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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DETERMINATION OF HONEY BEE (*Apis mellifera*) BACTERIAL FLORA, *CRY* GENE ANALYSIS AND HONEY BEE HEALTH

Bal Arısı (*Apis mellifera*) Bakteri Florasının Belirlenmesi, *Cry* Geni Analizi ve Bal Arısı Sağlığı

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ABSTRACT

Beekeeping provides important contributions to the agricultural economy and crop production through pollination both in Turkey and the world. It is evaluated that without bees, the plant production can decrease by 47%. Many factors affect honey production negatively. Among these reasons, besides diseases caused by microorganisms, diseases and dangers originating from organisms are at the forefront. Today, many methods are used in the control these pests and yet they are still unavoidable. Among these methods, the biological control method is not used commonly. The aim of the study is to create a basis for the development of biopesticides to control bee diseases. In this context, as a result of the study, 16 bacteria were isolated from honey bees. While, 12 bacteria belonging to the genus *Bacillus*, two bacteria belonging to the genus *Lysinibacillus*, one bacterium belonging to the genus *Paenibacillus* and one bacterium belonging to the genus *Pantoea* were obtained. Molecular and biochemical identifications of these bacteria were done and registered in GenBank and their accession numbers were obtained. *cry* gene analyzes of 15 bacteria belonging to the genus *Bacillus* were performed. As it is known, *cry* genes have the potential to be used against pests. In the future, these bacteria and their genes will have the potential to be used as biopesticides. According to these results, the *cry1* gene was observed in 8 bacteria and the *cry3* gene was observed in 3 bacteria. *cry2* and *cry4* genes could not be detected in these bacteria. Bacteria that including *cry* genes are of great importance for honey bee health. Bacteria have the potential to be developed as internal biopesticides and used against different bee diseases to improve honey bee health.

Keywords: ***Apis mellifera*, Bacteria, Microbiology, Honey bee health, *cry* genes**

ÖZ

Arıcılık gerek Türkiye'de gerekse dünyada tarım ekonomisine ve tozlaşma yoluyla bitkisel üretime önemli katkılar sağlar. Arıların olmadığı bir ortamda bitkisel üretimin %47 oranında azalabileceği değerlendirilmektedir. Arıcılık sektöründe birçok etken de bal üretimini olumsuz yönde etkilemektedir. Bu sebepler arasında mikroorganizma sebepli hastalıkların yanı sıra, organizma kaynaklı hastalıklar ve tehlikeler de ön sıralarda yer almaktadır. Günümüzde bu zararlılarla mücadelede birçok yöntem kullanılmakta olup halen önüne geçilememiş durumdadır. Bu yöntemler arasında biyolojik mücadele yöntemi kullanılmamaktadır. Buradan yola çıkarak, çalışmanın amacı, bal arılarının sağlığını korumak için biyolojik bir etmen kullanılarak hastalık ve zararlı organizmalarla mücadele konusunda biyopestisit geliştirilmesinde taban oluşturmaktır. Bu bağlamda çalışma sonucunda bal arılarından 16 adet bakteri izolasyonu gerçekleştirilmiştir. Elde edilen bakterilerden 12 tanesi *Bacillus* cinsine, iki tanesi *Lysinibacillus* cinsine, bir tanesi *Paenibacillus* cinsine ait iken bir tanesi de *Pantoea* cinsine aittir. Bu bakterilerin moleküler ve biyokimyasal tanımlamaları yapılarak GenBank a kayıt yaptırılmış ve kayıt

numaraları alınmıştır. On beş adet *Bacillus, Paenibacillus ve Lysinibacillus* cinslerine ait bakterinin *cry* gen analizleri yapılmıştır. Bilindiği üzere *cry* genleri hem zararlılara karşı kullanılma potansiyeline sahiptir hem de ileride bu bakteri ve genleri geliştirilerek biyopestisit kullanılma potansiyeli olabilecektir. Bu sonuçlara göre *cry1* geni 8 bakteride ve *cry3* geni de 3 bakteride gözlemlenmiştir. *cry2* ve *cry4* genleri bu bakterilerde tespit edilememiştir. Bu genleri taşıyan bakteriler bal arısı sağlığı açısından büyük önem taşımaktadır. Bakteriler biyopestisit olarak geliştirilerek başta organizma gibi zaralılar olmak üzere farklı arı hastalıklarına karşı kullanılma potansiyeline sahiptirler.

