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ECOPHYSIOLOGICAL PROFILING of *Ignatzschineria larvae* ISOLATED FROM AN AQUATIC INSECT: pH, SALINITY, THERMAL, RADIATION and ANTIBIOTIC TOLERANCE

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ABSTRACT

Ignatzschineria larvae are a recently characterized bacterium with limited ecophysiological data and are proposed as a potential microbial biomarker for insect colonization. We isolated *I. larvae* (strain MW602513) from the gut of an aquatic insect and assessed their tolerance and antibiotic susceptibility (Kirby-Bauer, CLSI/EUCAST) to pH (3-11), NaCl salinity (0.5-10 %), temperature (4-55°C) and ultraviolet radiation. Inhibition zones were interpreted according to current breakpoints. The isolate exhibited robust growth at pH 6-7, low salinity (≤ 2%) and moderate temperatures (30-37°C); there was no growth at 10°C or 50-55°C, and a significant growth reduction was observed at ≥ 5% NaCl. Bacteria were observed to not grow under UV radiation applied for 5 minutes. Resistance to various antibiotic groups tested was observed. The results demonstrate that the bacteria can adapt to mesophilic environments with low salinity and neutral to alkaline pH, highlighting their ecological resilience and potential impact on ecosystems.

1. INTRODUCTION

Biomarkers are biological indicators that can support diagnosis, assess disease severity or risk and guide clinical interventions [1]. Certain bacterial species have been proposed as potential biomarkers in various forensic contexts, including Sudden Infant Death Syndrome [2]. Moreover, biomarkers provide growing evidence of the long-term persistence of some microbial life forms in low-oxygen environments, offering valuable insights into microbial adaptation and survival mechanisms [3]. They may also serve as diagnostic and classification tools in disease detection, such as in lung cancer [4].

Insect colonies exhibit adaptive internal organization, and their reproductive success is influenced by factors such as overwintering, predation, and ecological competition [5]. The metabolic potential of microbial communities associated with these colonies tends to be stable and predictable in most ecosystems [6]. Biological control, supported by the activity of macroinvertebrates, offers a sustainable approach to maintaining this balance [7, 8]. Accordingly, insect-associated bacteria with biomarker and biocontrol potential have attracted increasing research interest [9–11]. Bacteria are the dominant and most ecologically relevant microorganisms in these niches [12].

Rapid emergence of antimicrobial resistance remains a global health concern [13–15]. Gram-negative bacteria, which possess two membrane bilayers separated by a periplasmic space, display distinctive resilience to these stressors compared to other bacterial groups. In addition to antibiotic exposure, environmental stressors such as radiation, salinity, and pH can significantly impact bacterial survival, persistence and community dynamics in aquatic environments [16-18].

The genus *Ignatzschineria* was first described by [19] and belongs to the class Gammaproteobacteria. *Ignatzschineria larvae* was originally isolated from the larval stages of *Wohlfahrtia magnifica* (Diptera:

Sarcophagidae), a fly species responsible for myiasis. Members of this genus are aerobic, Gram-negative, non-spore-forming, non-motile, rod-shaped bacteria [20]. Although several clinical and ecological case reports exist, there is limited information regarding their physiological and ecological characteristics.

In this study, *I. larvae* MW602513 was isolated from the intestinal microbiota of the aquatic insect *Hydrobius fuscipes* Linnaeus, 1758 [10]. To better understand its ecological adaptability, the isolate was subjected to pH, temperature, salinity and radiation tolerance tests, as well as antibiotic susceptibility assays, and the results were compared with previously described *I. larvae* strains. *I. larvae* is a relatively recently characterized bacterium with limited ecological and physiological data available. Therefore, investigating its tolerance to multiple environmental stress factors can provide important insights into its ecological niche and potential biomarker applications [21].

