Ordu Üniversitesi Bilim ve Teknoloji Dergisi
Ordu Üniv. Bil. Tek. Derg.

Ordu University Journal of Science and Technology
Ordu Univ. J. Sci. Tech.

E-ISSN: 2146-6459 2023, 13(1), 37-43 Araştırma Makalesi / Research Article
http://doi.org/10.54370/ordubtd.1274632

Bacterial Flora Analysis in Mud Nests of Alien Invasive Wasps (*Sceliphron curvatum* Smith, 1870)

Ömer Ertürk[1], Beyhan Taş[1]

[1]Ordu University, Faculty of Art and Science, Department of Molecular Biology and Genetics, Ordu

Geliş Tarihi / Received Date: 31.03.2023 Kabul Tarihi / Accepted Date: 24.05.2023

Abstract

Sceliphron curvatum (Smith, 1870), a solitary, invasive wasp species, builds a mud nest. This species is a new record for Türkiye. This study was carried out to determine the bacterial flora in mud nests made by *S. curvatum* wasps. Bacterial isolations were made from mud nests made by adult bees collected from the town of Perşembe in Ordu province. As a result of the study, five isolates were obtained and three of these isolates were identified. Isolate 1 was identified as *Staphylococcus vitulinus*, isolate 3 *Aeromonas sobria* and isolate 4 *Pseudomonas aeruginosa*. Identification percentages of the isolates were determined as 84% for *S. vitulinus*, 84% for *A. sobria* and 94% for *P. aeruginosa*, respectively. It has been determined that these bacterial species are important bacterial species that cause effective infections on humans and other living things.

Keywords: alien invasive, mud dauber wasp, mud-nest

Yabancı İstilacı Yaban Arılarının (*Sceliphron curvatum* Smith, 1870) Çamur Yuvalarında Bakteriyel Flora Analizi

Öz

Yalnız, istilacı bir yaban arısı türü olan *Sceliphron curvatum* (Smith, 1870) çamurdan yuva yapar. Bu tür Türkiye için yeni bir kayıttır. Bu çalışma, *S. curvatum* yaban arılarının yaptıkları çamur yuvalarında bakteri florasının belirlenmesi amacıyla yapılmıştır. Ordu ili Perşembe ilçesinden toplanan ergin arıların yaptığı çamur yuvalarından bakteri izolasyonları yapılmıştır. Çalışma sonucunda beş izolat elde edilmiş ve bu izolatlardan üç tanesi tanımlanmıştır. İzolat 1 *Staphylococcus vitulinus*, izolat 3 *Aeromonas sobria* ve izolat 4 *Pseudomonas aeruginosa* olarak belirlenmiştir. İzolatların tanımlama yüzdeleri sırasıyla *S. vitulinus* için %84, *A. sobria* için %84 ve *P. aeruginosa* için %94 olarak tespit edilmiştir. Bu bakteri türlerinin insanlar ve diğer canlılar üzerinde etkili enfeksiyonlara neden olan önemli bakteri türleri olduğu belirlenmiştir.

Anahtar Kelimeler: yabancı istilacı, çamur yuva yapan yaban arısı, çamur yuvası

Sorumlu Yazar / Corresponding Author: Beyhan Taş beyhantass@gmail.com

Introduction

The Sphecidae family includes solitary wasps of the order Hymenoptera, usually medium-large-bodied, in the suborder Apocrita and in the group Aculeata. The wasps of the genus *Sceliphron* Klug, known as potter wasps, are distinguished by their elongated body from 30 with 40 mm (15 to 25 mm) long, their black coloration with yellow or reddish spots, their very long legs, their small abdomen with a long petiole, and their habit of building mud cells that they supply with numerous spiders to feed the larvae (Bohart et al., 1976; Kohl, 1918). The genus *Sceliphron* is represented by few species in the New World and only two species are native to Argentina: *S. asiaticum* (L.) and *S. fistularium* (Dahlbom) (Van der Vecht & Van Breugel, 1968). These two species have a wide distribution in South America, where they are the only one's present. The natural area of distribution of *S. curvatum* spans India, Nepal, and Pakistan to Kazakhstan in Central Asia (Hensen, 1987). In the 1970s it was introduced in Austria and has now spread throughout much of Europe, covering the Mediterranean region from the south of France to Greece and north to Germany and the Czech Republic (Ertürk & Taş, 2022; Schmid-Egger, 2005).

