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Bioremediation of Emerging Contaminants Using Antarctic Ampicillin-Degrading Bacteria,

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The project was based on the study of a new Antarctic bacterial species, *P. violetae* (named in honor of Chilean artist and musician Violeta Parra (1917–1967)), isolated and identified in the Bionanotechnology Laboratory of Universidad Andrés Bello in Chile, from soil samples taken from the Unión Glacier in Antarctica. This strain is characterized by being part of the *Pseudomonas* genus, being a Gram-negative bacillus, and having a growth temperature range between 0 and 30°C. Finally, its resistance in a medium containing ampicillin and its capacity to degrade it were evaluated. The use of this bacterium is proposed as a feasible method to combat the emerging contamination of antibiotics in wastewater.

EXECUTIVE SUMMARY

Emerging contaminants are chemical substances that have begun to appear in the environment, originating from products not necessarily considered pollutants and which are now becoming a cause for concern. Some of these contaminants include pesticides, pharmaceuticals, personal care products, UV filters, hydrocarbons, illicit drugs, food additives and metabolites (Guzmán, 2019), perfumed products, cleaning agents, and microplastics (Tejada, 2014). Organisms can be affected by these compounds in various ways, such as by the disruption of endocrine functions or the blockage or disturbance of hormonal functions, which affects the health of both humans and animal species (Serrano, 2017).

The use of pharmaceuticals has significantly increased in livestock farming, the salmon industry, and the human population due to various needs such as prevention, treatment, and control of diseases, as well as the application of growth-promoting antibiotics to improve food use and production (Halden, 2015). In addition, the use of pharmaceuticals has risen due to self-medication, overprescription, misuse, lack of information, among other factors. These antibiotic medications, being toxic substances, can even reach drinking water reserves (Reinoso, 2017). Since antibiotics are incompletely metabolized, they enter wastewater through feces and urine, adding to the contamination caused by improper disposal, hospital effluents, and some industries, eventually reaching wastewater treatment plants (Meléndez, 2020). These waters are typically treated at wastewater treatment plants, but these were not designed to eliminate antibiotics, so in some cases these compounds are not completely removed and remain present in the effluents (Szymańska et al., 2019), which are ingested through drinking water, the irrigation of edible crops with wastewater, or through the consumption of beef or salmon.

In livestock or the salmon industry, antibiotics are administered to animals intended for human consumption or from which a product is obtained. Chile is the second largest salmon producer (38%) after Norway (39%) (FAO, 2018). Antibiotic residues accumulate in the organism, in the animal's musculature, which being a consumable product, means the presence of drug residues in foods of animal origin, especially meat, is considered by global food safety and security organizations as a risk factor that can affect public health, especially antibiotics (Ramírez, 2021).

The use of **antibiotics** poses a problem in ecosystems, contaminating food and water sources. In the environment, they can cause microbial resistance, skin irritation, endocrine disruption, increased incidence of allergies, altered metabolism of thyroid hormones, and the development of tumors (Serrano, 2017). In agriculture, at high concentrations, plants exhibit stress symptoms such as reduced photosynthetic capacity and pigment alteration. Additionally, these compounds can cause internal histo-biochemical changes that affect normal plant growth and development, which is reflected in growth inhibition and biomass increase (Maldonado, 2023). An alarming effect is the development of antibiotic-resistant bacteria capable of surviving these substances and transferring their resistance to other bacteria. When exposed to environmental bacteria, they can generate bacterial resistance genes, reduce microbial diversity, and be toxic to animals such as fish and invertebrates (García, 2020).

Ampicillin is one of the most commonly used antibiotics in livestock. It has been widely used in medicine and agriculture to treat bacterial infections and promote animal growth (Kuswandi, 2017).

Bacterial resistance is a constant battle and represents a public health problem. So much so that the World Health Organization (WHO) considers it one of its top health priorities due to its impact on health (with recent projections indicating that by 2050, more deaths will be caused by this than by cancer today), and its economic impact (which, according to a recent study in the United Kingdom, will cost the global economy an estimated $100 trillion annually) (Camacho, 2025). Faced with this large-scale issue, a solution to bacterial resistance and the indiscriminate use of antibiotics became necessary, which is the reason for carrying out this research.