Anahtar Kelimeler: *Apis mellifera*, Bakteri, Mikrobiyoloji, Bal arısı sağlığı, *cry* genleri

GENİŞLETİLMİŞ ÖZET

Amaç: Bal arıları ekonomik ve biyolojik yönden oldukça önemlidir. Bal arıları bal, propolis, arı sütü, polen, bal mumu, arı zehri gibi arı ürünleri sayesinde dünya pazarında önemli yer almaktadır. Arıcılık, Dünya'nın hemen hemen her yerinde yapılan tarımsal bir faaliyettir. Arıcılık faaliyetlerini engelleyen en önemli nedenlerden biri de arı zararlı ve hastalıklarıdır. Bu nedenle de arı sağlığını korumak büyük önem arz etmektedir. Günümüzde bu zararlılarla mücadelede birçok yöntem kullanılmakta olup halen önüne geçilememiş durumdadır. Bu yöntemler arasında biyolojik mücadele yöntemi kullanılmamaktadır. Çalışmanın amacı, bal arılarının sağlığını korumak için biyolojik bir etmen kullanılarak hastalık ve zararlı organizmalarla mücadele konusunda biyopestisit geliştirilmesinde taban oluşturmaktır.

Yöntem: Çalışma için gerekli olan bal arısı örnekleri Gümüşhane ili Kürtün ilçesinden elde edilmiştir. İlçedeki belirlenen sağlıklı arılık ve kovanlardan ortalama 20 şer arı toplanmıştır. Bu arılar steril taşıma kaplarına alınarak laboratuvar ortamına getirilmiştir. Laboratuvara getirilen arı örnekleri öncelikle %70'lik alkol ile yüzey strelizasyonu sağlanmıştır. Yüzey sterilizasyonunun ardından 500µl Nutrient broth besiyeri içerisinde arıların ezilerek parçalanması sağlanmıştır. Artık parçaları ortamdan uzaklaştırmak için steril filtre ile süzme işlemi gerçekleştirilip, geri kalan sıvı Nutrient agar besiyerine ekim yapılarak 30°C' de 2-3 gün inkübasyona bırakılmıştır. Bu süre sonunda besiyerinde oluşan bakterilerin saflaştırılması için ayrı ayrı besiyerilerine ekimleri yapılmıştır. Bu bakterilerin tanımlanması için moleküler karakterizayonu, biyokimyasal testleri, fiziksel ve morfolojik analizleri yapılarak belirlenmiştir ve bakteriler GenBank'a kayıt ettirilmiştir.

Sonuç: Çalışma sonucunda bal arılarından 16 adet bakteri izolasyonu gerçekleştirilmiştir. Elde edilen bakterilerden 12 tanesi *Bacillus* cinsine, iki tanesi *Lysinibacillus* cinsine, bir tanesi *Paenibacillus* cinsine ait iken bir tanesi de *Pantoea* cinsine aittir. Bu bakterilerin moleküler ve biyokimyasal tanımlamaları yapılarak GenBank a kayıt yaptırılmış ve kayıt numaraları alınmıştır. On beş adet *Bacillus, Paenibacillus ve Lysinibacillus* cinslerine ait bakterinin *cry* gen analizleri yapılmıştır. Bilindiği üzere *cry* genleri hem zararlılara karşı kullanılma potansiyeline sahiptir hem de ileride bu bakteri ve genleri geliştirilerek biyopestisit kullanılma potansiyeli olabilecektir. Bu sonuçlara göre *cry1* geni 8 bakteride ve *cry3* geni de 3 bakteride gözlemlenmiştir. *cry2* ve *cry4* genleri bu bakterilerde tespit edilememiştir.