2. MATERIAL AND METHOD

The present study provides an overview of the experimental design, as illustrated in Figure 1. The biological material consisted of an aquatic insect belonging to a genus classified among edible insect taxa (e.g., *Cybister* spp. Curtis, 1827; *Hydrophilus* spp. Geoffroy, 1762; *Agabus* spp. Leach, 1817). Intestinal tissues were aseptically dissected from live specimens to minimize external contamination, and bacterial strains were subsequently isolated from the gut microbiota. The isolates were then characterized using phenotypic and molecular identification methods to ensure accurate taxonomic classification.

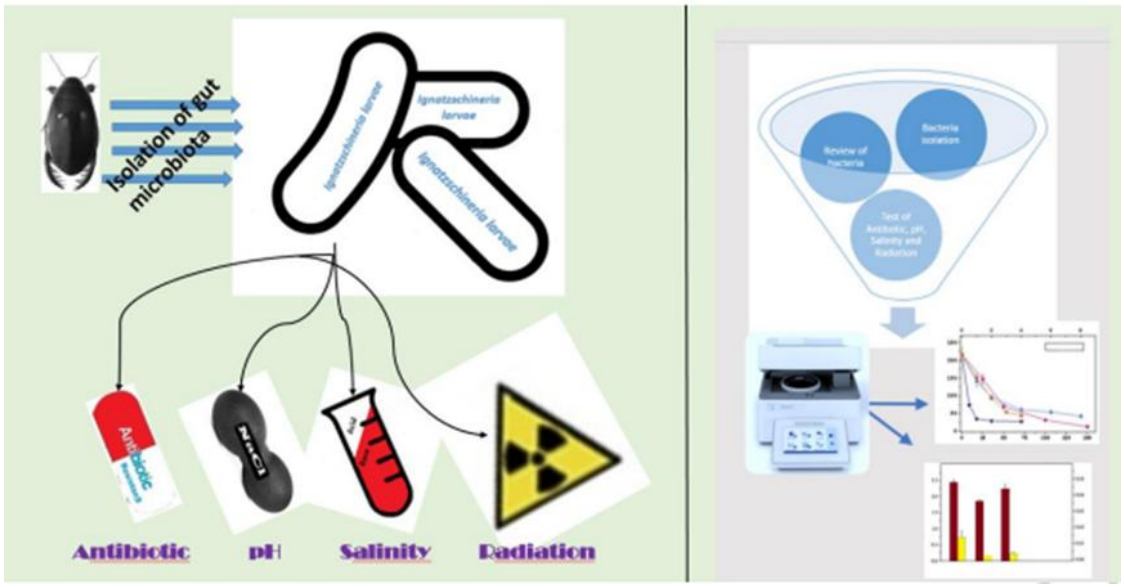

Figure 1. General working scheme.

2.1. Bacteria isolation from aquatic insects and revival of *I. larvae* bacteria

Bacteria isolation from aquatic insects and revival of *I. larvae* bacteria the insect specimens were first inactivated in closed boxes containing ethyl acetate-impregnated cotton wool [22]. Then, the appendages, including elytra and wings, were removed, and the entire outer surface of the insects was treated with 70% ethanol for 5 minutes to remove possible contaminant microorganisms [23]. The digestive tract of the insects was rinsed in autoclaved sterile distilled water to remove the alcohol and then examined under a binocular microscope in the laboratory under aseptic conditions. Dissected, separated and placed in 0.9% sterile physiological water without delay. Samples were brought to the microbiology laboratory for cultivation [24].

Insect digestive tracts were crushed in 0.9% sterile physiological water using a sterile glass homogenizer, homogenized by vortexing, and serial dilutions were prepared with sterile physiological water. Following inoculation on a general-purpose culture medium (TSA), the bacterial isolates were

subjected to phenotypic characterization and molecular identification. Molecular identification has been performed through 16S rRNA gene sequencing [25, 26].

2.2. Experimental methods

In this study, a series of tests was conducted to characterize the adaptation capacity of *I. larvae* by evaluating their tolerance to various environmental stress factors, such as pH, temperature, radiation and their antibiotic susceptibility profile.

