ISOLATION AND IDENTIFICATION OF POTENTIAL PATHOGENIC BACTERIA ON AQUATIC INSECTS – AN EXAMPLE STUDY OF ENVIRONMENTAL MICROBIOLOGY IN TÜRKİYE (ERZURUM PROVINCE)

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(Received 11th Jul 2024; accepted 3rd Dec 2024)

Abstract. Insects, leading the richest group in species diversity in the world of living organisms, display the largest diversity in feeding, habitat and adaptation to different living environments. In addition to studies related to the use of insects in the search for alternative foods in the world in general, the focus has been on toxicological and pathogenic research about insects. The habitats of aquatic beetles are the water sources used for both agricultural and drinking water, so aquatic beetles gain further importance. This study collected aquatic insects from different localities in Erzurum and its surroundings. After insect species were identified and their digestive tracts were dissected under aseptic conditions, microbiological analyses were performed. Isolates were identified by analyzing molecular and conventional data together. In addition to conventional analyses, 16S rRNA gene sequences were amplified with PCR reaction and DNA sequencing analyses were performed. GenBank accession numbers were obtained after BLAST analyses of the obtained DNA sequences. This study aimed to identify definite and potential human pathogens in bacteria isolated from the intestinal microbiota cultures, characterized by conventional and molecular methods of the aquatic insects.
Keywords: *bacterial isolation, DNA sequencing, freshwater, insect microbiome, Türkiye*

Introduction

Insects belong to the Arthropoda phylum, Insecta (Hexapoda) class and comprise the richest group of living organisms on earth in terms of biological diversity and animal biomass with an estimated 5.5 million different species. The Coleoptera order, also called as or hard wing case insects, is the most populous group including 40% of insect species (Engel and Maron, 2013; Bektaş, 2015; Bertola and Mutinelli, 2021).

Insects are one of the most promising alternative sources that can meet global protein requirements. Because insects were proven in studies to be better protein and fat sources compared to other nutrient sources (De Silva Lucas et al., 2020).

Currently, on a global scale, nearly 2000 insect species are consumed and including mostly insects from the Coleoptera order (31%) included most (31%) within this group. Additionally, it is the insect group with the highest fat content (33.4%) (Amiri, 2017; Bertola and Mutinelli, 2021).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

Edible insects appear to comprise a market in the world for increasing nutrient needs and protein supplementation. However, as they involve a range of chemical and biological hazards like micro toxins, pathogenic microorganisms, pesticide residues, and heavy metals, eating all insects is not safe in terms of human health. The microbiological safety for humans of microbiota, comprising 1-10% of insect biomass, has not gained full clarity and there are few studies on the topic (José et al., 2009; Douglas, 2015; Da Silva Lucas et al., 2020). Especially, migrating insects, may act as a mechanism for the derivation and spread of diseases carried in water (Wooldridge and Wooldridge, 1972; Evariste et al., 2019).

Aim of this study is to identify pathogenic bacteria from aquatic insects in wetlands area. Insects from this group were preferred because Coleoptera species are more abundant in aquatic ecosystems than other populations. In this study, the preference for the Helophoridae and Hydrophilidae families (Coleoptera) is due to the limited research available on their gut microbiota, which is essential for the understanding of their potential in bioconversion processes. The composition and function of the gut microbiota play a crucial role in the absorption of nutrients, the immune response and the general health of the insects, which directly influences their suitability for sustainable feed production. Expanding research in this area could uncover specific microbial interactions that enhance the digestive efficiency and bioavailability of nutrients, thus providing valuable insights for the optimization of insect-based feed formulations.

Materials and methods

Collection and species identification of insect

Aquatic insects were collected in samples from lakes, springs, streams, ponds, and water supply in regions close to the research area taken regularly every month from May to September 2022. The collected insect species and locality information are given in *Table 1*. After catching live insects, the locality information was noted and they were brought to Atatürk University, Faculty of Agriculture Entomology Laboratory for species identification under aseptic conditions. The general map of the research region and detailed information about the areas of the research are presented in *Figure 1*.

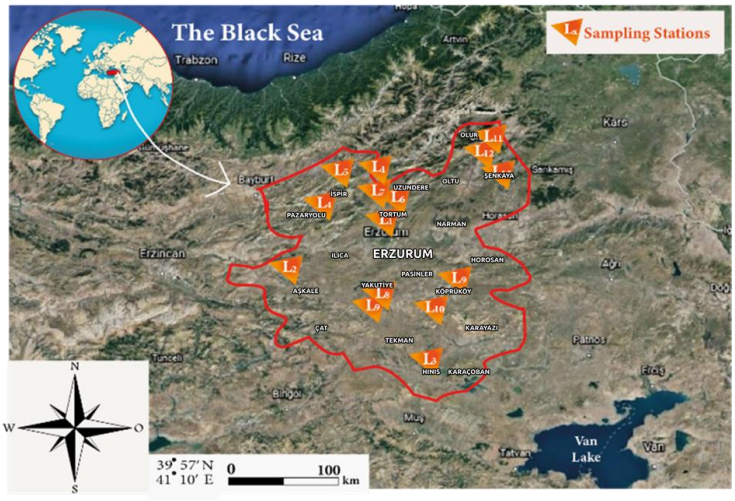

Figure 1. *Location of the study area on national map in Türkiye*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