INTRODUCTION

According to the 2020 data of the Ministry of Food, Agriculture and Livestock, Directorate of Beekeeping Research Institute, Turkey ranks at the forefront of the beekeeping sector in the world with an average of 8 million 128 thousand hives and 109,330 tons of honey production. Beekeeping provides important contributions to the agricultural economy and crop production through pollination both in Turkey and in the world. So honey bees (*Apis mellifera*) play a very important role in pollination in natural ecosystem and agricultural field (Evans et al. 2010). In recent years, bee population and colony losses have increased in the world. Pathogens (parasites, fungi, viruses and bacteria) and abiotic stress factors can adversely affect colony health. All these factors are affecting the bee ecosystem (Brutscher et al. 2015, Li et al. 2018, Larsen et al. 2019). Over time, insects have developed a strong and effective immune system. The immune system of insects combats various pathogens and conseuqently has become one of the most diverse and successful immune systems in the world. Insects defense mechanisms include cellular and humoral immunity (Evans and Armstrong 2005, Schmid et al. 2008, Wilson-Rich et al. 2008).

Epidemic infection risks in honey bee colonies is reduced by individual and social immunity (Ilyasov et al. 2012). Both the type of immunities together at various levels protects the bees from diseases and ectoparasites (DeGrandi-Hoffman and Chen 2015, Larsen et al. 2019).

Pathogens, acaricides, fungicides, herbicides and other insecticides affect the bee immune system and thus affects bee health (Ilyasov et al. 2021). The social immune defense strategy, individual bees, reduces the pressure on the immune system (Larsen et al. 2019).

Like every living creature, honey bees are also have a microflora (Gilliam 1997, Olofsson and Vásquez 2008). These bacteria can play a positive or negative role on honey bee health and it is also important of rbutrient aquisition from a healthy diet (Evans and Spivak 2010). Bacterial microflora in bees, can inhibit lime disease (Reynaldi et al. 2004) and American foulbrood (Evans and Armstrong 2005, Evans et al. 2006). With a healthy microbiome, the reproduction of beneficial microorganisms is supported and the reproduction of pathogens is prevented. (Evans et al. 2006). Honey bees both at the individual and colony level is capable of immunity defense. Beekeepers use antibiotics to control pathogens and parasites, so pesticide applications are done frequently. This situation results in residues in hive products and hive equipment leading to an increased antibiotic resistance problem.

One of the biggest problems in beekeeping is attempting to treat honeybee diseases with chemical treatments. Limited success is achieved after chemical treatment, and there are problems, such as a danger to human health with chemical residues in the honey (Barganska et al. 2011). Problems are experienced in export markets for honey from treated bees, so attempts are made to sell this honey in the domestic market. As a result, the products cannot be sold easily and at their proper value. For this reason, biological control methods gain importance. Generally, for biological control *Bacillus* genus bacteria are used. *B. thuringiensis*, which was first isolated from diseased silkworm larvae by Japanese researcher Ishiwata (Ishiwata, 1901) in 1901, is the most widely used microbial control agent (Lacey et al. 2001). The insecticidal activity of *B. thuringiensis* is carried out by toxins in protein structure called insecticidal crystal proteins (ICP, cry proteins). These proteins are encoded by the genes (*cry*) located on the plasmids. These insecticidal proteins produced during spore formation constitute approximately 30% of the total protein content of the bacteria (Höfte and Whiteley, 1989; Aronson, 1993). ICPs are found undissolved under normal conditions. Therefore, they do not pose a risk to humans and other vertebrate organisms. On the other hand, their soluble properties at pH 9.5. Thus, the ICPs in the structure of this protein dissolve in the insect gut and turn into protoxin. Then, protoxins are broken down by intestinal enzymes and active toxins are formed. Active toxins cling to the receptors of intestinal epithelial cells, paralyze the intestinal wall of the insect and destroy it, forming pores. The poisoned insect can die immediately due to toxin activity and die as a result of blood poisoning within 2-3 days (Knowles, 1994). In this context, cry gene analyses were performed because 15 of the bacteria obtained belonged to the genus *Bacillus*. These bacteria will pave the way for developing biopesticides for organisms and microorganisms that affect bee health. The ground will be prepared for development for different agricultural or forest pests. In this context, it is aimed to obtain bacterial flora by collecting healthy honey bees. Bacteria obtained from bacterial flora will have the potential to be used against organisms that cause harm to bees.