2.2.1. Antibiotic Susceptibility Testing

To determine the resistance patterns of *I. larvae* strains to multiple antibiotics and to assess the prevalence of antibiotic resistance in environmental isolates, antibiotic susceptibility testing was performed using the disk diffusion method [27] against antibiotics commonly used in clinical practice. This approach provided a comprehensive characterization of the bacterial resistance profile and highlighted its potential epidemiological significance.

2.2.2. pH tests

pH tests assess the tolerance of bacteria to different pH conditions, including highly acidic and alkaline environments, providing insight into their ability to survive under varying acidity levels. In this study, bacterial cultures were exposed to a range of pH values (3, 5, 7, 9 and 11) to evaluate their growth and survival potential under environmental stress. These pH levels were selected to mimic different ecological niches, thereby allowing the characterization of the adaptive capacity of *I. larvae* to extreme pH fluctuations (Figure 2).

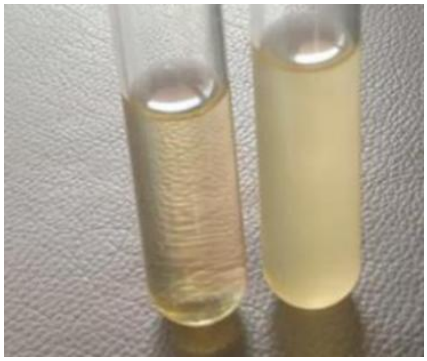

Figure 2. pH test applications (growth at pH 5 and pH 7).

2.2.3. Salinity tests

To assess the tolerance levels of the bacteria to different salt concentrations and to determine their resistance to osmotic stress under varying ecological conditions, their growth ability was tested at different NaCl concentrations (0.5%, 2%, 5%, 8% and 10%). These concentrations were selected to represent a broad range of environmental salinity conditions, from low to high osmotic stress.

2.2.4. Radiation tests

Radiation tolerance experiments were carried out in a microbiology laboratory under a biosafety cabinet to maintain a controlled and sterile environment. Petri dishes containing bacterial cultures were partially covered with aluminum foil, leaving half of the surface exposed to radiation while the remaining half was shielded. This experimental design enabled a direct comparison of bacterial survival between the irradiated and protected areas. Following exposure, the plates were incubated under standard laboratory conditions, and bacterial growth was evaluated to determine the level of radiation resistance [28].

2.2.5. Temperature Tolerance Test

Temperature tolerance experiments were conducted under controlled laboratory conditions to evaluate the ability of the bacteria to grow and survive at different temperature levels. To determine the optimal temperature for bacterial growth, cultures were incubated in separate petri dishes at 4°C, 25°C, 37°C, 45°C and 55°C.

3. RESULTS AND DISCUSSION

It is widely accepted that insect ecosystems are a significant source of bacterial species diversity. Insects are predicted to host thousands of new, as yet unidentified, microorganisms, and these microorganisms contribute significantly to ecological diversity [19]. The interaction between hosts (such as insects) and their endogenous microbiota plays a crucial role in the biology of many organisms, ranging from humans to plants [29]. Gut microbiota regulates host nutrition, digestion, metabolism, reproduction and immunity [30, 31]. In insects, gut microorganisms contribute to the synthesis of essential nutrients, thereby supporting host physiology [32]. Consequently, the gut microbiota plays a fundamental role in shaping insect physiology and can influence behavior, reproduction and susceptibility to pathogens [33].

In recent years, increasing attention has been given to the study of insect gut microbiota [9, 10, 14], reflecting its ecological importance and potential implications for public health. The overuse and misuse of antibiotics in both healthcare and agriculture have accelerated the emergence of antibiotic-resistant bacteria [34-36], increasing the need to identify and understand alternative bacterial reservoirs that may contribute to the spread of resistant or opportunistic strains.