Table 1. *Collected insect species and localities*

Insect species	Coordinates *(N: North; E: East)	Altitude (mt.)	Collection time	Stations of Collection
L_1 *Anacaena rufibes* (Guillebeau, 1896)	40°03”03’ N* 41°30”44’ E*	1850	23.06.2022	Karasu Stream/Yolgeçti village/Yakutiye/Erzurum
L_3 *Berosus spinosus* (Steven, 1808)	40°44”00’ N 41°18”06’ E	1555	19.06.2022	Karasu Stream/Küçükgeçit/Aşkale/Erzurum
L_{15} *Coelostoma orbiculare* (Fabricius, 1775)	41°11”04’ N 42°09”36’ E	1852	12.09.2022	Oltu Stream/Vişneli/Oltu/Erzurum
L_{10} *Enochrus fuscipennis* (Thomson, 1884)	40°44”20’ N 41°43”20’ E	1444	22.07.2022	Başköy Stream/Ovaçevirme/Hınıs/Erzurum
L_{12} *Enochrus quadripunctatus* (Herbst, 1797)	39°39”33’ N 41°02”18’ E	2130	03.09.2022	Çat Dam/Budaklar River/Çat/Erzurum
L_6 *Helophorus aquaticus* (Linnaeus, 1758)	40°21”05’ N 41°09”01’ E	2087	13.07.2022	Kuzgun dam connection water/Pazaryolu/Erzurum
L_{16} *Helophorus brevipalpis* (Bedel, 1881)	40°43”43’ N 42°39”55’ E	1860	12.09.2022	Narman Stream/Toprakkale/Oltu/Erzurum
L_8 *Helochares obscurus* (Müller, 1776)	40°47”39’ N 41°38”11’ E	1152	13.07.2022	Çoruh River floods/Aşağı Özbağ/İspir/Erzurum
L_9 *Hydrochara caraboides* (Linnaeus,1758)	40°38”39’ N 41°31”44’ E	1450	19.08.2022	Yellitepe River/Arılı/Tortum/Erzurum
L_4 *Hydrobius fuscipes* (Linnaeus, 1758)	39°30”11’ N 42°19”40’ E	1721	11.07.2022	Başköy Çayı/Ovaçevirme/Hınıs/Erzurum
L_{13} *Hydrophilus piceus* (Linnaeus, 1758)	40°17”50’ N 41°50”28’ E	2199	02.08.2022	Kızılgeçit River, Hürriyet Hometown/Tekman/Erzurum
L_{11} *Laccobius simlutatrix* (d’Orchymonth, 1932)	39°41”42’ N 41°08”10’ E	2160	11.09.2022	Çat Dam/near Karaşeyh village/Çat/Erzurum
L_{17} *Laccobius striatulus* (Fabricius, 1801)	41°15”39’ N 42°34”41’ E	1851	12.09.2022	Melikoğlu River/Akşar/Şenkaya/Erzurum
L_7 *Laccobius syriacus* (Guillebeau, 1896)	40°21”27’ N 41°08”02’ E	2101	13.07.2022	Kuzgun dam connection water/Pazaryolu/Erzurum
L_2 *Microlaccobius gracilis gracilis (*Moutschoulsky, 1855)	40°43”03’ N 41°18”56’ E	1554	26.05.2022	Karasu Stream/Küçükgeçit/Aşkale/Erzurum
L_{14} *Paracymus aeneus* (Germar, 1824)	40°18”50’ N 41°47”34’ E	2199	03.09.2022	Kızılgeçit Riveri, Hürriyet Hometown/Tekman/Erzurum

Dissection of insect digestive structure

For each trial, one insect was used from among adult and healthy insects collected from aquatic environments. Insect samples were first immobilized by being placed in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

closed boxes containing cotton soaked in ethyl acetate and then joints like the elytra and wings were removed and the outer surface of the insects was treated with 70% ethanol for 5 min to remove possible contaminant microorganisms. Then, alcohol was washed off by shaking in sterile distilled water and the digestive tract of the insects was dissected under a binocular microscope in an environment with aseptic conditions in the laboratory (*Fig. 2*). The digestive structures had cultures taken for microbiological identification on the same day.

Inoculation of samples and microbiological characterization with conventional methods

The insect digestive tract was pulverized with a sterile glass rod in 0.85% sterile NaCl and homogenized and serial dilutions were prepared (10^{-6}). From the prepared dilutions smear plate cultivated was performed according to aseptic rules on tryptic soy agar (TSA) medium (100 µl of the prepared dilutions were inoculated according to aseptic rules). Samples with inoculation performed were incubated at 30°C for three days under aerobic conditions. Because the isolation of pathogenic bacteria was aimed, only TSA medium was used in cultivation. Isolates with different features in terms of color, texture and colony morphology were passaged on new media to obtain pure cultures and stored at -70°C in Luria-Bertani (LB) containing 16% glycerol.

Characterization with API 20 E multi-test system

With to identify metabolic enzyme profiles of bacteria, analytical profile index (API) 20 E test strips suitable for enteric bacteria were used. The selected 25 Gram (-) isolates were analyzed according to the kit protocol (Anonymous, 2022). Twenty different biochemical properties were tested for the analyzed bacteria (*Biomeriux API 20E*).