MATERIAL AND METHODS

Sampling

Honey bee samples used in this study were collected from hives where are located in Gümüşhane province, distinct from Kürtün (It is located at latitude 40.748302 and longitude 38.984703). No chemical application has been made on the hives that samples were taken. The collected *Apis mellifera* were placed in sterile tubes and brought to the laboratory. The samples were collected twice a year, in the spring (June) and autumn (September) of 2020.

Isolation of Bacteria

Before bacterial isolation, honey bees were surface sterilized with 70% alcohol to remove possible contamination and then washed in sterile distilled water. The bee bodies were homogenized in 0.5 ml of sterilized Nutrient Broth (NB) using a glass tissue grinder and filtered. After preparing the homogenate for bacterial isolation, suspensions were diluted to 1×10-5 (Thiery and Frachon 1997) and 0.1 ml were spread on nutrient agar (Thiery and Frachon 1997). Plates were incubated at 30°C for 2-3 days. Isolates

were determined based on the color and morphology of the colonies. Individual colonies were isolated, sub-cultured twice to ensure purity and then stored in 15% sterilized glycerol at -80°C for further studies. Pure cultures of bacterial colonies were identified by their morphology, biochemical, physiological and molecular characteristics (16S rRNA).

Morphological, Physiological and Biochemical Characterisations of Isolates

Bacterial strains were selected based on morphological biochemical, physiological features according to Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986). Phenotypic characteristics of the strains include cell and colony shape on NA. Optimum pH was determined, after 16 h incubation at 30°C by measuring the densities using a spectrophotometer (Spectramax M2) at OD600 (Ben-Dov et al. 1995). Biochemical panel test system API 20E (bioMerieux, France) was handled according to the manufacturer's instructions. Then the panels were incubated for 18-24 h at 30°C. The results of the tests were performed by referring to the API 20E reading table.

16S rRNA Gene Sequence Analysis

Genomic DNA from all samples was extracted by phenol/chloroform procedures (Sambrook et al. 1989). PCR amplification of 16S rRNA genes of bacterial isolates was performed with the following universal primers (William et al. 1991); UNI 16S-L: 5_-ATTCTAGAGTTTGATCATGGCTCA-3_ as forward and UNI 16S-R: 5_ATGGTACCGTGTGTGACGGGCGGTG TGTA-3_ as the reverse. PCR conditions were adjusted according to William et al. (1991). Reactions were totally in 50 µl; 1 µl of template DNA was mixed with 5 µl reaction buffer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM (each) with primer and 0.5 U with Taq DNA polymerase. Amplification was performed with 30 cycle program (each cycle consisting of denaturation at 94°C for 3 min, annealing at 55°C for 60 s and extension at 72°C for 3 min), followed by a final extension step at 72°C for 5 min, by using a thermal cycler (BioRad). Each experiment was associated with negative (without DNA template) controls. PCR products were analyzed on a 1.2% agarose gel. Sequence analysis of 16S rRNA products samples were performed using 16S universal primers by SenteBiolab (Ankara/TURKEY). Using the NCBI GenBank database, determined sequences were used to perform BLAST searches (Altschul et al. 1990). Comparison of approximately 1.400 bp fragments of 16S rRNA gene sequences of each isolate with other 16S rRNA sequences in the NCBI GenBank database (Altschul et al. 1990) were performed.

