Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi
Afyon Kocatepe University – Journal of Science and Engineering
https://dergipark.org.tr/tr/pub/akufemubid

Araştırma Makalesi / Research Article
DOI: https://doi.org/10.35414/akufemubid.1348983
AKÜ FEMÜBİD **24** (2024) 011001 (01-07) AKU J. Sci. Eng. **24** (2024) 011001 (01-07)

Determination of Antimicrobial Effects of Secondary Metabolites of Different Bacteria Belonging to the Genus *Bacillus*

Makale Bilgisi / Article Info
Alındı/Received: 24.08.2023
Kabul/Accepted: 11.01.2024
Yayımlandı/Published: 27.02.2024

Bacillus Cinsine Ait Farklı Bakterilerin Sekonder Metabolitlerinin Antimikrobiyal Etkilerinin Belirlenmesi

Aydın YEŞİLYURT[1], Seda BİRYOL[1], Ali SOYDİNÇ[2], Sevda İŞIK[2], Mehtap USTA[3*]

[1] *Trabzon University, Tonya Vocational School, Department of Medical Services and Techniques, Trabzon, Türkiye*
[2] *Regional Directorate of Trabzon Forestry, Trabzon, Türkiye*
[3] *Trabzon University, Tonya Vocational School, Department of Health Care Services, Türkiye*

Abstract

Secondary metabolites of bacteria can be used to control microorganisms. In this study, the antimicrobial activity properties of *Bacillus* isolates from *Apis mellifera* and *Varroa destructor* have been determined. The antimicrobial activities of *Bacillus* species against some bacteria and pathogenic yeast (*Candida albicans*) were investigated according to the disc diffusion method. As a result of the research, secondary metabolites of *Bacillus* isolates used in the study inhibited the development of the tested microorganisms at different rates (1.1-8.4 mm inhibition zone). Two isolates GAP2 (*Bacillus subtilis*) and GAP9 (*Bacillus thuringiensis*) showed high antibacterial activity. Most of the metabolites isolated from bacterial isolates were shown to be sensitive to *Escherichia coli* ATCC2471 and *Serratia marcescens* ATCC13880 ($p<0.05$). It was determined that the products obtained from GV6, GV7, GAP7, GAP8, GAP11, GAP13, and GAP15 isolates did not affect any of the bacteria used in the experiments ($p<0.05$). It is thought that *Bacillus* strains producing secondary metabolites, especially GAP2 and GAP9 isolates, may have the potential to be used in various applications for saprophytic and pathogenic microbes in medicine, veterinary medicine, agriculture, and the food industry.

Anahtar Kelimeler: *Antimicrobial; Antifungal; Bacillus; Bacteria; Disc diffusion assay; Microbiology.*

Öz

Bakteriyal sekonder metabolitler, mikroorganizmaları kontrol etmek için kullanılabilir. Bu çalışmada *Apis mellifera* ve *Varroa destructor*'dan elde edilmiş olan farklı *Bacillus* izolatlarının antimikrobiyal aktivite özelliklerinin belirlenmesi amaçlanmıştır. Bacillus türlerinin bazı bakteri ve patojen mayalara (*Candida albicans*) karşı antimikrobiyal aktiviteleri disk difüzyon yöntemine göre araştırıldı. Araştırma sonucunda çalışmada kullanılan *Bacillus* izolatlarının sekonder metabolitleri, test edilen mikroorganizmaların gelişimini farklı oranlarda (1,1-8,4 mm inhibisyon bölgesi) inhibe etmiştir. GAP2 (*Bacillus subtilis*) ve GAP9 (*Bacillus thuringiensis*) yüksek antibakteriyel aktivite göstermiştir. Bakteriyel izolatlardan izole edilen metabolitlerin çoğunun *Escherichia coli* ATCC2471 ve *Serratia marcescens* ATCC13880'e duyarlı olduğu görüldü ($p<0,05$). GV6, GV7, GAP7, GAP8, GAP11, GAP13 ve GAP15 izolatlarından elde edilen ürünlerin deneylerde kullanılan bakterilerin hiçbirine etkisinin olmadığı belirlendi ($p<0,05$). GAP2 ve GAP9 izolatları başta olmak üzere sekonder metabolit üreten *Bacillus* suşlarının tıp, veterinerlik, tarım ve gıda endüstrisinde saprofitik ve patojenik mikroorganizmalara yönelik çeşitli uygulamalarda kullanılma potansiyeline sahip olabileceği düşünülmektedir