Molecular analyses and DNA isolation

For the identification of bacterial isolates, the region synthesizing 16S rRNA was selected and amplified in an *in vitro* environment. The master mix (*EcoTaq 2x PCR Master Mix*) and PCR program are given in *Table 2*. Amplicons were sequenced by BM labosis (Ankara/Türkiye). Analyses of the rRNA sequences amplified with PCR were obtained and after general assessment, they were compared with virtual library data for BLAST analyses (Anonymous, 2021) The sequences consisting of about 1255–1434 nucleotides (nt) of the 16S rRNA genes were determined (*EcoPURE Genomic DNA Kit*).

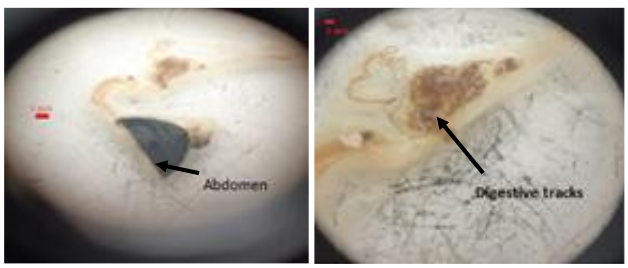

Figure 2. *Appearance of intestinal content of insects under the microscope (1 mm)*

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Table 2. *Applied PCR program and base sequence (Bektas et al., 2022)*

Target region	Base name and sequences of primers	Master Mix 1 sample (50 µL)		PCR program	
16S rRNA	27F (forward 5'- AGA GTT TGA TCC TGG CTC AG -3')	EcoTaq master mix	25 µL	Denaturation	98°C 30 s
		Primer 27F (10 µM)	2 µL	Denaturation (35 cycles)	94°C 10 s
		Primer 1492 R (10 µM)	2 µL	Binding	52°C 15 s
	1492R (reverse 5'- GGT TAC CTT GTT ACG ACT T-3')	Sterile purified water	20 µL	Extension	72°C 15 s
		Template DNA	1 µL	Final extension	72°C 1 min

Results

In this study, 17 different regions were determined in and around Erzurum, taking into account seasonal conditions and precipitation (*Table 1*) and insect samples were collected. As a result of isolation from all samples, a total of 140 bacterial isolates were purified. Then bacteria with different morphologies were distinguished according to their various phenotypic characteristics, named by genotypic methods and sequence numbers were obtained from Genbank. Among these 52 bacteria have been identified as potential pathogens in humans. These bacteria are from 18 different families, 30 of them are different species and belong to different genera. In the intestinal microbiota of the families that are the subject of the study; the density of environmental isolates such as *Aeromonas*, *Acinetobacter*, *Pseudomonas* and *Bacillus* have attracted attention. Considering the micro-environmental conditions of the insect gut, which can support bacterial growth, the frequency of environmental isolates of water and soil origin was considered a predictable result. In addition, separating an isolated environmental strain and a pathogen of the same species requires detailed analyses by revealing their virulence characteristics. The virulence characteristics of the bacteria isolated in the study were not analyzed and only potential pathogens were identified. In the obtained data, API 20 E analyses results are in *Table 3* and the distribution of isolated bacteria according to density and family are given in *Figure 3*.

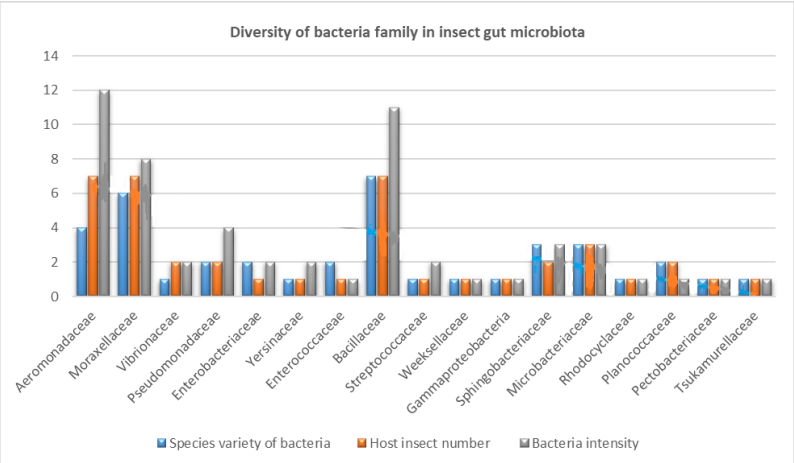

Figure 3. *Species diversity and distribution according to bacterial families isolated from insect species*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Table 3. *API 20 E analyses results*

Result & isolate codes	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX
Acinetobacter calcoaceticus (FO-108)	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	?
Acinetobacter calcoaceticus (FO/132)	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-
Acinetobacter baumanni\calcoaceticus (FO-110)	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-
Acinetobacter baumanni\calcoaceticus (FO-103)	-	-	-	-	-	-	-	-	-	+	-	+	?	-	-	-	-	+	-	+	-
Acinetobacter calcoaceticus (FO-26)	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-
Aeromonas veroni (FO-71)	+	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	+
Aeromonas hydrophilia\caviae (FO-74)	+	+	-	-	?	-	-	?	+	+	+	+	+	-	-	-	+	-	-	-	+
Aeromonas hydrophilia\cavia\sobria (FO-105)	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	+	-	-	+	+
Aeromonas hydrophili \cavia\sobria (F0-130)	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+
Aeromonas hydropflia (FO-96)	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	+	+
Escherichia coli (F0-31)	+	+	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	?
Escherichia coli (FO-77)	+	-	+	+	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	+	+
Pantoea spp. (FO-97)	+	-	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	-
Pseudomonas fluorescens\putida (F0-27)	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	+
Pseudomonas putida (FO-45)	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Vibrio cholera (FO-5)	+	-	+	+	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+
Vibrio cholerae (FO-51)	+	-	+	+	-	-	-	?	+	-	+	+	+	-	-	-	+	-	-	-	+