Ignatzschineria is some Gram-negative bacilli that belongs to the class Gammaproteobacteria and consists of three species: *I. indica*, *I. larvae* and *I. ureiclastica*. *Ignatzschineria* species, commonly associated with parasitic larvae like those of certain flies, are notable for their potential role as biomarkers in forensic and medical studies. The bacteria found within these larvae can provide insights into decomposition stages, aiding forensic investigations by helping estimate the time of death. Additionally, the presence of *Ignatzschineria* bacteria in infected wounds can serve as a biomarker for myiasis, a parasitic infection, offering diagnostic clues in both human and veterinary medicine [37, 38]. Table 1 shows the results.

Table 1. I. larvae antibiotic susceptibility result (Disc Diffusion Method)

Antibiotic name	Antibiotic code	Antibiotic dosage/brand (µg/Bioanalyse)	Antibiotic group	Antimicrobial susceptibility results*
Doripenem	DOR10	10µg	Carbapenem	S
Ertapenem	ETP10	10µ	Carbapenem	R
Imipenem	IPM1	10 µg	Carbapenem	S
Meropenem	MEM10	10 µg	Carbapenem	S
Gentamisin	CN10	10 µg	Aminoglikozid	20 mm
Tobramycin	TOB10	10 µg	Aminoglycoside	R
Amikacin	AK30	30 µg	Aminoglycoside	R
Ampicillin	AM10	10 µg	Penicillin	S
Ampicillin Sulbactam	SAM20	20 µg	Beta-lactam	S
Piperacillin / Tazobactam	TPZ110	100/10 µg	Beta-lactam	S
Ciprofloxacin	CIP5	5 µg	Quinolone	R
Levofloxacin	LEV5	5 µg	Quinolone	R
Nitrofurantoin	F300	300 µg	Nitrofuran	S
Trimethoprim / Sulfamethoxazole	SXT25	25 µg	Sulfonamide	R
Tetracycline	TE30	30 µg	Tetracycline	R
Minocycline	MI30	30 µg	Tetracycline	14 mm
Tigecycline	TGC15	15 µg	Tetracycline	S
Cefotaxime	CTX30	30 µg	Cephalosporin	S
Cefepime	FEP30	30 µg	Cephalosporin	17 mm
Ceftazidime	CAZ30	30 µg	Cephalosporin	R
Ceftriaxone	CRO30	30 µg	Cephalosporin	R
Cefazolin	CZ30	30 µg	Cephalosporin	21 mm
Aztreonam	ATM30	30 µg	Monobactam	R

The limited knowledge regarding *Ignatzschineria* species and the challenges associated with their accurate identification highlight the need for further research and the development of improved diagnostic and characterization techniques for this emerging pathogen.

I. larvae is an emerging opportunistic pathogen that has recently drawn growing clinical and scientific attention due to its association with human infections, particularly in cases involving myiasis and chronic-neglected wounds. Initially identified from the digestive tract of necrophagous flies, this bacterium has since been reported in an increasing number of clinical cases, underscoring its potential medical importance. In the present study, *I. larvae* was isolated from the intestinal tract of an aquatic insect, providing novel insights into its ecological distribution and suggesting that insect hosts may act as potential reservoirs for opportunistic human infections [39, 40].

3.1. Antibiotic Resistance and Forensic Relevance of *Ignatzschineria larvae*

The treatment of *Ignatzschineria* bacteremia constitutes a clinical challenge due to the limited number of reported cases and the difficulties associated with accurate identification of the organism. This reason has led to the absence of CLSI breakpoint guidelines and standardized therapeutic recommendations for this emerging pathogen [40]. The breakpoint tables established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) do not provide specific interpretive criteria for *I. larvae* [41]. Therefore, the antimicrobial susceptibility results obtained in this study were interpreted based on available data from the literature. For antimicrobial agents lacking published reference values, only inhibition zone diameters were reported.

3.2. Environmental Tolerance of *Ignatzschineria larvae*: Effects of pH, Salinity and Radiation

Variation in environmental factors such as pH, salinity, and radiation plays a critical role in shaping bacterial survival strategies and adaptive capacities [42]. pH analysis investigates how bacterial communities respond to different levels of acidity and alkalinity, thereby revealing both their optimal growth conditions and their potential to survive in extreme environments [43]. Salinity analysis evaluates bacterial tolerance to various salt concentrations, which is particularly important for understanding their capacity to persist in marine or hypersaline ecosystems [44].