Keywords: *Antimikrobiyal; Antifungal; Bacillus; Bakteri; Disk difüzyon testi; Mikrobiyoloji*

1. Introduction

Microbial secondary metabolites are small molecules with unique structures produced by bacteria at late growth stages, which are generally not essential for the growth of microbial cultures but are essential for human health, nutrition, and economy (Ruiz *et al*. 2010). The use of antibiotics and the potential for the discovery of new antibiotics has become even more important with the discovery of secondary metabolites (Spellberg 2014). However, the widespread and incorrect use of antibiotics brings the issue of antibiotic resistance to the forefront. Aside from preventing the development of resistance with increased usage of antibiotics, microorganisms have responded to this situation by developing many types of resistance, and as the use of antimicrobial drugs increases, so does the level and complexity of bacterial pathogen resistance mechanisms (Tenover 2006). Therefore, the discovery and development of novel antibiotics is critical. Despite their natural difficulty, new antimicrobial substances may be

*Sorumlu Yazar/Corresponding Author: Mehtap USTA e-posta/e-mail: mehtapyakupoglu@trabzon.edu.tr

identified by screening and isolating bacteria that generate them (Barsby *et al*. 2002, Ren *et al*. 2007).

Like each organism, insects have their microbial flora. This flora is contaminated by other microorganisms from the environment or other organisms called 'entomopathogens', which are bacteria, fungi, nematodes, and protozoa. These pathogens cause the death of insects by synthesizing compounds that will cause various diseases. There are many studies on insect pathogens (Miller *et al*. 2021, Steele *et al*. 2021, Usta 2021b). However, more information is needed on whether the secondary metabolites produced by these pathogens have lethal or growth-inhibitory effects.

Today, there is a need for more studies on the use of biological control agents to protect biological diversity and combat diseases naturally. Some compounds are naturally synthesized by bacteria and show antimicrobial activity on other bacteria. These compounds are generally short-chain proteins with low molecular weight (Akkoç *et al*. 2009). Unlike antimicrobial peptides, there are also secondary compounds with different structures synthesized by bacteria (Keswani *et al*. 2020, Sansinenea and Ortiz 2011).

As the use of antimicrobials increased, the resistance mechanisms introduced by pathogens increased and became more complex. The emergence of strains resistant to antimicrobials and the unconscious use of antibiotics has led to the search for natural substances (Bérdy 2005, Cowan 1999, Demain and Fang 2000, Keswani *et al*. 2020, Nabavi *et al*. 2014, Sansinenea and Ortiz 2011). *Bacillus*, *Lysinibacillus,* and *Brevibacillus* species are among the most studied organisms in terms of their ability to produce antimicrobial substances (Demirkan *et al*. 2021, Perez *et al*. 1993, Prashanthi *et al*. 2021).

Bacillus genus microorganisms of the Bacillaceae family are rod-shaped bacteria that create endospores, are normally gram-positive, have peritrack flagella and motile flagella, and are aerobic or facultative anaerobes (Turnbull 1996). In addition to the convenience of isolation and production of *Bacillus*, and the use of many enzymes produced by *Bacillus* in various industrial areas, the secondary metabolites they produce are the most striking (Rosovitz *et al*. 1998, Johnvesly *et al*. 2002).

The primary goal of this study is to identify the antibacterial properties of secondary metabolites from various *Bacillus* species. The aim is to investigate and evaluate the potential antibacterial properties of secondary metabolites derived from these bacteria against a variety of harmful pathogens. The findings suggest that antimicrobial chemicals derived from *Bacillus* might be used as alternative treatment and preventative measures in future medicinal or agricultural applications and for the discovery of antibiotics. Furthermore, this research can help to further the discovery of antimicrobial chemicals derived from natural sources and increase biological control.

2. Materials and Methods

2.1. Bacterial strains

The bacterial strains in this study have been previously isolated and determined (Usta 2021b, 2021a). Supernatant samples containing secondary metabolites were obtained from these previously described bacteria (Table 1).