Sphecids are found in all zoogeographic regions except glaciers and are particularly common and diverse in temperate regions. Adult sphecids help pollinate flowering plants by feeding mostly on nectar. Larvae are carnivores. It attacks the adults or nymphs of other insects and spiders, stings it, paralyzes it, and carries it to it nest in the soil it digs. Then they lay eggs on the prey and come out and close the mouth of the hole. The bee larva that emerges from the egg develops by eating the prey and becomes an adult. Because they are predators, they keep the populations of insects and spiders they hunt under control and thus contribute to the maintenance of the biological balance in nature (Bohart et al., 1976)

S. curvatum is an anthropophilic species. They especially prefer to build their mud nests in houses. Their nests are usually attached to various parts of the houses such as ceilings, curtains, walls, clothes, bedspreads, books, closets, and frames (Bitsch & Barbier, 2006; Ertürk & Taş, 2022). In this study, *S. curvatum* mud nests were obtained from village houses in Ordu (Ertürk & Taş, 2022). Each nest is filled with paralyzed spiders that serve as food for the larvae. Like all *Sceliphron* species, *S. curvatum* is not aggressive unless threatened (Bogusch et al., 2004; 2005; Gepp & Bregant, 1986). Considering the current world distribution, it is expected that this species has spread to different regions in Türkiye. The first records of the *S. curvatum* species in Türkiye are quite recent, and it has been reported to be present in the provinces of Tokat, Samsun, Ordu from Central Black Sea Region and Izmit from East Marmara Region in northern Anatolia (Ertürk &Taş, 2022; Gülmez & Can, 2015)

It has been reported that the nest made by the wasp *S. curvatum* contains very different materials from other bee nests (Ertürk et al., 2019). This study investigates the isolation and characterization of bacteria from the sludge-origin nest of *S. curvatum* wasp and reveals the relationship of these microorganisms with honeybees. Also, this work is targeted to expose the available knowledge regarding the determination of the bacterial flora characteristics of the nests. The current study is the first study on the nest bacterial flora of *S. curvatum* in Turkey and will form the basis for future studies.

Materials and Methods

Sampling of Mud Dauber Wasp Nests

The nests of *S. curvatum* samples was gathered from the province of Ordu, Persembe (Figure 1). Exemplification locations: Ten wasp nests, the nests were constructed in a wooden village summer home on the bed cloth. 41°06'47" N, 37°46'03" E coordinates, Ordu, Persembe, Mersin District, 27 m above sea level. Four wasp nests were taken from behind a picture frame and over a curtain in a reinforced concrete house located at 41°03'16" N, 37°40'56" E coordinates at an altitude of 429 m above sea level in Ordu, Persembe, Ortatepe District.

Figure 1. Mud Nests of Sphecid Wasp (Ertürk & Taş, 2022).

Bacterial Isolation from Mud Dauber Wasp Nests

While mud nest samples were taken for bacterial isolation, the insides of the nests were emptied without any damage. The samples were crushed in a sterile environment. To isolate bacteria from collected wasp sludge nest samples, 5 sludge nests were selected and placed in separate sterile tubes. Surface sterilization of the nests placed in the tubes was made with 70% alcohol (Poinar & Thomas, 1978). After this procedure, the samples were washed three times with sterile distilled water under aseptic conditions. In this method, 10 mL of sterile distilled water was added to the tubes and the samples were crushed in distilled water until they became homogeneous. After this process, 100 µL of nutrient agar was added and spread plate cultivation was performed. Petri dishes prepared by this method were incubated at 28°C for 5-6 days. Colonies formed on nutrient agar at the end of incubation were separated according to colony morphology and colony color. Colonies that were different from the separated colonies were determined and planted on nutrient agar by sequential sowing method and thus pure cultures were obtained. At the end of this method, 5 different bacterial isolates were obtained.

Identification of Bacteria

The morphologically diverse samples were stained using various techniques. As a result of staining, the model bacteria in terms of shape and color were chosen to serve as the experimental material. First, basic staining was done to identify the cell morphologies of the isolated organisms. Afterwards, crystal, endospore, and Gram stains were carried out.