ONPG: Beta-Galactosidase Test; ADH: Antidiuretic Hormone Test; LDC: Lysine Decarboxylase Test; ODC: Ornithine Decarboxylase Test; CIT: Combined Infection Training Test; H_2S: Hydrogen Sulfide Test; URE: Urease Test; TDA: Tryptophan-deaminase Activity; IND: Indole Test; VP: Voges-Proskauer Test; GEL: Gel Test; GLU: Glucose Broth Tubes Test; MAN: Mannoz Test; INO: Inositol Test; SOR: Sorbitol Test; RHA; Reiter Haemagglutination Test; SAC: Sample Adequacy Controls; MEL: D-MELibiose Test; AMY: Amylase Test; ARA: Arabinose Test; OX: Oxidase Test

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

Discussion

Entomophagy is defined as the use of insects for food involves a range of chemical and biological hazards. Some microbial agents in the insect microbiota may be potential disease vectors in humans through the consumption of insects. For this reason, it is important to research the microbiota of insects, especially those offered for human consumption (Van der Spiegel et al., 2013; Makkar et al., 2014; Garofalo et al., 2017).

Bacteria are common microorganisms in insect microbiota. Insect-bacteria interaction may be symbiotic and pathogenic. Most symbiont bacteria in the insect intestine comprise environmental samples. Literature information obtained from studies about insect intestinal assemblages reported that the *Pseudomonas* and *Bacillus* taxa are dominant and dependent to a large extent on diet (Broderick et al., 2004; Robinson et al., 2010). In this study, environmental isolates like *Acinetobacter, Aeromonas, Pseudomonas,* and *Bacillus* were densely observed. Considering the micro environmental conditions of the insect intestine, it is a predicted outcome that environmental isolates with water and soil origin are frequently observed.

Bacteria in the Enterobacteriaceae family are known as pathogen and opportunistic pathogens and are frequent parameters for the assessment of enteric contamination in food (Barco et al., 2014; Stoops et al., 2016). The presence of these bacteria in insects indicates that the intestines cannot be cleaned or the pollution of the aquatic habitat. *E. coli*, which is in the enteric bacteria group and has an important place in the human intestinal flora, was isolated from two different insect species *Laccobius syriacus* (Guillebeau, 1896), *Hydrophilus piceus* (Linnaeus, 1758) in this research. This bacteria, in a compatible relationship with the host organism, it may cause disease in situations with emplacement in organs outside the intestines or in the intestines of another host. *Enterococcus* species bacteria include species adapting at high rates to different habitats like humans, animals, insects, plants, soil, water and fermented foods. Bacteria from this family are important as they have shown antibiotic resistance in nosocomial infections since the 1970s to the present day. The inclusion of insects as a reservoir for antibiotic-resistant *Enterococcus* is worrying. In our study, the *E. rivorum* species from this family was identified (Lebreton et al., 2014).

Two *Vibrio cholerae* were identified with API 20E and molecular methods isolated from the *Laccobius syriacus* (Guillebeau, 1896) and *Helophorus brevipalpis* (Bedel, 1881) insect species. *Vibrio* is found in abundant amounts in seawater and river mouth environments. They may cause sporadic gastroenteritis and severe disease by proliferating on phytoplankton and zooplankton surfaces (Traore et al., 2014; Cabral, 2010). Consumption of insects from water containing sewage waste is especially a risk factor in terms of *V. cholera.* Virulence factors of *V. cholera* should be compared with the pathogen species causing intestinal infection with its toxins in humans and the pathogenicity of its environmental species.

Microorganisms belonging to the Pseudomonadaceae family commonly live in soil and water. These microorganisms have pathogenic or opportunistic pathogen roles for insects and humans. Additionally, it has clinical importance due to frequently developing resistance against antibiotics. It was identified in edible fresh locust and mealworm larvae samples marketed in Belgium (Stellato et al., 2015; Stoops et al., 2016). In this study, four *Pseudomonas* isolates were identified. While three of them are defined as *Pseudomonas putida* one is not identified at the species level. *P. putida* has

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both pathogenic and biotechnological importance. It is an opportunistic pathogenic microorganism in patients with suppressed immunity and was seen to colonize these individuals (Molina et al., 2016; Fernandez et al., 2015).