Table 1. Bacteria whose secondary compounds are used in the disk diffusion susceptibility test.

Isolate codes	Bacterium Name	Host	References
GV1	*Pantoea dispersa*	*Varroa destructor*	Usta 2021a
GV3	*Lysinibacillus macroides*	*Varroa destructor*	Usta 2021a
GV4	*Bacillus mycoides*	*Varroa destructor*	Usta 2021a
GV5	*Lysinibacillus fusiformis*	*Varroa destructor*	Usta 2021a
GV6	*Pseudomonas lutea*	*Varroa destructor*	Usta 2021a
GV7	*Lysinibacillus varians*	*Varroa destructor*	Usta 2021a
GAP1	*Bacillus cereus*	*Apis mellifera*	Usta 2021b
GAP2	*Bacillus subtilis*	*Apis mellifera*	Usta 2021b
GAP4	*Bacillus megaterium*	*Apis mellifera*	Usta 2021b
GAP6	*Bacillus nakamurai*	*Apis mellifera*	Usta 2021b
GAP7	*Bacillus mobilis*	*Apis mellifera*	Usta 2021b
GAP8	*Bacillus pacificus*	*Apis mellifera*	Usta 2021b
GAP9	*Bacillus thuringiensis*	*Apis mellifera*	Usta 2021b
GAP11	*Bacillus vallismontis*	*Apis mellifera*	Usta 2021b
GAP13	*Bacillus velezensis*	*Apis mellifera*	Usta 2021b
GAP14	*Bacillus flexus*	*Apis mellifera*	Usta 2021b
GAP15	*Bacillus paramycoides*	*Apis mellifera*	Usta 2021b

2.2. Antimicrobial activity

The disc diffusion assay method was used for the detection of the antimicrobial activity of strains (Table 1). Antimicrobial activity of the strains was evaluated against *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloaceae* ATCC2468, *Enterococcus faecalis*

ATCC51299, *Escherichia coli* ATCC2471, *Klebsiella pneumoniae* ATCC700603, *Bacillus thuringiensis* ATCC10792, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus epidermidis* ATCC14990, *Staphylococcus aureus* ATCC25923, *Streptococcus faecalis* ATCC 9790, *Yersinia pestis* ATCC 19428 and *Candida albicans* ATCC10351. These selected bacteria are also clinically important as they are human pathogens. Firstly, Mueller-Hinton Agar (MHA) (1038720500, Merck) and Mueller-Hinton Broth (MHB) (1102930500, Merck) mediums were prepared in accordance with the manufacturer's instructions for bacterial strains and the fungal strain were used Potato Dextrose Agar (PDA)(Merck 70139) and Potato Dextrose Broth (PDB)(Merck P6685). After the media were autoclaved, MHA/PDA was dispensed in sterile circular Petri dishes with a diameter of 90 mm and a thickness of 4 ± 0.5 mm, while the liquid one (MHB/PDB) was directly stored in a 4 ˚C fridge after cooling. The pH was adjusted to 7.2-7.4 for bacterial media and 5.3 for fungal media. Bacteria from Table 1 were initially resurrected by getting them from -80 stock and inoculating a single colony into a Nutrient agar (NA) medium. Bacterial strains were inoculated in 3 ml Mueller Hinton broth (MHB) and incubated at 30 ˚C for 48 hours. The supernatants were collected by centrifugation at 13000 rpm for 15 min. Then, the supernatant was filtered by a membrane filter (0.22 µm) and stored at 4 °C. The test microorganisms were grown in Nutrient Broth (NB)/PDB and at 37 °C / 30 °C for 16-18 h.

The disc diffusion test was used for the detection of antibacterial activity. The experiments in the current study were performed based on Kirby-Bauer's method (Barry *et al*. 1970, Bauer *et al*. 1966). A hundred microliter of each test microorganism suspension adjusted at 10^8 cfu/ml was spread on MHA/PDA. The previously sterilized discs (5 mm diameter, Whatman no 1) were placed on the same plates. A 100 µl of filtered supernatant of each sample was absorbed into discs. The plates were incubated at 37 °C for 16-18 h and *Candida albicans* was incubated at 30 °C for 16-18 h. In the study, 10 mg/ml Kanamycin was utilized as a positive control for all bacterial groups, while 10 mg/ml Penicillin solutions were employed in *Candida albicans* studies. The inhibition zone diameters were recorded (Sharma *et al*. 2014) (Figure 1).

