yellow strain, which is under the pressure of many abiotic and biotic factors, is in the struggle to maintain its generation (personal information by Mrs. Emel Duman and Mr. Fikret Duman). Although there are very serious deaths in larval populations due to microbial infections, no studies have been conducted on the microbial natural enemies of this strain. In one of the observations and examinations carried out to determine the microbial natural enemies of the strain in Hatay yellow production facility, which is produced as a family business by only one family in Turkey, a significant and intense bacterial infection and a large number of deaths were detected in the growing trays of the strain.

The demand for silk fibers, one of the indispensable textile fibers with high added value, which today as throughout history are gaining in value and importance, is increasing day by day, and in parallel production is being increased to meet the demand. In addition to all these, farmers are faced with many problems due to the contamination of silkworm with various microbial diseases (Mishra, 2017; Sharma et al., 2020; Chopade et al., 2021). One of the most virulent microorganisms infesting *Bombyx mori* is the entomopathogenic bacteria, which spread very rapidly among individuals in the population and cause mass deaths, and therefore precautions should be taken (Karthikairaj et al., 2013). Although governments and various organizations are forming various programs and providing support to inform farmers and overcome these diseases, crop loss is not yet controlled as expected.

Bacterial flora, microbial diversity and facultative microorganism density of arthropods are extremely important for their survival and viability. Under normal conditions, the relationships between microorganisms and their hosts are in a state of equilibrium. This balance is occasionally disturbed in favor of microorganisms, threatening the life of the host. This situation enabled the use of microorganisms in the biological control of agricultural and forest pests, and very important successes were achieved in this field (Demir et al., 2012; Secil et al., 2012; Sevim et al., 2012; Eski et al., 2018). However, this situation is extremely bad and negative for beneficial insects. In order to sustain the lives of beneficial insects, it is necessary to determine both the microbial pathogens and the ways of being strong against these pathogens.

Hatay yellow strain can be considered as Türkiye's extremely important local and beneficial biological wealth. In a study on Hatay yellow strain, Ulaşlı et al. (2021) investigated some biological characteristics of Hatay yellow strain. The only study to date on microbial pathogens of this local strain identified fungal pathogens as a major problem in the process of growing insect culture (In Review-Unpublished data). There is no study in the literature on bacterial pathogens of Hatay yellow strain, which is an extremely sensitive compared to hybrid.

In this study, the entomopathogenic bacteria of Hatay yellow strain were studied for the first time and the bacteria isolated from the insect were identified and their lethal effects on silkworm were determined.

MATERIALS and METHODS

Collection of larvae

The infected and dead larvae, cocoon and adults of Hatay yellow strain (*Bombyx mori* L.) were collected from Hatay, Türkiye, between 2020 and 2021, and transfered to the laboratory in sterile falcon tubes (50 mL). The signs of bacterial infection were investigated by examining the larvae,cocoon and adults macroscopically and under light microscope (Figure 1).

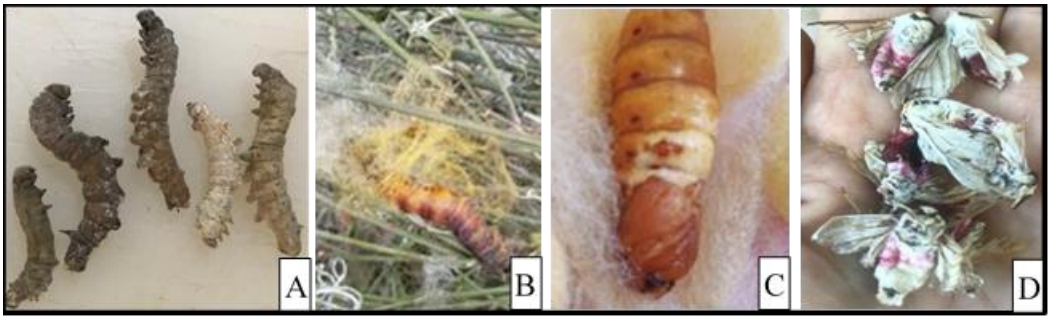

Figure 1. Morphological images of Hatay yellow strain cadavers that died due to bacterial infection. Deaths in the larval period (A). Larva that died before entering the cocoon (B). Death in cocoon (C). Dead adult (D)