Similarly, radiation analysis examines bacterial resistance to different levels of exposure, providing valuable insights into their mechanisms of environmental stress tolerance and their potential applications in biotechnology, bioremediation and even space research [45]. In this context, examining the responses of *I. larvae* to these environmental variables offers a better understanding of their ecological adaptability and survival potential in diverse habitats. Presented in the figure below is the isolate's growth response under different environmental conditions, including temperature, salinity and pH levels. (Figure 3 and Table 2).

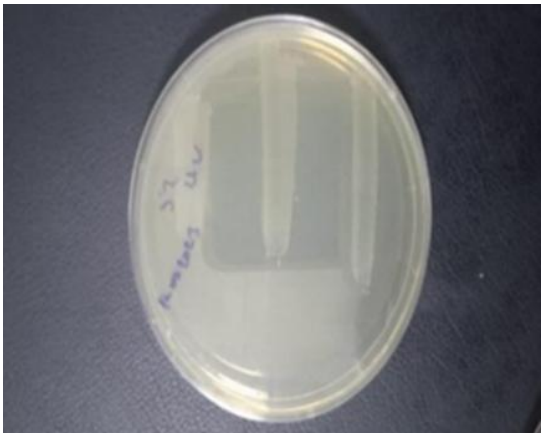

Figure 3. Radiation resistance.

The data provided on the temperature parameter highlights the optimal growth range for the organism in question, showing that it thrives between 25-37°C. This suggests that the bacteria or larvae being studied are mesophyll, which are organisms that grow best at moderate temperatures, common for many

pathogenic species. The lack of growth at 4°C indicates that the organism cannot survive or reproduce in colder environments, while the inability to grow at 55°C suggests it is heat-sensitive and does not possess thermophilic adaptations. When examining the reproduction characteristics in saltwater, the organism shows intensive reproduction at lower salinity levels (0.5%), which aligns with many freshwater or mildly saline environment dwellers. The absence of reproduction at higher salinity levels (5, 8, 10) suggests the organism is not halotolerant and cannot thrive in highly saline environments. This is crucial for understanding its ecological limits and potential habitats, indicating that areas with moderate to high salinity would not be conducive to its spread.

Table 2. Observations on temperature, salt concentrations and pH tests

Parameter	Range / Value	Observation	Interpretation
Temperature (°C)	25 – 37	Growth observed	Optimal growth under moderate temperature conditions
Temperature (°C)	4 and 55	No growth	No adaptation to extreme cold or heat
Salt concentration (%)	0.5	Intensive reproduction	Favors growth in low-salinity environments
Salt concentration (%)	2-5	Weak growth	Limited halotolerance
pH Level	pH 6 – pH 11	Intensive reproduction	Broad tolerance to neutral and alkaline pH
pH Level	pH 5	Weak growth	Mildly acidic conditions reduce but do not support growth at acidic pH ≤ 5

The pH results underscore the organism's environmental adaptability. The absence of reproduction at pH 3 and suggests that it cannot tolerate highly acidic conditions, which could be useful in controlling its spread in certain environments. However, the weak reproduction observed at pH 5 implies that it can survive, albeit with reduced vigor, in mildly acidic conditions. The reproduction between pH 6-11 demonstrates a broad pH tolerance in neutral to alkaline environments, which provides a competitive advantage in diverse ecological niches (Table 2). Overall, these findings indicate that the organism prefers moderate temperatures, low salinity and neutral to alkaline pH environments for optimal growth. Understanding these parameters is crucial for predicting its ecological range and it provides valuable information for developing strategies to control its spread by manipulating environmental factors such as temperature, salinity and pH (Table 2).

In recent years, cases of *I. larvae* bacteremia have been reported. These cases were clearly associated with fly larval infestations and myiasis, particularly in patients with poor hygiene and neglected wounds. Researchers have noted a significant association between *Ignatzschineria* bacteremia and chronic wounds infested with maggots in patients living under poor hygienic conditions [21].