On nutrient medium plates, stock culture strains of bacteria obtained from wasp mud nests were subcultured to ensure their purity. Gram stain was initially used to distinguish between Gram-positive and Gram-negative bacteria in all bacterial isolates. After that, it was examined for biological responses. Using VITEK® 2 bacterial identification kits (product no. 21341 and 21342, BioMerieux, Craponne, France), Gram-negative and Gram-positive bacteria isolated were identified. In accordance with the manufacturer's recommendations, ID-GNB cards were used to create identification with the VITEK® 2 system. There are 41 tests on the 64-well plastic ID-GNB cards, including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 tests for decarboxylase, and 3 other tests (for urease, malonate utilization, and tryptophane deaminase). The cards are manually placed into the VITEK® 2 reader-inoculator module after being spontaneously signed and transfused with a 0.5 McFarland suspension of the organism from an 18–20-hour Columbia sheep blood agar license plate (BioMérieux) using vacuum equipment. Every 15 minutes, fluorescence is assessed, and results are evaluated with ID-GNB database after 3 hours. The features of these tests were also evaluated because the VITEK® system requires oxidase (Gram-negative) and catalase (Gram-positive) test results for identification (Barry et al., 1982).

Results and Discussion

In this study, the bacterial flora of *S. curvatum* mud nests collected from the town of Persembe in Ordu province was tried to be determined. As a result of the studies, a total of 5 pure cultures were obtained. While 3 Gram-negative bacteria were obtained in these, 2 of our species could not be identified. Isolates were coded and numbered (Table 1). No spore-forming bacteria were found in any of them. VITEK® 2 fully automatic bacterial identification system installed in Samsun Training and Research Hospital Microbiology Laboratory was used to determine the species of isolates. According to VITEK® 2 results, 3 of the isolates were identified at the species level. As a result of the study from these isolates, isolate No. 1 was determined as *Staphylococcus vitulinus* (Table 1).

Table 1. The VITEK® 2 Bacterial Identification System Identified Bacterial Species According to Their Names and Identification Percentages of Organisms

Isolate No.	Bacteria Name	Source	% Possibility
1	*Staphylococcus vitulinus*	In the mud nest of *S. curvatum*	84%
3	*Aeromonas sobria*	In the mud nest of *S. curvatum*	84%
4	*Pseudomonas aeruginosa*	In the mud nest of *S. curvatum*	94%

Honeybee colonies can be influenced by biotics such as pathogens, parasites, and abiotic factors such as environmental pollution and insecticide implementation for agricultural aim. Honeybees are constantly submitted to pathogenic microorganisms due to distinct sources. These organisms cause major infections in honeybees and affect honeybee biology. Some of these bacteria are also pathogenic for honeybees. *S. vitulinus* is a Gram-positive, coagulase-negative associate of the bacterial genus *Staphylococcus* consisting of clustered cocci. The varieties were essentially isolated from food (beef, chicken, lamb, and other meats) and animals (mammals with the inclusion of horse, vole, and whale) and was denominationed *S. vitulus* (Webster et al., 1994). The name was later altered to *S. vitulinus* for correct Latin grammar (Trüper & de' Clari, 1998). The varieties *Staphylococcus pulvereri*, originally isolated from humans and from a diseased chicken carcass (Zakrzewska-Czerwińska et al., 1995) was afterwards decided to be synonymous with *S. vitulinus* (Švec et al., 2004).

Aeromonas sobria was found as isolate 3 in our research (Table 1). A species of Gram-negative, facultative anaerobic, rod-shapedier bacteria known as *Aeromonas* resembles members of the Enterobacteriaceae family morphologically. The majority of the 14 identified variants have been associated with diseases that affect humans. The most significant pathogens are *A. hydrophila*, *A. caviae*, and *A. veronii* biovar sobria. They can be found in both fresh and brackish water (Martinez-Murcia et al., 1992).

Pseudomonas aeruginosa was identified as isolate number 4 in our study (Table 1). In most habitats, such as soil, plants, and animal tissue, *P. aeruginosa* can be isolated as a Gram-negative aerobic rod-shaped bacterium. With its potent binding components, including flagella, pili, and biofilms, this bacterium can survive on water, various surfaces, and medical equipment. As a result, *P. aeruginosa* is widespread in both natural and man-made settings, such as lakes, hospitals, and domestic sink drains (Diggle & Whiteley, 2020; Remold et al., 2011; Stover et al., 2000). *P. aeruginosa* is a multidrug-resistant bacterium with major medical significance. It is known for being widespread, having innately improved antibiotic resistance mechanisms, and working with serious disorders like ventilator-associated pneumonia and various sepsis syndromes. Bees from many different habitats, particularly those contaminated with some common bacterial strains such as *P. aeruginosa* and *Serratia marcescens*, die and are dismembered from septicemia. Adult bees with the bacterial illness septicemia are likely to get a secondary, deadly infection (Bailey, 1968). *P. apiseptica* is one of the most frequent pathogens of this disease. In one study, honeybees kept in hives were experimentally infected by immersion in a bacterial suspension of *Pseudomonas aeruginosa* ATCC 27014 (*P. apiseptica*), which is known to cause septicemia. The highest mortality rate (66.8%) was reported to occur 10-50 hours after infection. The number of bacteria isolated in the hemolymph of diseased

honeybees was found to be approximately 10^6-10^9 CFU per hemolymph (Papadopoulou-Karabela et al., 1992). Due to the presence of this bacterium in the mud nest of *S. curvatum*, we think that these bees may cause infection if they encounter honeybees.