Optimum pH Range of All Bacteria

In order to determine the pH range in which the bacteria grows optimally, media at different pH values (pH3, pH4, pH5, pH6, pH7, pH8, pH9 and pH10) were prepared and incubated at 30°C for 24 hours.

Determination of *cry* Genes of Bacteria

PCR amplification of *cry* genes was performed of the toxin genes using *cry1*, *cry2*, *cry3* and *cry4* primers (Table 1). PCR was constructed according to the following conditions: pre-amplification 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52-59°C for 60 s, and elongation at 72°C for 3 min. Reactions were totally in 50 µl; 1 µl of template DNA was mixed with 5 µl reaction buffer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM (each) with primer and 0.5 U with Taq DNA polymerase. PCR products were analyzed on a 1% agarose gel.

Table 1. *Cry* gene primers

Primer Name	Primer sequence	Sequence lenght (PCR amplification)	Tm (°C)
cry1Fw	CATGATTCATGCGGCAGATAAC	277bp	55
cry1Rv	TTGTGACACTTCTGCTTCCCATT		
cry2Fw	GTTATTCTTAATGCAGATGAATGGG	701bp	52
cry2Rv	CGGATAAAATAATCTGGGAAATAGT		
cry3Fw	CGTTATCGCAGAGAGATGACATTAAC	604bp	54
cry3Rv	CATCTGTTGTTTCTGGAGGCAAT		
cry4Fw	GCATATGATGTAGCGAAACAAGCC	439bp	59
cry4Rv	GCGTGACATACCCATTTCCAGGTCC		

RESULTS

In this study, 16 bacterial isolates; *Bacillus cereus* (GAP1), *Bacillus subtilis* (GAP2), *Bacillus wiedmannii* (GAP3), *Bacillus megaterium* (GAP4), *Pantoea rodasii* (GAP5), *Bacillus nakamurai* (GAP6), *Bacillus mobilis* (GAP7), *Bacillus pacificus* (GAP8), *Bacillus thuringiensis* (GAP9), *Lysinibacillus fusiformis* (GAP10), *Bacillus vallismontis* (GAP11), *Paenibacillus odorifer* (GAP12), *Bacillus velezensis* (GAP13), *Bacillus flexus* (GAP14), *Bacillus paramycoides* (GAP15) and *Lysinibacillus sphaericus* (GAP16) from *Apis mellifera* were isolated and characterized. The optimum pH range in which bacteria can grow was also determined (Table 2). When the pH results of the bacteria obtained in the study in Genbank are examined, it is seen that they confirm each other (https://www.ncbi.nlm.nih.gov/genbank/).These results are used to confirm the accuracy of the bacteria.

Table 2. Optimum pH range of bacteria.

pH	GAP 1	GAP 2	GAP 3	GAP 4	GAP 5	GAP 6	GAP 7	GAP 8	GAP 9	GAP 10	GAP 11	GAP 12	GAP 13	GAP 14	GAP 15	GAP 16
pH3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH5	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
pH6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH7	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+
pH8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Some biochemical characteristics (API20E) of bacterial isolates are summarized in Table 3, including which growth medium is suitable for their growth.

Table 3: Biochemical characteristics of bacteria (API20E).

API20E Tests	GAP 1	GAP 2	GAP 3	GAP 4	GAP 5	GAP 6	GAP 7	GAP 8	GAP 9	GAP 10	GAP 11	GAP 12	GAP 13	GAP 14	GAP 15	GAP 16
GEL	-	-	+	+	-	+	-	-	+	+	-	+	-	-	+	-
GLU	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
MAN	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
INO	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
SOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RHA	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
SAC	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
MEL	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+
AMY	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
ARA	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
ONPG	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
ADH	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
LDC	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
ODC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIT	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+
H2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
URE	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
TDA	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
IND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-

When the biochemical test results of the bacteria obtained in the study in Genbank are examined, it is seen that they confirm each other (https://www.ncbi.nlm.nih.gov/genbank/).These results are used to confirm the accuracy of the bacteria.