Microscopically, this bacterium was identified as some Gram-negative bacilli; however, it could not be accurately identified using standard laboratory procedures. In the case reported by Gigante et al. (2025), biochemical testing initially identified the organism as *Pasteurella canis* with a 95% probability, but subsequent molecular analyses confirmed its identity as *Ignatzschineria larvae*. The authors emphasized the critical importance of close collaboration between clinical and laboratory teams for the accurate diagnosis of such rare and emerging pathogens [41].

In 2025, Mielke et al. reported that *I. larvae* isolated from a patient in the United States were susceptible to levofloxacin, ciprofloxacin, trimethoprim–sulfamethoxazole, and cefepime. In contrast, our study isolates exhibited resistance to levofloxacin, ciprofloxacin, and trimethoprim–sulfamethoxazole. Since fewer than ten cases of *I. larvae* bacteremia have been documented worldwide, there is currently no standardized treatment protocol for this pathogen. Previous studies have indicated that *I. larvae* generally shows susceptibility to most antibiotics used in the treatment of Gram-negative bacteremia. However, other *Ignatzschineria* species have been reported to display resistance to carbapenems and beta-lactams. Consistent with these findings, our study also revealed resistance to more than one class of antibiotics (Carbapenem, Aminoglycoside, Quinolone). These results provide valuable data to the literature regarding potential therapeutic strategies and highlight the need for the establishment of standardized treatment guidelines. Table 1 shows the results. Demurtas et al. (2023) further reported that

their *I. larvae* isolate was susceptible to ertapenem, ceftriaxone, levofloxacin and trimethoprim–sulfamethoxazole, due to the lack of specific treatment guidelines, the duration of ertapenem therapy was extended. In fewer than ten reported cases of bacteremia worldwide, patients have been treated with various antibiotics and differing treatment durations. In the present study, antimicrobial susceptibility data were obtained for 23 bacterial isolates, contributing to the growing body of evidence for the optimal management of *I. larvae* infections [38, 46, 47].

Taken together, these findings emphasize that *I. larvae* should not be regarded merely as an uncommon clinical isolate, but rather as a microorganism with a dual ecological and pathogenic role. Its environmental adaptability enables persistence and dissemination in freshwater ecosystems, while its resistance profile underscores its potential clinical impact. The data obtained highlight the importance of integrated ecological, clinical, and forensic surveillance to better understand and mitigate the risks posed by this emerging species. Future studies incorporating quantitative stress tolerance assays, biofilm formation capacity, oxidative stress responses, and genomic analysis would further elucidate its ecological strategies and potential health implications.

4. CONCLUSION AND SUGGESTIONS

Biomarkers, exemplified by *I. larvae*, show considerable promise in facilitating interdisciplinary research across the domains of health, ecology, and the environment due to their molecular and microbial adaptability. The resilience of *I. larvae* to diverse stressors, including antibiotics, salinity, pH and radiation, underscores its potential for early detection and targeted interventions in the management of invasive species and the promotion of ecological balance. Such insights not only advance the understanding of the role of gut microbiota in host regulation but also pave the way for innovative strategies in disease diagnostics and environmental control. These findings suggest that *I. larvae* could serve as a valuable model organism and biomarker. This would be beneficial for developing integrated approaches linking microbial ecology with environmental and public health monitoring.

A review of the literature [48] indicates that, although no cases of *Ignatzschineria* bacteremia have been reported in our country to date, this organism should not be overlooked, particularly in cases of myiasis. In complex diagnostic situations, identification methods based on 16S rRNA gene amplification and sequencing should be preferentially employed to ensure accurate detection.

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Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Contributions of the Authors

Writing (original draft), methodology and conceptualization were carried out by Mehmet Bektaş. Visualization and validation were carried out by Figen Orhan. Conceptualization, supervision, writing (review and editing) were carried out by Özlem Barış.

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