Aeromonas are mostly isolated in this study, which is an expected situation since it is a members of aquatic environments. Within this group, *A. hydrophila, A. caviae* and *A. veronii* biotype sobria are defined as human pathogens. They may cause gastrointestinal and extraintestinal infectious diseases in humans. Especially they can cause serious wound infections in patients receiving medical leech therapy. In this study, 8 human pathogen *Aeromonas* species were isolated (4 individual *A. veroni* and 4 individual *A. hydrophilia*). *A. veronii* biotype sobria was frequently reported in clinical isolates and this bacterium is the etiological agent of traveler's diarrhea (Vila et al., 2003; Al-Fatlawy and Al-Hadrawy, 2014). Another bacterium isolated from *A. hydrophila* is a potential agent in gastroenteritis, septicemia, meningitis, and wound infections, and was accepted as an opportunistic pathogen in recent years. *A. hydrophila* was reported to be the main cause of an epidemic occurring in people consuming oysters in Louisiana. Additionally, it was identified as a pathogenic bacterial species in fish and crab farms in China (De Silva et al., 2021; Cabral, 2010). A significant increase in resistance to β-lactam populations is observed in these bacteria, not only in isolated clinical isolates but also in environmental isolates. For this reason, it is a bacteria group that should be taken seriously (Saavedra et al., 2007).

Acinetobacter was the bacteria identified lots this study due to being an isolate with environmental sources. Significant species are clinically *A. baumnnii, A. nosocomial, A. pitii* and *A. calcoaceticus*. In this study, three *A. pitti* and one *A. calcoaceticus* were identified among pathogenic species. Attracting attention in recent years, especially as the most frequent cause of antibiotic resistance and nosocomial infections. *Acinetobacter* species are the most frequent causes of ventilator-associated pneumonia, blood circulation, urinary tract and intra-abdominal infections in intensive care units (Esen and Gözalan, 2020).

Another isolated bacterium was *Exiguobacterium* sp. species from this genus are rarely associated with human infections. Additionally, bacteremia and skin infection cases were documented. Additionally, as there is a trend toward mistaken identification of the microorganism with routine commercial methods, deficient detection or reporting rates are high (Chen et al., 2017). *Tsukamurella inchonensis,* which was detected in the insect species *Berosus spinosus* (Steven, 1808) is an environmental saprophyte first isolated from soil, arthropods, water and mud. Especially in hospitalized immunocompromised patients, it causes infection by passing through water and medical devices. This bacterium, which is difficult to identify by phenotypic methods, it was named by genotypic methods in our study (Tang et al., 2022).

Currently, *Lactobacillus* spp. is the most commonly used probiotic bacteria. It is found in large-scale habitats such as gastrointestinal systems and environmental environments. In the study, *Lactococcus lactis* bacteria isolated from *Laccobius syriacus* (Guillebeau, 1896) insect species is a microorganism accepted as having unknown virulence factors and being very safe. However, Rostagno et al. (2013) reported that these bacteria may be a source of a variety of infections (liver-brain abscesses, cholangitis, peritonitis, osteomyelitis and deep neck infection) linked to consumption of raw milk and milk products by humans in the last twenty years.

Ignatzschineria larvae identified in *Hydrobius fuscipes* (Linnaeus, 1758) insect species were first identified by isolation from larvae of *Wohlfahrtia magnifica* (spotted

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DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

flesh fly) by Toth et al. (2007). Because this bacterium is a newly identified human and animal pathogen, an appropriate phylogenetic classification has not yet been made (Montecillo, 2022). Although few cases have been described in the literature until now, it has been recommended that clinicians consider *Ignatzschineria* bacteremia as a potential complication of myiasis, especially in patients presenting with sepsis (Maniam and Argentine, 2022). It was of interest to isolate recently identified pathogens from aquatic insects in this study.

Apibacter raozihei isolated from *Helophorus aquaticus* (Linnaeus, 1758) insect species is a facultative anaerobic bacterium. In this study, it is reproduced on the 4th day in the aerobic conditions and for a shorter duration (2 days) in the anaerobic conditions. These bacteria were first isolated from honeybees in and were defined as microaerobic members of bee intestine (Kwong et al., 2018). There is no literature information about pathogenicity. Morever, *Sphingobacterium* sp. isolated from *Helophorus aquaticus* and *Laccobius sulcatulus* insect species. Biotechnological importance in addition to being a bacterium that may be a rare infectious vector (Cai et al., 2019).

Accurate laboratory techniques have critical importance in the identification of infectious syndromes linked to microorganisms with uncertain pathogenicity that are rarely isolated. 16S rRNA gene sequencing allows the opportunity to accurately identify potential pathogenic bacteria. *Lysinibacllus,* generally accepted as an environmental pollutant when isolated in clinical microbiology laboratories, was documented to have pathogenic potential in humans. *Lysinibacillus sphaericus,* identified in the study, was reported to cause 12 (2%) out of 469 bacteremia attacks in children with cancer during a 10-year duration in a pediatric cancer hospital in Italy (Wenzler et al., 2015).

Another bacterium obtained in the study was *Kurthia gibsonii* which is accepted as a zoonosis transmitted by sexual routes. It is commonly found in soil polluted by sewage, animal-sourced products and animal feces. This bacterium with the ability to survive in difficult living conditions was isolated from human feces in acute diarrhea and is accepted as pathogenic in most cases due to causing gastroenteric diseases. However, pathogenicity of this bacteria has not yet been confirmed with clinical evidence and virulence factors are unknown (Pawar et al., 2012; Kövesdi et al., 2016; Cucini et al., 2020). In the study, three bacteria from *Microbacterium* species were identified. *Microbacterium* spp. is found in soil, waste-water, hospital pools and humidifiers. It is mainly known as environmental isolate. Frequency of reports as a potential pathogenic agent in humans, especially patients with weak immune systems, has increased (Woo et al., 2010).