Şekil 1. Bakteriyel enfeksiyon nedeniyle ölen Hatay sarı ırkı kadavralarının morfolojik görüntüleri. Larva dönemindeki ölümler (A). Kozaya girmeden ölen larva (B). Kozadaki ölüm (C). Ergin ölüm (D)

Isolation and purification of the bacteria

Hatay yellow strain larvae, cocoon and adults were individually placed in 70% ethanol and they were left to surface sterilization for 3 min. Afterwards, the larvae, cocoon and adults were cleaned from alcohol by washing 2-3 times with sterile distilled water in the tube. Then, they were became homogeneous in tube including 1 mL of nutrient broth. The mixture was filtered through a sterile cheesecloth to remove coarse particles, and 100 µL of the filtrate were spread onto nutrient agar medium. Plates were incubated for 2-3 days at 30°C. Pure cultures were obtained from bacterial colonies growing at the end of incubation according to their size, color and morphology (Ozkan-Cakici et al., 2015).

Morphological properties

The colony morphology of the isolates grown on Nutrient Agar plates was observed with a binocular microscope. The motility and shape of bacterial cells were also examined with a light microscope. Gram and spore staining were performed according to the Claus, (1992) and Reynolds et al., (2009). Their characteristics were evaluated according to Bergey's Manual of Systemic Bacteriology 1 and 2 (Krieg, 2001; Sneath, 2001).

Biochemical properties

The biochemical characteristics of the isolates were determined using API 20E panel test systems. API test strips were performed according to the manufacturer's instructions (bioMerieux SA Marcy l'Etoile, France). Stock cultures were seeded on nutrient agar to obtain single colonies for each bacterial isolate and the amount of bacteria was adjusted to 1 McFarland. 200 mL of this solution was transferred to each well of the panels and the panels were incubated at 30 °C for 24-48 hours (Gökçe et al., 2010).

Molecular identification

Bacteria were inoculated in nutrient broth and at the end of the growth period, genomic DNA was extracted from the bacteria using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research) according to the manufacturer's protocols. The 16S rRNA genes of the bacterial isolates were amplified by PCR using universal bacterial forward and reverse primers (Weisburg et al., 1991).

PCR reactions were performed in a final volume of 50 µL containing DNase-free water, 10 µL of 5X phusion reaction buffer, 200 µM dNTPs, 1 µL (0.5 µM) of each primer, and 0.02 U/µL unit of phusion DNA polymerase (Phusion™ High-Fidelity DNA Polymerase). The amplification program included an initial step of denaturation at 98 °C for 1 min, followed by 30 cycles at 98 °C for 50 s, 53 °C for 30 s, 72 °C for 50 s, and a final extension at 72 °C for 15 min. The obtained amplicons were evaluated by 0.8% (w/v) agarose gel electrophoresis stained with ethidium bromide and excised from the gel using the NucleoSpin Gel and PCR Clean-up Kit. Gel-purified 16S rRNA gene fragments were cloned directly into the PUC vector cloning system. Sequencing of the amplicons was performed by MACROGEN sequencing service, Amsterdam. Sequences were checked for vector contamination by NCBI Vecscreen tool and compared with known 16S rRNA gene sequences in the NCBI (http://www.ncbi.nlm.nih.gov/ BLAST) (Altschul et al., 1990).

Phylogenetic analysis

Multiple sequence alignment was performed, and phylogenetic trees for the 16S rRNA gene were constructed using MEGA X software, version 7.0.26 (Kumar et al., 2018) and phylogenetic analysis was performed to compare them to similar species (Benson et al., 2013). The robustness of the Neighbor-Joining tree was tested by bootstrapping analysis of 1000 replicates.