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was run to determine any significant differences in the study groups by Duncan multiple range test was performed through SPSS (Statistical Package for Social Sciences, version 28, Chicago, IL, USA), and the significance level was determined at $p < 0.05$.

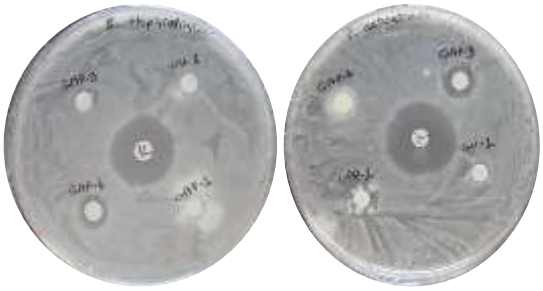

Figure 1. Determination of the inhibition effect of *Bacillus* sp. seconder metabolites against some human pathogen bacteria and fungus by disc diffusion method.

3. Results

In this study, the total extracellular products with secondary compounds produced by 16 bacterial isolates were used to determine the effects on some other pathogenic bacteria. These bacterial isolates, which were identified in the previous studies, are given again in the material and method section. It was determined that the compounds produced by GAP2 and GAP9 isolates affected all applied bacterial strains except the GAP2 and GAP9 products on *Salmonella typhimurium* ATCC13311 ($p<0.05$, $F(16,36) = 16.39$). It was determined that the products obtained from GV6, GV7, GAP7, GAP8, GAP11, GAP13, and GAP15 isolates did not affect any of the bacteria used in the experiments ($p<0.05$) (Table 2).

The results revealed that the pathogenic bacterium *Serratia marcescens* ATCC13880 formed the most zones from the total secondary metabolites obtained from GAP2, and there was a significant difference when compared to the control group ($p<0.05$, $F(16.36) = 91.059$) (Table 2). Most of the metabolites isolated from bacterial isolates were shown to be sensitive to *Escherichia coli* ATCC2471 and *Serratia marcescens* ATCC13880 ($p<0.05$). Total secondary metabolites obtained from GV1 (*Pantoea dispersa*) were found to be effective against the test microorganisms *Escherichia coli* and *Serratia marcencens* ($p<0.05$) (Table 2). Of the metabolites isolated from GV3 (*Lysinibacillus macroides*), it was determined to be effective only on *Escherichia coli* and was determined as zone 2.2 mm ($p<0.05$, $F(16.36) = 22.801$) (Table 2). When the results of GV4 (*Bacillus mycoides*) are evaluated, except for *Pseudomonas aeruginosa* ATCC27853 and *Enterobacter cloaceae* ATCC2468, it was determined that it is effective on *Escherichia coli* ATCC2471, *Klebsiella pneumoniae*

ATCC700603, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus epidermidis* ATCC14990 and *Staphylococcus aureus* ATCC25923, and especially *Serratia marcescens* ATCC13880 in the most susceptible pathogen bacterium (inhibition zone 6.3 mm) ($p<0.05$, F (16.36) =16.694) (Table 2). The extracts from GV5 (*Lysinibacillus fusiformis*) were recorded to act on *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC2471, *Serratia marcescens* ATCC13880 and *Staphylococcus aureus* ATCC25923 and create a 7.1 mm zone of inhibition, especially in *Serratia marcescens* ATCC13880 ($p<0.05$, Table 2). GAP4 (*Bacillus megaterium*) isolates, *Pseudomonas aeruginosa* ATCC27853 and *Enterobacter cloaceae* ATCC2468 were found to be effective, and their zones of inhibition were similar ($p>0.05$, Table 2).