Another group found densely in insect intestines and gaining importance due to spores and being pathogenic is *Bacillus*. In this study, 9 *Bacillus* species were isolated and with *B. cereus* isolated in two different insect species. The important human pathogen of *B. cereus* is a common cause of food-sourced gastroenteritis. Firstly, accepted as a harmless pollutant, this bacterium is known to be the etiologic agent for a variety of intestinal and extra intestinal diseases since the 1960s (Tuipulot et al., 2020).

Despite our study being an *in vitro* study characterizing cultured bacteria, the bacterial density is remarkable. However, it is possible to identify much more bacteria in the insect gut microbiota with more advanced techniques such as metagenomics analyses that define the entire gut microbiome (Bektaş et al., 2021). For example, some fastidious microorganisms defined with molecular methods in this study could not be isolated with traditional culture-based methods due to having specific growth

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DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

requirements. For this reason, the use of methods independent of culture in research about pathogenic microorganisms in insect microbiota is very important. Also, using culture-independent methods such as metagenomics or microbiome studies to make any correlation between insects and isolated bacteria can be recommended (*Fig. 3*; *Table 3*). The findings obtained in this study show that insect microbiota in the families studied contain many bacterial assemblages including microorganisms present in soil and water.

While some of bacteria in these microorganism assemblages, including permanent and temporary microbiota elements, protect insect against a variety of pathogens, some may play the role of opportunistic pathogen in humans, while others have phytopathogenic properties. Outcome of this study, two bacteria (*Pectobacterium* sp., *Bacillus mojavensis)* without clear pathogenicity but accepted as phytopathogens were isolated. Interest in insects as an alternative candidate for sustainable nutrient sources in the future will increase further on. Since some of the bacteria isolated in our study have clinical importance in humans it is seen that raw consumption of insects belonging to these families is a risk factor for human health (*Fig. 3; Table 3*). Additionally, broad ecologic and taxonomic diversity of insects makes it difficult to generalize about intestinal microbiota and there is a need for more research on this topic. Food safety of insect consumption has not been fully determined and it is thought the findings obtained in the study will provide current data about insect microbiota for literature and contribute to future research.

Author contributions. Orhan, F. conceived of the study, designed the experimental protocols. Bektaş, M. was responsible for data collection, statistical analyses, literature search, the experimental protocols and drafting the manuscript. Baris, O. was involved in literature search, data interpretation and critical revision for important intellectual content. The final version of the manuscript was read and approved by all the authors.

Acknowledgements. This study was funded by the Scientific and Technical Research Council of Turkey (TUBITAK) (Tubitak Project No. 119Z236). The authors wish to thank the Scientific and Technological Research Council of Turkey (TUBITAK) for its financial support. This article was published as a preprint under the title "*Isolation and identification of pathogenic bacteria in the digestive microbiota of some aquatic insect*" at https://www.researchgate.net/publication/362445715_Isolation_and_identification_of_pathogenic_bacteria_in_the_digestive_microbiota_of_some_aquatic_insect#full-text.

Conflict of interests and ethics statement. The authors declare no conflict of interest. This study complies with research and publishing ethics and rules.

REFERENCES

[1] Al-Fatlawy, H. N. K., Al-Hadrawy, H. A. (2014): Isolation and characterization of *A. hydrophila* from the Al-Jadryia River in Baghdad (Iraq). – American Journal of Educational Research 2: 658-662. DOI: epdf/10.2903/sp.efsa.2014.EN-636.

[2] Amiri, A. (2017): An investigation on some edible insects as source of human food and animal feed. – PhD thesis, Near East University, Cyprus Town, Cyprus.

[3] Anonymous (2021): EcoPURE Genomic DNA Kit. – www. https://www.ecotechbiotech.com (accessed15 01202).

[4] Anonymous (2022): Antibiyotik Direnç Yönetimi. – www.biomerieux.com.tr (accessed 15 01 2022).

[5] Barco, L., Belluco, S., Roccato, A., Ricci, A. (2014): *Escherichia coli* and Enterobacteriaceae counts on poultry carcasses along slaughter processing line, factors

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

influencing the counts and relationship between visual faecal contamination of carcasses and counts: a review. – EFSA Supporting Publication 636: 1-107. DOI: 10.2903/sp.efsa.2014.EN-636.

[6] Bektaş, M. (2015): Hydrophilidae, Helophoridae and Hydrochidae (Coleoptera) fauna of Gaziantep, Hatay, Kahramanmaraş, Kilis and Osmaniye province. – Dissertation, Ataturk University, Erzurum, Türkiye.

[7] Bektaş, M., Orhan, F., Barış, Ö. (2021): A new approach and a model study in a floating island microbial biodiversity and endosymbionts on the digestive structure of an aquatic insect. – Fresenius Environmental Bulletin 30: 13250-13263.

[8] Bektas, M., Orhan, F., Baris, O. (2022): Isolation of biological control agents and biotechnological bacteria from aquatic insect gut microbiota (Coleoptera: Helophoridae, Hydrophilidae). – Biology Bulletin 49: 596-608.