16S rRNA gene sequence analysis results of isolates are given in Table 4. The 16S rRNA partial gene sequences generated in this study have accession numbers MZ097346, MZ097347, MZ097348, MZ097349, MZ097350, MZ097351, MZ097352, MZ097353, MZ097354, MZ097355, MZ097356, MZ097357, MZ097358, MZ097359, MZ097360 and MZ097361, respectively. In the Table 4, the isolate name is the code that we gave in this study, the bacterium name is from the 16S identifiation, accession numbers are provided from GenBank and the similarity score is presented from GenBank for each strain identification.

As seen in Figure 1, cry gene analyzes were made and *cry 1* gene was only found in 8 bacteria, and *cry 3* gene in 3 bacteria. *cry 2* and *cry 4* genes were not observed in any bacteria. *cry 1* gene has 277 bp and *cry 3* gene has 604 bp. In the first lane (M) there is marker (1kb) and the bands are 250 bp, 500 bp, 750 bp, 1000bp, 1500 bp, 2000 bp and 2500 bp, respectively (from the bottom to top). The bands under the marker bands, which are seen in all four gel images (A, B, C and D), are the bands belonging to the primers, and their appearance indicates the quality of the gel. According to these results and looking at the marker, the bands corresponding to around 250 bp in A shows the presence of *cry1* gene. According to the results, bands seen around 500 bp in the B also indicates the presence of *cry3* gene. In addition, one sample of each of these bands was sent to the sequence and were confirmed. On the other hand, the bands seen in the gel images of *cry 2* and *cry 4* genes belong to the primers. As can be seen, these bands are below the marker bands.

Table 4. 16S results of bacteria.

Isolate name	Bacterium name	Accession number	Similarity
GAP1	*Bacillus cereus*	MZ097346	99%
GAP2	*Bacillus subtilis*	MZ097347	99%
GAP3	*Bacillus wiedmannii*	MZ097348	99%
GAP4	*Bacillus megaterium*	MZ097349	99%
GAP5	*Pantoea rodasii*	MZ097350	99%
GAP6	*Bacillus nakamurai*	MZ097351	99%
GAP7	*Bacillus mobilis*	MZ097352	99%
GAP8	*Bacillus pacificus*	MZ097353	99%
GAP9	*Bacillus thuringiensis*	MZ097354	99%
GAP10	*Lysinibacillus fusiformis*	MZ097355	99%
GAP11	*Bacillus vallismontis*	MZ097356	99%
GAP12	*Paenibacillus odorifer*	MZ097357	99%
GAP13	*Bacillus velezensis*	MZ097358	99%
GAP14	*Bacillus flexus*	MZ097359	99%
GAP15	*Bacillus paramycoides*	MZ097360	99%
GAP16	*Lysinibacillus sphaericus*	MZ097361	99%