Insecticidal activity

Bioassays were carried out to determine the insecticidal effect of the bacterial isolates from Hatay yellow strain against hybrid *Bombyx mori* larvae that was taken Kozabirlik in Bursa. The Hatay yellow strain is a sensitive and less produced breed. Since it will be used in large numbers for the experiment, the larvae of *Bombyx mori* obtained from Kozabirlik were used in the experiment.

Bacteria were incubated in nutrient broth at 30°C for 18 h, and their density were adjusted at 1.89 at OD_{600}. Mulberry leaves contaminated with bacterial suspensions were used in bioassays. Contaminated leaves were placed into individual sterile plastic box including 4-5 hours starved 30 second instar larvae , also clean leaves were placed in control group (Figure 2). The leaves were replaced with fresh ones every day. Mortality were recorded every day until 10 days after inoculation (Demir et al., 2012). Then, mortality ratios were calculated according to Abbot's formula (Abbott, 1925).

RESULTS and DISCUSSIONS

Sixteen bacterial isolates belonging to nine different genera were isolated from Hatay yellow strain cadavers and identified based on phenotypic, genotypic, and phylogenetic characteristics. Colony color of the isolates were cream (BM-1, BM-9, BM-10, BM-17, BM-19, BM-20, BM-21, BM-23), yellow (BM-5, BM-7, BM-22, BM-25), white (BM-8, BM-16, BM-18) and red (BM-24) on the agar plates. It was observed that the color characteristics of the colonies were reflected in the shapes and height levels of the colonies. In the Gram reaction, while 11 of the isolates (BM-1, BM-5, BM-7, BM-8, BM-9, BM-10, BM-16, BM-18, BM-19, BM-21, BM-25) were positive, the others gave negative reactions. It was determined that BM-8, BM-9 and BM-10 produced spores during the culture process. It was determined that eight of the isolates (BM-8, BM-9, BM-10, BM-17, BM-20, BM-22, BM-23, BM-24) were rod-shaped and the others were round-shaped, and all of the rod-shaped were mobile. All bacterial isolates caused

turbidity in nutrient broth (Table 1). The species belonging to the *Staphylococcus* and *Bacillus* genera turned out to be the most common species in bacterial density.

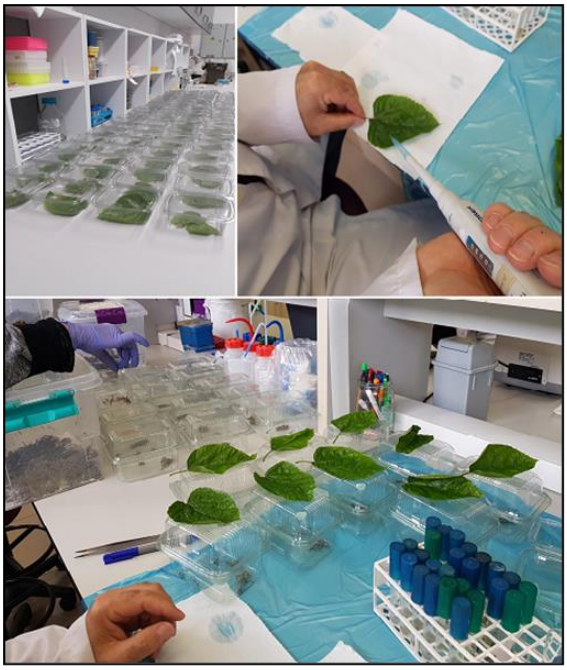

Figure 2. Bioassay applicationas on hybrid *Bombyx mori larvae*
Şekil 2. Hybrid *Bombyx mori larvalarında biyoassay uygulaması*