[9] Bertola, M., Mutinelli, F. (2021): A systematic review on viruses in mass-reared edible insect species. – Viruses 13: 2280. DOI: 10.3390/v13112280.

[10] Broderick, N. A., Raffa, K. F., Goodman, R. M., Handelsman, J. (2004): Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. – Applied and Environmental Microbiology 70: 293-300. DOI: 10.1128/AEM.70.1.293-300.2004.

[11] Cabral, J. P. (2010): Bacterial pathogens and water. International journal of environmental research and public health. – Water Microbiology 7: 3657-3703. DOI: 10.3390/ijerph7103657.

[12] Cai, X., Wang, R., Hu, S., Li, Y., Chen, T., He, J., Tan, S., Zhou, W. (2019): Complete genome sequence of *Sphingobacterium* sp. strain CZ-2T isolated from tobacco leaves infected with wildfire disease. – Research Square 1: 26. DOI: 10.21203/rs.2.15235/v1.

[13] Chen, X., Wang, L., Zhou, J., Wu, H., Li, D., Cui, Y., Lu, B. (2017): *Exiguobacterium* sp. A1b/GX59 isolated from a patient with community-acquired pneumonia and bacteremia: genomic characterization and literature review. – BMC Infectious Diseases 17: 1-7. DOI: 10.1186/s12879-017-2616-1.

[14] Cucini, C., Leo, C., Vitale, M., Frati, F., Carapelli, A., Nardi, F. (2020): Bacterial and fungal diversity in the gut of polystyrene-fed *Alphitobius diaperinus* (Insecta: Coleoptera). – Animal Gene 17(1): 1-8. DOI: 10.1016/j.angen.2020.200109.

[15] Da Silva Lucas. A. J., Oliveira, L. M., Da Rocha, M., Prentice, C. (2020): Edible insects: an alternative of nutritional, functional and bioactive compounds. – Food Chem 311: 126022. DOI: 10.1016/j.foodchem.2019.126022.

[16] De Silva, L. A. D. S., Wickramanayake, M. V. K. S., Heo, G. J. (2021): Virulence and antimicrobial resistance potential of *Aeromonas* spp. associated with shellfish. – Letters in Applied Microbiology 73: 176-186. DOI: 10.1111/lam.13489.

[17] Douglas, A. E. (2015): Multiorganismal insects: diversity and function of resident microorganisms. – Annual Review of Entomology 60: 17-34. DOI: 10.1146/annurev-ento-010814-020822.

[18] Engel, P., Moran, N. A. (2013): The gut microbiota of insects—diversity in structure and function. – FEMS Microbiol. Rev. 37: 699-735. DOI: 10.1111/1574-6976.12025.

[19] Esen, B., Gözalan, A. (2020): *Acinetobacter calcoaceticus - Acinetobacter baumann* (abc) and new species. – Kocatepe Medical Journal 21: 211-216. DOI: 10.18229/kocatepetip.545268.

[20] Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., Pinelli, E. (2019): Gut microbiota of aquatic organisms: a key endpoint for ecotoxicological studies. – Environmental Pollution 248: 989-999.

[21] Fernandez, M., Porcel, M., Torre, J., Molina-Henares, M. A., Daddaoua, A., Llamas, M. A., Duque, E. (2015): Analyses of the pathogenic potential of nosocomial *Pseudomonas putida* strains. – Frontiers in Microbiology 6: 871. DOI: 10.3389/fmicb.2015.00871.

[22] Garofalo, C., Osimani, A., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., Clementi, F. (2017): The microbiota of marketed processed edible insects as revealed by