A method was applied to evaluate bacterial strains from the Antarctic continent, which were selected for their great genetic potential and their ability to adapt as extremophilic bacteria due to the extreme climatic conditions of the Antarctic continent. The bacteria *Pseudomonas violetae*, *Rhodobacter navarretei*, and *Pseudomonas veronii NanoPratt* were selected because of their potential to inhabit areas with extreme characteristics such as drought, strong winds, and low temperatures. In addition to these bacteria, a strain of *E. coli* sensitive to ampicillin was included as a negative control and *E. coli* with the pHEN6 plasmid, which confers resistance to ampicillin, as a positive control.

These bacteria were cultured on plates with rich culture medium (LB – Luria-Bertani) with ampicillin at a concentration of 100 µg/ml to assess their tolerance to ampicillin. Subsequently, bacteria identified as resistant were cultured in liquid LB medium with a concentration of 100 µg/mL of ampicillin to evaluate their ability to **degrade** the antibiotic.

The growth of *E. coli* in some filtered media indicated that the ampicillin had been degraded, as the growth of a sensitive bacterium is evidence that the medium no longer contained high concentrations of antibiotics. This result confirmed that the Antarctic bacterium *P. violetae* degrades ampicillin, allowing the growth of *E. coli* without the pHEN6 plasmid in the treated media.

The results obtained will allow the development of a biofilter based on these ampicillin-degrading bacteria, being a feasible and innovative method, as it could reduce antibiotic concentrations in water and consequently remediate the negative effects of emerging contaminants such as antibiotics. This new bacterial species has the potential to survive longer and under better conditions in the environment, even in adverse climatic conditions. **Key Concepts:** Emerging contaminants, antibiotics, Antarctic bacteria, degradation.

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1. INTRODUCTION AND BACKGROUND

Antarctica, also known as the White Continent, is the fifth largest continent on the planet, covering an area of approximately 14 million square kilometers (National Geographic, 2024). The Antarctic continent has characteristics that make it unique, inhospitable, and difficult to access due to extreme environmental conditions such as strong winds, excessively low temperatures, and high solar radiation during the summer (Jiménez de las Heras, 2020). With an average temperature of -50°C in winter and -12°C in summer, Antarctica is the coldest, driest, windiest continent and has the highest average elevation (National Geographic, 2024). However, despite these adverse conditions, Antarctica is home to a wide variety of wildlife, including penguins, seals, whales, and a large number of bird species. Because these animals must live in extremely cold conditions, they have developed different bodily adaptations (Chilean Antarctic Institute [INACH], 2010).

Antarctica is also a place of great scientific importance, as its unique climate and geology offer a unique opportunity to study the planet's history and climate change. This continent represents one of the most extreme environments on the planet for life. This ecosystem is characterized by high-stress environmental conditions such as low temperatures, limited access to nutrients, and high levels of ultraviolet radiation (Chilean Antarctic Institute [INACH], 2025). In this sense, Antarctica's severe conditions—low temperatures, high salinity, and natural formation of perchlorates—present extreme environments analogous to Mars, which can be favorable for the growth and development of native bacteria (Acevedo, 2021).

The conditions in which these organisms live give them highly interesting evolutionary potential, classifying these bacteria as extremophilic. They not only survive but thrive in these hostile environments. These extraordinary organisms defy biological expectations and offer us a unique view into the limits of life (Official Blog of the College of Biologists of the Community of Madrid [COBCM Blog], 2024). This potential has previously been tested in the laboratory where this research was conducted, showing that some of these bacteria have the ability to degrade substances such as diesel or chloroform. Among these bacteria is *P. violetae*, which has a particularly interesting ability to degrade TNT, demonstrating its potential for degrading other substances (Pérez, 2023).

Due to the unique characteristics of Antarctic microorganisms, we aimed to test their potential to degrade a specific emerging contaminant. The term emerging contaminants refers to compounds of diverse origin and chemical nature whose presence in the environment is not yet considered significant in terms of distribution and concentration, so they often go unnoticed. However, due to increasing detection rates, their potential ecological impact, and the risks they pose to human health, they are drawing growing concern (Jacobo, 2021). Among the most representative contaminants are antibiotics, analgesics, and anti-inflammatory drugs (Sánchez, 2018).