Table 1. Phenotypic properties of the bacterial isolates
Çizelge 1. Bakteri izolatlarının fenotipik özellikleri

Isolates	Colony Appearance		Cell Typical				
	Color	Elevation	Gram's reaction	Spore	Shape	Motility	Turbidity
BM-1	Cream	Convex	+	-	Coccus	-	Turbid
BM-5	Yellow	Convex	+	-	Coccus	-	Turbid
BM-7	Yellow	Convex	+	-	Coccus	-	Turbid
BM-8	White	Raised	+	+	Rod	+	Turbid
BM-9	Cream	Umbonate	+	+	Rod	+	Turbid
BM-10	Cream	Raised	+	+	Rod	+	Turbid
BM-16	White	Convex	+	-	Coccus	-	Turbid
BM-17	Cream	Umbonate	-	-	Rod	-	Turbid
BM-18	White	Convex	+	-	Coccus	-	Turbid
BM-19	Cream	Convex	+	-	Coccus	-	Turbid
BM-20	Cream	Raised	-	-	Rod	+	Turbid
BM-21	Cream	Convex	+	-	Coccus	-	Turbid
BM-22	Yellow	Raised	-	-	Rod	+	Turbid
BM-23	Cream	Convex	-	-	Rod	+	Turbid
BM-24	Red	Umbonate	-	-	Rod	+	Turbid
BM-25	Yellow	Convex	+	-	Coccus	-	Turbid

All metabolic and biochemical results from API 20E are given in Table 2. API 20E test was used to determine the metabolic and biochemical properties of the isolates. According to the test results, the biochemical characters differed from each other. In the test, while H_2S production was positive only in BM-17 coded isolate, β-galactosidase

Table 2. API 20E test results of the bacterial isolates

Çizelge 2. Bakteri izolatlarının API 20E test sonuçları

Tests	**Activities**	**Isolates**															
		BM-1	**BM-5**	**BM-7**	**BM-8**	**BM-9**	**BM-10**	**BM-16**	**BM-17**	**BM-18**	**BM-19**	**BM-20**	**BM-21**	**BM-22**	**BM-23**	**BM-24**	**BM-25**
ONPG	β-galactosidase	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Arginine	Arginine dihydrolase	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-
Lysine	Lysine decarboxylase	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-
Ornithine	Ornithine decarboxylase	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-
Citrate	Use of citrate	-	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-
Nathiosulfate	H_2S Production	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Urea	Urea hydrolysis	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-
Tryptophan	Deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
İndole	İndole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Napyruvate	Acetone production	+	-	-	+	+	+	-	+	+	-	-	+	+	-	+	-
Coal gelatin	Gelatinase	-	-	-	+	+	+	-	+	+	-	+	-	+	-	+	+
Glucose	Fermentation/oxidation	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+
Mannitol	Fermentation/oxidation	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-
Inositol	Fermentation/oxidation	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
Sorbitol	Fermentation/oxidation	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-
Rhamnose	Fermentation/oxidation	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Sucrose	Fermentation/oxidation	+	+	+	-	-	-	-	+	+	+	-	-	+	-	+	+
Melibiosis	Fermentation/oxidation	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-
Amygdalin	Fermentation/oxidation	+	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+
Arabinose	Fermentation/oxidation	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+

enzyme production was positive in all isolates except BM-8 and BM-25. Deaminase enzyme and indole production did not occur in any of the isolates. It has been found that fermentation reactions in API 20E are generally negative. In the reactions, inositol was fermented by BM-17 and BM-24, while rhamnose was fermented only by BM-22 and BM-23. Other test results in the API 20E test were obtained as positive or negative, depending on whether the isolates were gram positive or gram negative.

An approximately 1,400 bp fragment of the 16S rRNA gene regions sequenced for further characterization were used for blast in NCBI for molecular identification of the isolates. The isolates showed similarity to its counterpart in GenBank at different rates (Table 3).