Table 2. Antibacterial activity of some bacterial isolates against human pathogen bacteria

Isolate Codes	*Pseudomonas aeruginosa* ATCC27853	*Enterobacter cloaceae* ATCC2468	*Escherichia coli* ATCC2471	*Klebsiella pneumoniae* ATCC700603	*Salmonella typhimurium* ATCC13311	*Serratia marcescens* ATCC13880	*Staphylococcus epidermidis* ATCC14990	*Staphylococcus aureus* ATCC25923
GV1	NZ	NZ	1.1±0.28a	NZ	NZ	6.1±0.97fg	NZ	NZ
GV3	NZ	NZ	2.2±0.57b	NZ	NZ	NZ	NZ	NZ
GV4	NZ	NZ	4.0±1.15d	4.2±1.15de	4.0±1.21d	6.3±0.17fg	5.2±0.34ef	3.2±1.21c
GV5	5.1±0.57ef	NZ	3.0±0.47c	NZ	NZ	7.1±1.62gh	NZ	4.0±0.07d
GV6	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GV7	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GAP1	NZ	NZ	3.0±0.57c	NZ	NZ	6.0±0.09f	NZ	5.1±0.76ef
GAP2	4.0±0.67d	4.1±1.15de	5.0±1.15e	5.0±0.61e	4.0±1.34d	8.4±0.54h	7.1±0.87g	4.3±0.27de
GAP4	3.1±0.41c	3.0±0.58c	NZ	NZ	NZ	NZ	NZ	NZ
GAP6	NZ	NZ	2.0±0.57b	NZ	4.0±1.46d	2.0±0.13b	NZ	NZ
GAP7	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GAP8	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GAP9	5.1±0.53ef	6.0±1.15f	5.0±0.51e	3.0±0.45c	NZ	8.0±0.85h	4.1±0.57de	5.1±0.67ef
GAP11	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GAP13	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
GAP14	NZ	NZ	NZ	NZ	NZ	5.2±0.13ef	NZ	NZ
GAP15	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Control	8.0±0.57h	7.0±0.33g	8.1±0.55h	10.0±0.45ı	10.0±1.15ı	12.0±0.16j	11.05.1±0.57i	8.1±1.15h

Values are mean±standard errors. Mean values within the column followed by the different letter are significantly different at the $p<0.05$ probability level using the Duncan test NZ: Non-Zone

In particular, metabolites derived from GAP2 (*Bacillus subtilis*) and GAP9 (*Bacillus thuringiensis*) were found to be effective on pathogenic bacteria and were close to the inhibition zones that occurred when compared with positive control ($p<0.05$, Table 2). *S. marcescens* which is the kanamycin antibiotic is the most effective, which indicates that it is the most sensitive strain of the selected bacteria. Therefore, the secondary compounds of GAP2 showed the greatest zone as 8.4 mm on this bacterium, showing approximately 700 µg antibiotic effects. The smallest zone diameter of 1.1 mm was formed in *Escherichia coli* due to secondary compounds produced by GV1, which means that these bacterial compounds have about 100 µg antibiotic effect as an equal amount. The amounts of the secondary compound mixtures used in bacteria that have no zones can be examined again by concentrating.

4. Discussion and Conclusion

Throughout history, there has been a constant war between humans and the microorganisms that cause diseases. As the use of antimicrobials has increased, the resistance mechanisms revealed by pathogens have increased and become more complex (Reygaert 2018). Considering the emergence of strains resistant to antimicrobials, the unconscious use of antibiotics, and the economic dimension, studies have been directed to the search for natural substances (Davies and Davies 2010). Therefore, it is important to investigate from nature new microorganisms that produce large and powerful antibiotics. *Bacillus* species are among the most studied organisms in terms of their capacity to produce antibiotics (Perez *et al*. 1993).

Since in our preliminary studies, it was determined that there was no effect on bacterial groups from 24-hour secondary metabolite production of bacterial isolates, we determined the increase in the effects of metabolites, especially after 48 hours of growth. Generally, in *Bacillus*, the time of antibiotic activity is between 24-72 hours of incubation. The time at which the maximum antibiotic activity occurs changes, depending on the particular species of *Bacillus*. This phenomenon may be observed because different species have different metabolic pathways (Hosoya *et al*. 1998).