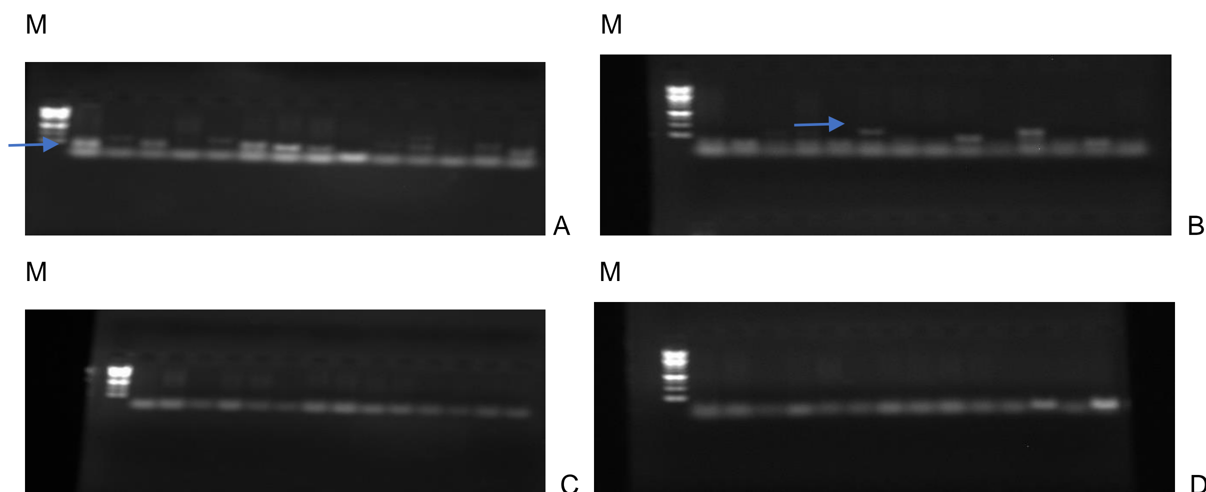

Figure 1: *cry* gene analysis of bacteria. A: *cry 1* gene PCR results, B: *cry 3* gene PCR results, C: *cry 2* gene PCR results, D: *cry 4* gene PCR results.

DISCUSSION

As a result of the study, 12 bacteria belonging to the genus *Bacillus*, two bacteria belonging to the genus *Lysinibacillus*, one bacterium belonging to the genus *Paenibacillus* and one bacterium belonging to the genus *Pantoea* were obtained. *Pantoea* species, which are common in plants and soil, belong to the Enterobacteriaceae family. *Pantoea* species are members of the normal flora of the human gastrointestinal tract and can be found in water, wastewater, soil, plants and foods such as fruit/vegetables. Many *Pantoea* species are known as plant disease agents and are used as biopesticides in the agricultural industry (Cruz et al. 2007). *Pantoea* species, known as plant pathogens, are microorganisms that develop following injuries with plant spines, and the most frequently isolated species is *Pantoea rodasii*, which was also identified from the GAP5 isolate (Kurşun et al. 2012).

The *Paenibacillus* genus includes some strains previously found in the *Bacillus* and *Clostridium* genera. *Paenibacillus odorifer* bacteria identified in GAP12 isolate are found in soil, water and various foods (Beno et al. 2020).

Members of the Bacillaceae family, which are important in terms of biological control, are Gram-positive, motile or non-motile rod-shaped bacteria that produce endospores. *Bacillus* and *Clostridium* genera in this family contain important insect pathogen species and are mostly separated from each other according to their oxygen needs. Species belonging to the genus *Bacillus* are aerobic or facultative anaerobic, while species belonging to the genus *Clostridium* are anaerobic. Both genera have rod-shaped cells that form chains (Tanada and Kaya 1993). Insect pathogens known in the genus *Clostridium* reproduce only in the insect gut and cause disease and never pass into insect hemocele. The genus *Bacillus* contains important insect pathogenic species. The most important of these is *Bacillus thuringiensis* bacteria, which is in the *Bacillus cereus* group. *B. thuringiensis* is a spore-forming soil bacterium that produces toxin in crystalline structure and has an insecticidal effect mostly against insects in Lepidoptera, Diptera and Coleoptera groups (Beegle and Yamamoto 1992). According to studies conducted in recent years, it has been found to be lethal among the insect groups of Hymenoptera, Homoptera, Orthoptera and Mallophaga, as well as on nematodes, ticks and protozoa (Feitelson et al. 1992, Feitelson 1993). The fact that insecticidal products obtained from *B. thuringiensis* bacteria do not cause infection on humans, non-target organisms and beneficial insects has increased the effective use of these products in the control harmful insects (Lacey et al. 2001, Seigel 2001). *B. thuringiensis*-derived products constitute 95% of the world biopesticide market. Many commercial companies have introduced products from *B. thuringiensis*. According to 1998 figures, more than 200 products of *B. thuringiensis* origin are used against pests only in the