Table 3. Taxonomic identification of the bacterial isolates

Çizelge 3. Bakteri izolatlarının taksonomik tanımlaması

Isolates	**Very likely identical taxonomic genus and species**	**Family**	**16S rRNA similarity (%)**	**Accession number**
BM-1	*Staphylococcus* sp.	Staphylococcaceae	97.91	KC951997.1
BM-5	*Staphylococcus xylosus*	Staphylococcaceae	97.48	MT353655.1
BM-7	*Staphylococcus succinus*	Staphylococcaceae	97.41	KX959978.1
BM-8	*Bacillus thuringiensis*	Bacillaceae	98.10	KM401866.1
BM-9	*Bacillus subtilis*	Bacillaceae	97.80	KC433738.1
BM-10	*Bacillus* sp.	Bacillaceae	97.85	MW012645.1
BM-16	*Staphylococcus saprophyticus*	Staphylococcaceae	98.58	OP028003.1
BM-17	*Klebsiella* sp.	Enterobacteriaceae	97.16	HQ204283.1
BM-18	*Staphylococcus arlettae*	Staphylococcaceae	96.97	OK618378.1
BM-19	*Staphylococcus saprophyticus*	Staphylococcaceae	90.78	MN603663.1
BM-20	*Pseudomonas aeruginosa*	Pseudomonadaceae	96.34	MT393981.1
BM-21	*Enterococcus mundtii*	Enterococcaceae	88.13	MH644178.1
BM-22	*Pantoea agglomerans*	Erwiniaceae	90.42	MT605813.1
BM-23	*Kluyvera intermedia*	Enterobacteriaceae	98.86	MT102139.1
BM-24	*Serratia* sp.	Yersiniaceae	88.46	MN874174.1.
BM-25	*Mammaliicoccus sciuri*	Staphylococcaceae	94.57	OK412723.1

According to phenotypic and genotypic analysis, bacterial diversity in the cadavers of Hatay yellow strain were determined as *Staphylococcus* sp. (BM-1), *Staphylococcus xylosus* (BM-5), *Staphylococcus succinus* (BM-7), *Bacillus thuringiensis* (BM-8), *Bacillus subtilis* (BM-9), *Bacillus* sp. (BM-10), *Staphylococcus saprophyticus* (BM-16), *Klebsiella* sp. (BM-17), *Staphylococcus arlettae* (BM-18), *Staphylococcus saprophyticus* (BM-19), *Pseudomonas aeruginosa* (BM-20), *Enterococcus mundtii* (BM-21*), Pantoea agglomerans* (BM-22), *Kluyvera intermedia* (BM-23), *Serratia* sp. (BM-24) and *Mammaliicoccus sciuri* (BM-25). The 16S rRNA gene partial sequences of isolates were deposited in the GenBank database under the accession numbers given in the Table 3. In addition, phylogenic analysis matched up with phenotypic and genotypic identification (Figure 3).