Among pharmaceuticals classified as emerging contaminants, we were especially drawn to antibiotics: these serve the function of killing or inhibiting the growth of bacteria and are used to treat bacterial infections. They are extremely useful since infectious diseases continue to be one of the leading causes of death globally in the early 21st century (Lozano, 2012). However, they still have a negative impact on both our health and the environment.

A clear example of the negative effects of these substances has been observed in the dairy industry, as shown in a study conducted in Paraguay by Fernando Pizarro in 2020. In this study, due to the wide variety of diseases, antibiotics are extremely useful tools, making their use difficult to avoid. However, due to the application of antibiotics, residues have been found in milk, affecting both dairy production and milk quality (Pizarro, 2020).

In Chile, this industry is primarily concentrated in regions such as Los Lagos, accounting for 39.1% of the industry with 1,215,221 head of cattle, Los Ríos with 21.5% and 666,943 head, and La Araucanía with 13.9% and 432,612 head (González, 2019). Livestock in these areas consists mainly of bovines, ovines, porcines, and caprines, mostly raised in grassland areas that occupy a large part of the total surface. In addition to human use, antibiotics are widely used in veterinary settings to control bacterial infections and as growth promoters (Meléndez, 2020). This livestock, like that in other countries, is administered antibiotics, which constitutes a problem because these compounds are not biodegradable and may remain in the environment for years. Moreover, the residues from animals exposed to pharmaceuticals may be even more toxic than the original compound, accumulating in the environment. An example of this is antibiotic resistance—the phenomenon that occurs when microbes no longer respond to antibiotics designed to eliminate them (World Health Organization [WHO], 2020). This has been known since the early days of antibiotics in the 20th century, but today many members of the scientific community consider it an epidemic. This was made evident in 2016, when the United Nations General Assembly declared antibiotic resistance the greatest global health threat (Cars, 2021).

Given the potential of extremophilic bacteria and the problem posed by antibiotic pollution, this project explored the ability of these bacteria to bioremediate antibiotics. Three Antarctic bacterial strains were selected: *P. veronii NanoPratt*, *P. violetae*, and *R. navarretei*, along with two control bacteria: *E. coli* with the pHEN6 plasmid (+), and *E. coli* (-) (antibiotic-sensitive). These strains were grown in the presence of ampicillin, a common antibiotic in livestock farming, to assess the bacteria's tolerance.

Subsequently, *P. violetae* was cultured alongside both control strains in liquid LB media with the antibiotic. The bacteria were then filtered out of the medium, which was used to culture a sensitive *E. coli* strain. If this sensitive strain was able to grow, it indicated that the medium no longer contained high concentrations of ampicillin, thus demonstrating that the previously present bacterium had the ability to degrade the contaminant. If the resistant bacterium inhibited the growth of the sensitive one, it suggested that the resistance was due to antibiotic expulsion via an efflux pump.

P. violetae proved capable of both surviving in a medium containing the antibiotic and enabling the growth of the sensitive strain, demonstrating its ability to degrade ampicillin.

It has been shown that extremophilic bacteria such as *P. violetae* are capable of degrading various compounds. The use of these new Antarctic bacterial strains represents a potential tool for wastewater treatment. *P. violetae* could help reduce the negative effects of ampicillin in water and marks the beginning of feasible methods to clean water sources from a wide range of contaminants.

The research is currently in the phase of evaluating quantitative data and creating the first prototypes of filters using these bacteria to reduce antibiotic concentrations in water, along with tests to determine whether *P. violetae* can use the antibiotic as a carbon source.