Conclusion

As a result, the VITEK® 2 system has been evaluated as a reliable, fast, sensitive, and species-level identification system. According to the results obtained, it was determined that some bacteria were present in the nests of mud nest wasps, but the number of bacterial species was low. Due to a lack of quantitative information on the topic, the interpretation of these findings is limited by the discovery of three separate bacterial isolates in the wasp mud nests. When wasps built their mud nests, they may have brought in bacteria-contaminated water or water-contaminated soil. It has been determined that the bacterial species detected in mud nests are important bacterial species that cause effective infections in humans and other living things. The VITEK® 2 system has been proven to be a trustworthy, quick, sensitive, and species-level identification method as a consequence.

Author Contribution

Beyhan Taş, collected nest samples and prepared them for analysis in the laboratory. *Ömer Ertürk*, determined the bacterial isolates. The authors co-authored, read, and approved the article.

Ethic

There are no ethical issues with the publication of this article.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID

Ömer Ertürk https://orcid.org/0000-0001-5837-6893

Beyhan Taş https://orcid.org/0000-0001-6421-2561

References

Bailey, L. (1968). Honey bee pathology. *Annual Review of Entomology*, *13*(1), 191–212. https://doi.org/10.1146/annurev.en.13.010168.001203

Barry, A., Gavan, T.L., Badal, R. E., & Telenson, M. J. (1982). Sensitivity, specificity, and reproducibility of the Auto Microbic system (with the Enterobacteriaceae-plus Biochemical Card) for identifying clinical isolates of Gram-negative bacilli. *Journal of Clinical Microbiology*, *15*(4), 582–588. https://doi.org/10.1128/jcm.15.4.582-588.1982

Bitsch, J., & Barbier, Y. (2006). Répartition de l'espèce invasive Sceliphron curvatum (F. Smith) en Europe et plus particulièrement en France (Hymenoptera, Sphecidae). *Bulletin de la Société Entomologique de France*, *111*(2), 227–237. https://doi.org/10.3406/bsef.2010.2838

Bogusch, P., Liška, P., Lukáš, J., & Dudich, A. (2005). Spreading and summary of the knowledge of the invasive sphecid wasp *Sceliphron curvatum* (Smith 1870) in the Czech Republic and Slovakia (Hymenoptera:nApocrita, Sphecidae). *Linzer Biologische Beiträge*, *37*(1), 215–221. https://doi.org/10.1128/jcm.15.4.582-588.1982

Bogusch, P., Straka, J., & Srba, M. (2004). The sphecid wasp from the Botanical Garden in Prague. *Živa*, *51*(3), 121–122. https://www.zobodat.at/pdf/LBB_0037_1_0215-0221.pdf

Bohart, R. M., Bohart, R. M., & Menke, A. S. (1976). *Sphecid wasps of the world: a generic revision*. University of California Press.

Diggle, S.,& Whiteley, M (2020). Microbe profile: Pseudomonas aeruginosa: Opportunistic pathogen and lab rat. *Microbiology*, *166*(1), 30–33. https://doi.org/10.1099%2Fmic.0.000860

Ertürk, Ö., & Taş, B. (2022). Distribution and a new locality record of the invasive Asian wasp, *Sceliphron curvatum* (F. Smith, 1870) (Hymenoptera: Sphecidae) in Europe and Turkey. *Pakistan Journal of Zoology*, *54*(1), 315–321. http://doi.org/10.17582/journal.pjz/20180723220732

Ertürk, Ö., Taş, B., Şahin, H., & Çaldaş, A. (2019). Determination of some structural features of the mud nest materials of *Sceliphron curvatum* (Smith, 1870), (Hymenoptera: Sphecidae) in Turkey. *Journal of the Entomological Research Society*, *21*(2), 157–166. https://www.entomol.org/journal/index.php/JERS/article/view/1506