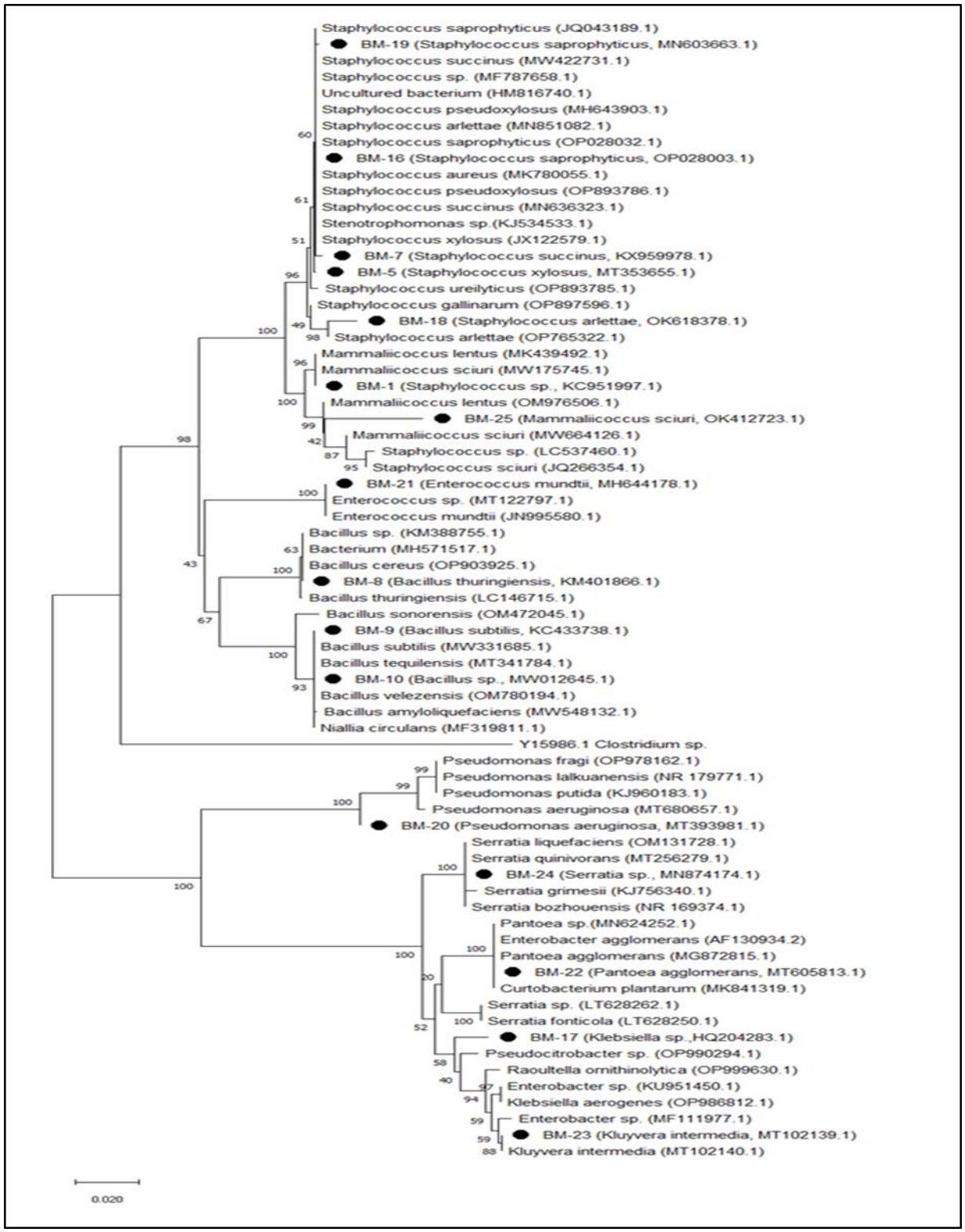

Figure 3. The neighbor-joining tree of the bacterial isolates and their closely related bacterial species. The approximately 1400-bp sequence of the 16S rRNA gene was used to construct the dendrogram. Bootstrap values based on 1000 replicates were indicated above nodes

Şekil 3. Bakteri izolatlarının ve bunların yakından ilişkili bakteri türlerinin komşu birleştirme ağacı. Dendrogramı oluşturmak için 16S rRNA geninin yaklaşık 1400 bp'lik dizisi kullanıldı. 1000 kopyaya dayalı önyükleme değerleri, düğümlerin üzerinde belirtilmiştir

Bacterial isolates were not caused remarkable mortalities against hybrid *Bombyx mori* larvae that was obtained from Kozabirlik. Mortality rates; BM-1: 3.3%, BM-5: 10%, BM-7:10%, BM-8: 3,3%, BM-9: 13.33%, BM-10: 6.6%, BM-16: 6,6%, BM-16: 6,6%, BM-17: 0%, BM-18: 6,6%, BM-19: 0%, BM-20: 6,6%, BM-21: 0%, BM-22: 0%, BM-23: 0%, BM-24:0%, BM-25: 0%. The highest mortality was obtained from BM-9 (13.33%) (Figure 4).