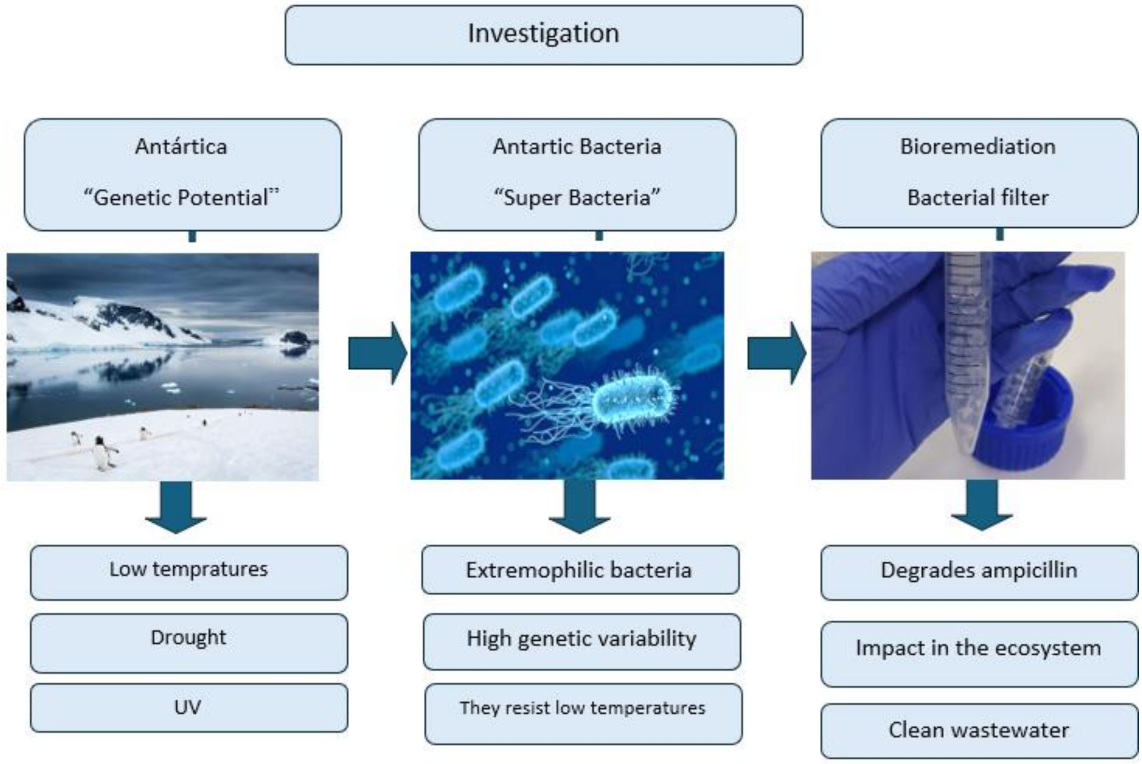

Image N° 1: Research outline

Source: Prepared by the authors

a. **Problem Formulation:**

● Research Question:

Are there Antarctic bacteria capable of reducing emerging contaminants such as ampicillin?

● Hypothesis:

There are Antarctic bacteria that possess the ability to degrade ampicillin, an emerging contaminant in wastewater.

● General Objective:

To evaluate the capacity of Antarctic bacteria to degrade ampicillin.

● Specific Objectives:

1. To evaluate the tolerance capacity of three Antarctic bacteria in LB medium with ampicillin.
2. To evaluate the resistance mechanism of the bacteria to ampicillin, determining whether it is due to an enzyme or the expulsion via an efflux pump.
3. To evaluate the Antarctic bacterium with the greatest performance in degrading antibiotics such as ampicillin.

● Final Planning and Timeline of Activities:

Activities / Month	May	July	August	September	October	November
Information search						
Topic selection						
Formulation of hypothesis and goals						
Theoretical background						
Reformulation of objectives						
Laboratory work						
Obtaining results						
Drafting of the report for national competition submission						
Final report editing and improvement						

Table No. 1: Final project planning and timeline

Source: Prepared by the authors

2. METHODOLOGY

• Study Area:

The research was carried out in the Bionanotechnology Laboratory of Universidad Andrés Bello. Scientists from the Nanolab, led by Dr. José Manuel Pérez Donoso, collected soil, water, and ice samples from Unión Glacier in the Antarctic territory during the Antarctic Scientific Expedition (ECA) and later analyzed the samples to isolate and identify bacterial strains, among them *P. violetae*.