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

high-throughput sequencing. – Food Microbiology 62(1): 15-22. DOI: 10.1016/j.fm.2016.09.012.
[23] José, M., Julieta, R. E., Victor, C. (2009): Edible aquatic coleopteran of the world with an emphasis on Mexico. – Journal of Ethnobiology and Ethnomedicine 5: 1-13. DOI: 10.1186/1746-4269-5-11.
[24] Kövesdi, V., Stercz, B., Ongrádi, J. (2016): *Kurthia gibsonii* as a sexually transmitted zoonosis: from a neglected condition during World War II to a recent warning for sexually transmitted disease units. – Indian Journal of Sexually Transmitted Diseases and AIDS 37: 68-71.
[25] Kwong, W. K., Steele, M. I., Moran, N. A. (2018): Genome sequences of *Apibacter* spp., gut symbionts of Asian honey bees. – Genome Biol. Evol. 10(1): 1174-1179. DOI: 10.1093/gbe/evy076.
[26] Lebreton, F., Willems, R. J. L., Gilmore, M. S. (2014): *Enterococcus* Diversity, Origins in Nature, and Gut Colonization. – In: Gilmore, M. S., Clewell, D. B., Ike, Y., Shankar, N. (eds.) Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Eye and Ear Infirmary, Boston, MA.
[27] Makkar, H. P. S., Tran, G., Heuzé, V., Ankers, P. (2014): State of the art on use of insects as animal feed. – Anim. Feed Sci. Tech.197: 1-33. DOI: 10.1016/j.anifeedsci.2014.07.008.
[28] Maniam, K., Argentine, S. (2022): A case of sepsis due to a rare carbapenem-resistant *Ignatzschineria* species. – IDCases 27. DOI: 10.1016/j.idcr. 2021.e01354.
[29] Molina, L., Udaondo, Z., Duque, E., Fernandez, M., Bernal, P., Roca, A., Torre, J., Ramos, J. L. (2016): Specific gene loci of clinical *Pseudomonas putida* isolates. – PLoS One 11: 2-24. DOI: 10.1371/journal.pone.0147478.
[30] Montecillo, J. A. V. (2022): Phylogenomics and comparative genomics analyses support the creation of the novel family *Ignatzschineriaceae* fam. nov. consisting of the genera *Ignatzschineria* and *Wohlfahrtiimonas* within the order Cardiobacteriales. – Research in Microbiology 103988. DOI: 10.1016/j.resmic.2022.103988.
[31] Pawar, K. D., Banskar, S., Rane, S. D., Charan, S. S., Kulkarni, G. J., Sawant, S. S., Shouche, Y. S. (2012): Bacterial diversity in different regions of gastrointestinal tract of Giant African Snail (*Achatina fulica*). – Microbiologyopen 1: 415-426. DOI: 10.1002/mbo3.38.
[32] Robinson, C. J., Schloss, P., Ramos, Y., Raffa, K., Handelsman, J. (2010): Robustness of the bacterial community in the cabbage white butterfly larval midgut. – Microbial Ecology 59: 199-211. DOI: 10.1007/s00248-009-9595-8.
[33] Rostagno, C., Pecile, P., Stefàno, P. L. (2013): Early *Lactococcus lactis endocarditis* after mitral valve repair: a case report and literature review. – Infection 41: 897-899. DOI: 10.1007/s15010-012-0377-8.
[34] Saavedra, M. J., Perea, V., Fontes, M. C., Martins, C., Murcia, A. (2007): Phylogenetic identification of *Aeromonas* strains isolated from carcasses of pigs as new members of the species *Aeromonas allosaccharophila*. – Antonie Van Leeuwenhoek 91: 159-167.
[35] Stellato, G., De Filippis, F., La Storia, A., Ercolini, D. (2015): Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy processing environment. – Appl. Environ. Microbiol. 81: 7893-7904. DOI: 10.1128/AEM.02294-15.
[36] Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B., Van Campenhout, L. (2016): Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. – Food Microbiology 53: 122-127. DOI: 10.1016/j.fm.2015.09.010.
[37] Tang, L., Huang, Y., Li, T., Li, Y., Xu, Y. (2022): Bacterial peritonitis caused by *Tsukamurella inchonensis* in a patient undergoing continuous ambulatory peritoneal dialysis. – Infection and Drug Resistance 15: 2475.
[38] Toth, E. M., Borsodi, A. K., Euzeby, J. P., Tindall, B. J., Marialigeti, K. (2007): Proposal to replace the illegitimate genus name *Schineria* Toth et al. 2001 with the genus name

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305

Ignatzschineria gen. nov. and to replace the illegitimate combination *Schineria larvae* Toth et al. 2001 with *Ignatzschineria larvae* comb. nov. – International Journal of Systematic and Evolutionary Microbiology 57: 179-180. DOI: 10.1099/ijs.0.64686-0.

[39] Traore, O., Martikainen, O., Siitonen, A., Traoré, A. S., Barro, N., Haukka, K. (2014): Occurrence of *Vibrio cholerae* in fish and water from a reservoir and a neighboring channel in Ouagadougou, Burkina Faso. – The Journal of Infection in Developing Countries 8: 1334-1338. DOI: 10.3855/jidc.3946.

[40] Tuipulotu, D. E., Mathur, A., Ngo, C., Man, S. M. 2020. *Bacillus cereus*: epidemiology, virulence factors, and host–pathogen interactions. – Trends in Microbiology 28: 458-471. DOI: 10.1016/j.tim.2020.09.003.

[41] Van der Spiegel, M., Noordam, M. Y., Van der Fels-Klerx, H. J. (2013): Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. – Comprehensive Reviews in Food Science and Food Safety 12: 662-78. DOI: 10.1111/1541-4337.12032.

[42] Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L., Figueras, M. J., Gascon, J. (2003): *Aeromonas* spp. and traveler's diarrhea: clinical features and anticipation resistance. – Emerging Infectious Ailments 9: 552.

[43] Wenzler, E., Kamboj, K., Balada-Llasat, J. M. (2015): Severe sepsis secondary to persistent *Lysinibacillus sphaericus*, *Lysinibacillus fusiformis*, and *Paenibacillus amylolyticus* bacteremia. – International Journal of Infectious Disease 35: 93-95. DOI: 10.1016/j.ijid.2015.04.016.

[44] Woo, H. I., Lee, J. H., Lee, S. T., Ki, C. S., Lee, N. Y. (2010): Catheter-related bacteremia due to *Microbacterium oxydans* identified by 16S rRNA sequencing analyses and biochemical characteristics. – Korean Journal of Clinical Microbiology 13(1): 173-177. DOI: 10.5145/KJCM.2010.13.4.173.

[45] Wooldridge, D. P., Wooldridge, C. R. (1972): Bacteria from the digestive tract of the phytophagous aquatic beetle *Tropisternus lateralimbutus* (Coleoptera: Hydrophilidae). – Environmental Entomology 4: 533-534. DOI: 10.1093/ee/1.4.533.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_12931305