According to the results of this study, all the treated bacteria were affected by secondary compounds belonging to *Bacillus* species, especially *B. subtilis* (GAP2)

and *B. thuringiensis* (GAP9). *E. cloacae*, *K. pneumonia*, *S. typhimurium,* and *S. epidermidis* are affected only by secondary compounds of *Bacillus* species. These results are not surprising because the genus *Bacillus* is already known to produce bioactive substances that have the potential to be used against agricultural pests, in the pharmaceutical industry, and in the production of biosurfactants (Kaspar *et al*. 2019, Stoica 2019, Wang *et al*. 2015).

Gram-positive and Gram-negative bacteria showing antibacterial activity by *Bacillus* strains have been reported to include *Yersinia enterocolitica*, *Micrococcus flavus*, *Staphylococcus aureus* (Chatterjee *et al*. 1992, Aslım and Yucel 2008), *Escherichia coli* (Perez *et al*. 1992, Aslım and Yucel 2008*), Pseudomonas aeruginosa* (Perez *et al*. 1992) and *Micrococcus luteus* (Perez *et al*. 1993). Especially in our study, it was shown to be effective on *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*.

Serratia marcescens is a harmful pathogen that causes mortality, particularly in preterm and low birth-weight infants, and it is known to develop antibiotic resistance (Atmaca *et al*. 2018). Furthermore, it can be fatal owing to infections in the eye and urinary system (Zivkovic *et al*. 2023). It was discovered to be especially susceptible to secondary metabolites produced by GAP2 (*Bacillus subtilis*). It may also be utilized as a probiotic for *B. subtilis* in humans (Hong *et al*. 2005, Hong *et al*. 2023). The results of the research support the literary theory. GAP2 metabolites have the potential to be employed both for *S. marcescens* infections and as probiotics.

The results of this study showed that many strains of the *Bacillus* community from natural isolates have antimicrobial activity against clinically important bacteria. *Bacillus* sp. is increasing bacteria resistance to conventional antibiotics. As a result, there is an increasing interest in using metabolites generated by bacteria as antimicrobials against human pathogenic microorganisms. *Bacillus* strains that produce secondary metabolites can be employed in a variety of applications for saprophytic and pathogenic microbes in medicine, veterinary medicine, agriculture, and the food industry.

Declaration of Ethical Standards

The authors declare that they comply with all ethical standards.

Credit Authorship Contribution Statement

Author 1: Investigation, Methodology / Study design, Writing – original draft, Conceptualization

Author 2: Investigation, Methodology / Study design, Formal analysis, Writing – original draft

Author 3: Investigation, Methodology / Study design

Author 4: Investigation, Methodology / Study design

Author 5: Investigation, Methodology / Study design, Formal analysis, Writing – original draft, Writing – review and editing

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the content of this article.

Data Availability

All data generated or analysed during this study are included in this published paper.

Acknowledgement

We would like to thank Trabzon Regional Directorate of Forestry and Biological Control Laboratory against Forest Pests for making use of the infrastructure facilities.

5. References

Akkoç, N., Şanlibaba, P. and Akçelik, M., 2009. Bakteriyosinler: Alternatif Gıda Koruyucuları. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, **25**(1), 59–70.

Aslim, B. and Yucel, N., 2008. In vitro antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp. *Food chemistry*, **107**(2), 602-606. https://doi.org/10.1016/j.foodchem.2007.08.048

Atmaca, S., Özekinci, T., Yakut, S., Akpolat, N. and Gül, K., 2018. *Serratia* Türlerinin İdentifikasyonu, Klinik Dağılımı, Antibiyotik Duyarlılığı. *Ankem Dergisi*, **32**(2), 62-71. https://doi.org/10.5222/ankem.2018.062

Barry, A. L., Garcia, F. and Thrupp, L. D., 1970. An Improved Single-disk Method for Testing the Antibiotic Susceptibility of Rapidly-growing Pathogens. *American Journal of Clinical Pathology*, **53**(2), 149–158. https://doi.org/10.1093/ajcp/53.2.149

Barsby, T., Michael, T. and Kelly, M.T., 2002. *Tupuseleiamides* and *Basiliskamids*, new acyldipeptides produced in culture by a *Bacillus laterosporus* isolate obtained from a tropical marine habitat. *Journal of Neuroscience Research*. **65**(10), 1447-1451. https://doi.org/10.1021/np0201321

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Technical Bulletin of the Registry of Medical Technologists*, **36**(3), 49–52.