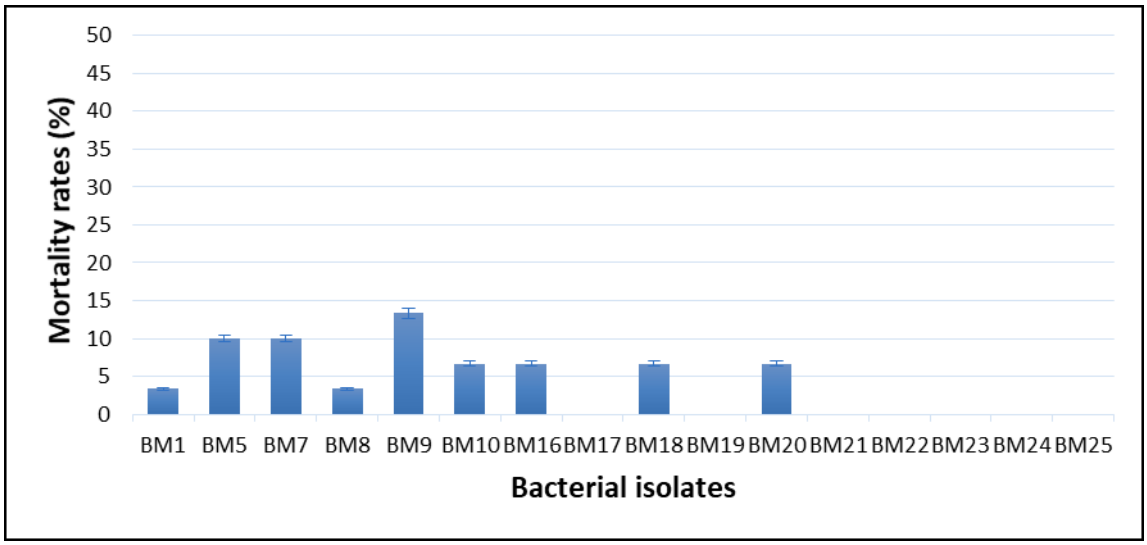

Figure 4. Mortality rates caused by bacterial isolates on hybrid *Bombyx mori* larvae obtained from Kozabirlik

Şekil 4. Bakteri izolatlarının Kozabirlik'den temin edilen hibrid Bombyx mori larvaları *üzerinde neden oldukları ölüm oranları*

Growth retardation was observed in infected larvae and darkening was detected in larval tissues. Observations revealed that some bacterial isolates caused larval growth stages to be longer than normal (Figure 5).

Figure 5. Growth retardation in infected hybrid *Bombyx mori* larvae in the experimental group

Şekil 5. Deney grubunda enfekteli hibrid Bombyx mori larvalarında büyüme geriliği

The bacterial diversity of Hatay yellow strain cadavers is highly compatible with the results of similar studies on both harmful and beneficial insects such as silkworms (Eski et al., 2018; Demir et al., 2012). Numerous studies on many agricultural and forest pest insects have shown that bacteria belonging to the genus *Bacillus* such as *B. thuringiensis* and *B. subtilis* are the most common in pests and the most effective bacterial agents on pests (Eski et al., 2018; Eski et al., 2019). Due to their high insecticidal effects, these bacteria have been developed as biological control preparations and are used in pest management programs. *Staphylococcus* species are opportunistic pathogens that are occasionally lethal and are common in insect microbiomes (Ayoade et al., 2014). *Serretia* with its characteristic red pigment, is widely found in the insect microbiomes, but is occasionally pathogenic (Pineda-Castellanos et al., 2015). *Pseudomonas aeruginosa* is also an opportunistic pathogenic species commonly found in insect microbiomes (Banerjee & Danger, 1995). Bacteria belonging to the Enterobacteriaceae family detected in the Hatay yellow strain are also among the species commonly found in the normal flora of insects.