Gepp, J., & Bregant, E. (1986). Zur biologie der synanthropen, in Europa eingeschleppten orientalischen mauerwespe *Sceliphron* (*Prosceliphron*) *curvatum* (Smith, 1870) (Hymenoptera: Sphecidae). *Mitteilungen des Naturwissenschaftlichen Vereines für Steiermark*, *116*, 221–240. https://www.zobodat.at/pdf/MittNatVerSt_116_0221-0240.pdf

Gülmez, Y., & Can, İ. (2015). First record of *Sceliphron* (*Hensenia*) *curvatum* (Hymenoptera: Sphecidae) from Turkey with notes on its morphology and biology. *North-Western Journal of Zoology*, *11*(1), 174–177. https://biozoojournals.ro/nwjz/content/v11n1/nwjz_142201_Gulmez.pdf

Hensen, R. V. (1987). Revision of the subgenus *Prosceliphron* van der Vecht (Hymenoptera, Sphecidae). *Tijdschrift voor Entomologie*, *129*, 217–261. https://www.biodiversitylibrary.org/part/69311

Kohl, F. F. (1918). Die Hautflüglergruppe "Sphecinae". IV. Die natürliche Gattung *Sceliphron* Klug (= *Pelopaeus* Latr.). *Annalen des k.k. Naturhistorischen Hofmuseums*, *32*, 1−171. https://doi.org/10.5962/bhl.part.2204

Martinez-Murcia, A. J., Benlloch, S., & Collins, M. D. (1992). Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. *International Journal of Systematic and Evolutionary Microbiology*, *42*(3), 412–421. https://doi.org/10.1099/00207713-42-3-412

Papadopoulou-Karabela, K., Iliadis, N., Liakos, V., & Bourdzy-Hatzopoulou, E. (1992). Experimental infection of honeybees by *Pseudomonas aeruginosa*. *Apidologie*, *23*(5), 393–397. https://doi.org/10.1051/apido:19920501

Poinar, G., & Thomas G. (1978). *Diagnostic manual for the identification of insect pathogens*. Plenum Press.

Remold, S. K., Brown, C. K., Farris, J. E., Hundley, T. C., Perpich, J. A., & Purdy, M. E. (2011). Differential habitat use and niche partitioning by *Pseudomonas* species in human homes. *Microbial Ecology*, *62*, 505–517. https://doi.org/10.1007/s00248-011-9844-5

Schmid-Egger, C. (2005). *Sceliphron curvatum* (F. Smith 1870) in Europa mit einem Bestimmungsschlüssel für die europäischen und mediterranen Sceliphron-Arten (Hymenoptera, Sphecidae). *BembiX*, *19*, 7–28. http://bembix.de/publicationen_pdf/Schmid-Egger%202005%20Sceliphron%20curvatum%20in%20Europa.pdf

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., ... & Olson, M. V. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959–964. https://doi.org/10.1038/35023079

Švec, P., Vancanneyt, M., Sedláček, I., Engelbeen, K., Štětina, V., Swings, J., & Petráš, P. (2004). Reclassification of *Staphylococcus pulvereri* Zakrzewska-Czerwińska et al. 1995 as a later synonym of *Staphylococcus vitulinus* Webster et al. 1994. *International Journal of Systematic and Evolutionary Microbiology*, *54*(6), 2213–2215. https://doi.org/10.1099/ijs.0.63080-0

Trüper, H. G., & de' Clari, L. (1998). Taxonomic note: erratum and correction of further specific epithets formed as substantives (nouns) 'in apposition'. *International Journal of Systematic Bacteriology*, *48*, 615–616. https://doi.org/10.1099/00207713-48-2-615

Van der Vecht, J., & Van Breugel, F. M. A. (1968). Revision of the nominate subgenus *Sceliphron* Latreille (Hymenoptera, Sphecidae) (Studies on the Sceliphronini, Part I). *Tijdschrift voor Entomologie*, *111*(1), 185–255. https://www.biodiversitylibrary.org/part/65807

Webster, J. A., Bannerman, T. L., Hubner, R. J., Ballard, D. N., Cole, E. M., Bruce, J. L., ... & Kloos, W. E. (1994). Identification of the *Staphylococcus sciuri* species group with Eco RI fragments containing rRNA sequences and description of *Staphylococcus vitulus* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *44*(3), 454–460. https://doi.org/10.1099/00207713-44-3-454

Zakrzewska-Czerwińska, J., Gaszewska-Mastalarz, A., Lis, B., Gamian, A., & Mordarski, M. (1995). *Staphylococcus pulvereri* sp. nov., isolated from human and animal specimens. *International Journal of Systematic and Evolutionary Microbiology*, *45*(1), 169–172. https://doi.org/10.1099/00207713-45-1-169