Bérdy, J., 2005. Bioactive microbial metabolites. *The Journal of Antibiotics*, **58**(1), 1–26. https://doi.org/10.1038/ja.2005.1

Chatterjee, S., Chatterjee, S., Lad, S. J., Phansalkar, M. S., Rupp, R. H., Ganguli, B. N. and Kogler, H., 1992. Mersacidin, a new antibiotic from *Bacillus* fermentation, isolation, purification and chemical characterization. *The Journal of Antibiotics*, **45**(6), 832-838. https://doi.org/10.7164/antibiotics.45.832

Cowan, M. M., 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**(4), 564–582. https://doi.org/10.1128/CMR.12.4.564

Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, **74**(3):417-33. https://doi.org/10.1128/MMBR.00016-10

Demain, A. L. and Fang, A., 2000. The natural functions of secondary metabolites. *Advances in Biochemical Engineering/Biotechnology*, **69**, 1–39. https://doi.org/10.1007/3-540-44964-7_1

Demirkan, E., Aybey, A. and Ak, A. U., 2021. Optimization of culture conditions for antibacterial substance production from newly isolated *Brevibacillus laterosporus* EA62. *The European Research Journal*, **7**(2), 152–158. https://doi.org/10.18621/eurj.603491

Hong, H.A., Duc, L.H. and Cutting, S.M., 2005. The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews,* **29**, 813–835. https://doi.org/10.1016/j.femsre.2004.12.001

Hong, G., Li, Y., Yang, M., Li, G., Jin, Y., Xiong, H. and Hou, X., 2023. Baseline gut microbial profiles are associated with the efficacy of *Bacillus subtilis* and *Enterococcus faecium* in IBS-D. *Scandinavian Journal of Gastroenterology*, **58**(4), 339-348. https://doi.org/10.1080/00365521.2022.2136013

Hosoya, Y., Okamoto, S., Muramatsu, H. and Ochik, K., 1998. Acquisition of certain streptomycin resistance (Str.). *Antimicrobial Agents and Chemotherapy*, **42**(8), 2041-2047. https://doi.org/10.1128/AAC.42.8.2041

Johnvesly, B., Manjunath, B.R. and Naik, G.R., 2002. Pigeon pea waste as a novel, inexpensive, substrate for production of a thermostable alkaline protease from thermoalkalophilic *Bacillus* sp. JB-99. *Bioresource Technol*ogy, **82**(1): 61-64. PMID 11848379. https://doi.org/10.1016/S0960-8524(01)00147-X

Kaspar, F., Neubauer, P. and Gimpel, M., 2019. Bioactive Secondary Metabolites from *Bacillus subtilis*: A Comprehensive Review. *Journal of Natural Products*, **82**(7), 2038–2053. https://doi.org/10.1021/acs.jnatprod.9b00110

Keswani, C., Singh, H. B., García-Estrada, C., Caradus, J., He, Y. W., Mezaache-Aichour, S., Glare, T. R., Borriss, R. and Sansinenea, E., 2020. Antimicrobial secondary metabolites from agriculturally important bacteria as next-generation pesticides. *Applied Microbiology and Biotechnology*, **104**(3), 1013–1034. https://doi.org/10.1007/s00253-019-10300-8

Miller, D. L., Smith, E. A. and Newton, I. L. G., 2021. A bacterial symbiont protects honey bees from fungal disease. *Host Microbial Interactions*, **12**(3). https://doi.org/10.1128/mBio.00503-21

Nabavi, S. M., Marchese, A., Izadi, M., Curti, V., Daglia, M. and Fazel Nabavi, S., 2014. Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. *Food chemistry*, **173**, 339-347. https://doi.org/10.1016/j.foodchem.2014.10.042

Perez, J., Dela Rubia, T., Moreno, J. and Martinez, J., 1992. Phenolic content and antibacterial activity of olive oil waste waters. *Environmental Toxicology and Chemistry: An International Journal*, **11**(4), 489-495. https://doi.org/10.1002/etc.5620110406