Bacteria that cause the death of silkworms have been the subject of some studies in different countries and geographies (Ayoade et al., 2014; Chopade et al., 2021). The findings obtained from the current study are consistent with the results of these studies in the literature, although the strains are different. Silkworms infected by bacterial pathogens showed symptoms such as cessation of feeding, flaccidity, loss of body brightness, formation of brown spot on body, swelling of thorax, sluggishness of silkworms with slow growth, oral and anal discharge, straightened appearance of body, liquefaction of inner organs, rupturing of skin and rupturing of skin and oozing of bad smelling sluggish brown liquid, depending on the infectious agent and the breeding season (summer and rainy season) (Zhang et al., 2013). These signs of infection were consistent with the signs of infection in Hatay yellow silkworm strains used in bacterial isolation in the present study (Figure 1). Silkworm bacterial diseases known as flacherie, and collected in three groups such as bacterial septicemia, bacterial toxicosis and bacterial gastro-intestinal diseases. The agents that cause these diseases are *Streptococcus faccalis*, *Streptococcus liquifacions*, *Staphylococcus acire*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Pseudomonas* sp., *Micrococcus* sp. and *Bacillus* sp. (Karthikairaj et al., 2013; Ayoade et al., 2014). As in the literature, in the current study, the most common bacterial disease agent in silkworm is *Staphylococcus* species. Six of the sixteen bacterial disease agents detected and identified in Hatay yellow strain seem to be included in the *Staphylococcus* genus. Species of this genus are the most important pathogens of this local species in our country. Another dominant genus that causes disease and death in Hatay yellow is *Bacillus*. In the present study, three species belonging to this genus were identified. *Serratia* genus, which shows its presence both on cadavers and on agar medium with its characteristic red color, is a common pathogen in silkworm diseases. Apart from the ones mentioned, some other pathogenic bacteria are also detected in different areas and in different silkworm strains.

In the current study, most *Staphylococcus* and *Bacillus* isolates isolated from cadavers showed that have a low level of pathogenic activity against the silkworm strain distributed by Kozabirlik. That means, hybrid *Bombyx mori* from taken Kozabirlik is a potent and commercially productive strain against the pathogens isolated in this study.

Temperature, high humidity, and unsuitable growing conditions cause bacterial diseases to spread rapidly among silkworms (Nataraju et al., 2005). As seen in the literature, although the bacteria causing flacherie are present in the insect population, the epidemic will be prevented if proper hygiene is provided by the farmers and the insects are raised under optimum conditions for productivity (Ayoade et al., 2014; Saad et al., 2019). In addition to combating bacterial pollution, it is necessary to use leaves with high nutritional value and quality in production facilities in order to be resilient during the development process of insects.

Bacterial flora and bacterial diversity are extremely important for the survival of insects, as in all living things. Bacterial diversity and burden in insects are effectively influenced by their feeding preferences and habits and living conditions (He et al., 2013). Depending on ecological conditions and food preferences, the degree of relationships between microorganisms and their hosts can vary between mutualistic and parasitic. In this study, culture-dependent and nucleic acid-based techniques were used to reveal the bacterial diversity of cadavers of Hatay yellow strain to determine the bacteria causing the death of the host. According to the literature and written records, this is the first

study to determine the bacterial diversity and bacterial load associated with Hatay yellow strain cadavers. As a result, the fact that Hatay yellow strain, whose bacterial disease agents were determined for the first time in Türkiye, is extremely sensitive to these agents which is also extremely worrying for the continuity of the generation of this local strain. In order to achieve success, the above-mentioned recommendations should be applied more precisely and carefully for Hatay yellow strain.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

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