USA (Schnepf et al. 1998). In addition, many *B. thuringiensis*-derived products are susceptible to synthetic chemical pesticides, obtained at a lower cost. Some other species belonging to the genus Bacillus are also used in the control of harmful insects. *Bacillus popilliae* (Dutky) is used in the control of some species belonging to the Scarabaeidae family, while *Bacillus sphaericus* Neide is used in the control of mosquito larvae (Klein and Jackson 1992). *B. popilliae* needs to be produced *in vivo*, and lower than expected levels of infection in many field applications reduces the potential of this bacterium to be used in large areas (Klein and Kaya 1995). Compared to *B. thuringiensis*, *B. sphaericus* is resistant to polluted habitats and environmentally friendly. Although it is more resistant to various factors, its biggest disadvantage is that the host spectrum is very narrow (Lacey and Undeen 1986, Charles et al. 1996, Nicolas et al. 1994). However, some fly species are resistant to this bacterium. It has been reported (Rao et al. 1995, Nielsen-Leroux et al. 1997).

The most important factor in *Bacillus* species are crystal (*cry* genes) genes. In the study, 16 *Bacillus* genus bacteria were obtained and *cry* gene analyzes were performed. In this context, the bacteria obtained are of great importance. According to the results of *cry* gene analysis 8 bacteria have *cry1* genes and 3 bacteria have *cry3* genes were obtained in bacteria. *cry2* and *cry4* genes could not be obtained in these bacteria. The most common *cry* gene types in nature are *cry1* and *cry3*. According to these results, the potential of using these bacteria against bee pests seems high.

For the honeybees to be healthy, under the chemical treatment practices, antibiotics are frequently used against bacterial diseases (Mutinelli, 2003). Antibiotic use weakens the immune system of the bees and leads to antibiotic-resistant bacterial pathogens (Doğaroğlu and Samancı, 2006; Barganska et al. 2011). Unfortunately, the fight against these bacteria is self-defeating. In previous studies, positive effects have been shown by the resistance of the bacterial flora in the bodies of bees against disease (Gilliam, 1997).Thus, the idea arises that if the naturally occurring microbial flora in the bodies of the bees are supported, the bees may be more resistant to disease (Tajabadi et al. 2013) In particular, the bacteria with probiotic properties found in the honey stomachs or intestines of honeybees have been observed to provide resistance against other bee pathogens (Forsgren et al. 2010).

In this study, it was aimed to determine the general bacterial flora of honey bees and the optimal growth conditions. We found that bacteria can be partially detected by using selective growth media.

Both similar and different bacteria were obtained previously from the honey bee intestinal flora, so we suspect that there could be regional differences of honey bee gut bacteria based on their floral diet selection (Yarılgaç, 2016). For future research, we aim to obtain and characterize local isolates to determine their biological significance in terms of improving honey bee health.

Conclusion

As a result, considering the definitions of the bacteria obtained in the study, 8 bacteria with the *cry 1* gene and 3 bacteria with the *cry 3* gene have the potential to be used in future biopesticide development studies. GAP6 (*Bacillus nakamurai*) bacteria, in which both *cry* genes (*cry 1* and *cry 3*) are common, should be studied in more detail. It is thought that it may have the potential to be a more potent biopesticide with the activity of both *cry* genes.

According to isolated bacteria and gene analyses, these bacteria should be tested primarily for use in maintaining honey bee health. Previously, some of these bacteria have been effective for manageing *Varroa* mites and especially *Galleria mellonella* pests. In addition to honey bee pests, future research can be carried out to develop bacterial biopesticides for different agricultural and forest pests in general.

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