Perez, C., Suarez, C. and Castro, G. R., 1993. Antimicrobial activity determined in strains of *Bacillus circulans* cluster. *Folia Microbiologica*, **38**(1), 25–28. https://doi.org/10.1007/BF02814544

Prashanthi, R., Shreevatsa, G. K., Krupalini, S. and Manoj, L., 2021. Isolation, characterization, and molecular identification of soil bacteria showing antibacterial activity against human pathogenic bacteria. *Journal, Genetic Engineering & Biotechnology,* **19**(1). https://doi.org/10.1186/s43141-021-00219-x

Ren, Z.Z., Zheng, Y. and Sun, M., 2007. Purification and properties of an antimicrobial substance from marine *Brevibcillus laterosporus* LH-1. *Acta Micobiological Slinica*, **47**(6):997-1001.

Reygaert, W. C., 2018. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS*

microbiology, **4**(3), 482. https://doi.org/10.3934/microbiol.2018.3.482

Rosovitz, M.J., Voskuil, M.I. and Chambliss, G.H., 1998. Topley and Wilson's Microbiology and Microbial Infections, Systematic Bacteriology. In: Collier L, Balows A, and Susman editors. *Bacillus*. 9nd edn. Volume 2, New York, USA: *Oxford University Press*, pp. 709-730.

Ruiz, B., Chávez, A., Forero, A., García-Huante, Y., Romero, A., Sánchez, M. and Langley, E., 2010. Production of microbial secondary metabolites: regulation by the carbon source. *Critical Reviews in Microbiol*ogy, **36**(2):146-67. https://doi.org/10.3109/10408410903489576

Sansinenea, E. and Ortiz, A., 2011. Secondary metabolites of soil *Bacillus* spp. *Biotechnology Letters*, **33**(8), 1523–1538. https://doi.org/10.1007/s10529-011-0617-5

Sharma, S., Verma, H. N. and Sharma, N. K., 2014. Cationic bioactive peptide from the seeds of *Benincasa hispida*. *International Journal of Peptides*, **14**, 156-160. https://doi.org/10.1155/2014/156060

Spellberg, B., 2014. The future of antibiotics. *Critical care*, **18**(3): 228. https://doi.org/10.1186/cc13948

Steele, M. I., Motta, E. V. S., Gattu, T., Martinez, D. and Moran, N. A., 2021. The Gut Microbiota Protects Bees from Invasion by a Bacterial Pathogen. *Microbiology Spectrum*, **9**(2). https://doi.org/10.1128/Spectrum.00394-21

Stoica, R.-M., 2019. Antimicrobial compounds of the genus *Bacillus*: A review. *Romanian Biotechnological Letters*, **24**(6), 1111–1119. https://doi.org/10.25083/rbl/24.6/1111.1119

Tenover, C.F., 2006. Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*. **119**, 3-10. https://doi.org/10.1016/j.amjmed.2006.03.011

Turnbull, P.C.B., 1996. "*Bacillus*". In: Baron S (Ed.). Barron's Medical Microbiology. *Univ of Texas Medical Branch*. ISBN 978-0-9631172-1-2.

Usta, M., 2021a. 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). *Uludağ Arıcılık Dergisi,* **21**(2), 157–167. https://doi.org/10.31467/uluaricilik.954479.

Usta, M., 2021b. Isolation and determination of bacterial microbiota of *Varroa destructor* and isolation of *Lysinibacillus* sp. from it. *Egyptian Journal of Biological Pest Control*, **31**(1), 1–8. https://doi.org/10.1186/s41938-021-00482-7

Wang, T., Liang, Y., Wu, M., Chen, Z., Lin, J. and Yang, L., 2015. Natural products from *Bacillus subtilis* with antimicrobial properties. *Chinese Journal of Chemical Engineering*, **23**(4), 744–754. https://doi.org/10.1016/j.cjche.2014.05.020

Zivkovic Zaric, R., Zaric, M., Sekulic, M., Zornic, N., Nesic, J., Rosic, V. and Canovic, P., 2023. Antimicrobial Treatment of *Serratia marcescens* Invasive Infections: Systematic Review. *Antibiotics*, **12**(2), 367. https://doi.org/10.3390/antibiotics12020367