As students from Liceo 1 Javiera Carrera, we approached the laboratory with concerns regarding the presence of emerging contaminants in wastewater and proposed exposing an Antarctic bacterium (due to its resistance to extreme conditions) to an emerging contaminant to observe whether it could degrade it.

We are grateful for the opportunity to use the lab's facilities and materials, the guidance provided to carry out our proposal, and above all, the learning experience based on collaborative teamwork.

- **Materials:**

The following table shows the list of materials used in the Bionanotechnology Laboratory of Universidad Andrés Bello.

	Materials
Bacterial Strains	• *E. coli* strain (negative control) • *E. coli* strain with pHEN6 plasmid (positive control) • R. *navarretei* strain • P. *veronii Nanopratt* strains • P. *violetae* strain
Instruments	• Incubator (T° 37°C/28°C) (biobase) • Laminar flow hood (Streamline)
Disposable Materials	• PTFE syringe filter, 0.22 µM pore size (NEST) • Pipette tips (NEST) • Eppendorf tubes (NEST)
Volumetric Materials	• Micropipette (Axygen) • Pipette (Normax)
Non-volumetric Materials	• LB agar culture plates with ampicillin (US Biological media, Sigma Aldrich ampicillin) • Culture plates (Boeco) • Test tubes (Boeco)

Table No. 2: Research Materials. *Source: Prepared by the authors*

- **Methods**:

1. Selection and cultivation of bacteria on LB agar plates with ampicillin

Five bacterial strains were selected, corresponding to:

- *E. coli*
- *E. coli* with the pHEN6 plasmid, modified to produce a beta-lactamase enzyme
- *R. Navarrete*
- *P. violetae*
- *P. veronii Nanopratt*

Our *Escherichia coli* strain was selected as a negative control due to its sensitivity to ampicillin, which means it will not grow in the presence of this antibiotic. On the other hand, *E. coli* with the pHEN6 plasmid was chosen as the positive control because of its ability to resist the antibiotic. Its growth would indicate resistance to ampicillin.

The Antarctic bacteria were selected for the following reasons: *R. navarretei* is a recently discovered species with interesting genetic potential, prompting us to test it. *P. violetae*, discovered two years ago, has the ability to degrade TNT, making it a beneficial candidate and a native species of Chile. Finally, *P. veronii* was selected due to its notable genetic ability to degrade difficult-to-break-down compounds such as diesel.

These bacteria were plated on LB + ampicillin medium at a concentration of 100 µg/mL. Plates with *E. coli* and *E. coli* with pHEN6 were incubated at 37°C (ideal growth temperature) for 48 hours, while the other bacteria were incubated at 28°C (optimal for Antarctic bacteria) for the same time period, to observe which strains were tolerant to ampicillin.

2. Cultivation of bacteria in liquid LB medium with ampicillin

The growth of bacteria identified as resistant on the plates was examined. Those showing growth were cultured in liquid LB medium with 100 µg/mL ampicillin. Depending on the ideal growth temperature of each bacterial strain, they were incubated at either 37°C or 28°C for 48 hours in a shaker incubator. In parallel, a control medium with ampicillin but without bacteria was prepared to serve as an untreated control.

3. Filtration of culture media

After the incubation period, the culture media were filtered to remove the bacteria using PTFE syringe filters with a pore size of 0.22 µm, aiming to retain the bacteria and obtain only the culture medium with residual ampicillin.

4. Incorporation of E. coli into the supernatants

A colony of *E. coli* was introduced into the filtered liquid culture media with ampicillin, then incubated at 37°C or 28°C (depending on the bacterial growth requirements) for 48 hours. If *E. coli* grew, it would indicate that the previously present bacterium had degraded the ampicillin. If *E. coli* did not grow, it would mean the antibiotic was not degraded by the previous bacterium.

5. Analysis of culture growth

After the incubation period, *E. coli* growth in the culture medium was observed to assess ampicillin degradation and compare results with control media and variables.

Growth of *E. coli* in a medium previously exposed to a resistant bacterium indicates that the prior bacterium had the ability to degrade the antibiotic. If *E. coli* fails to grow, it suggests the resistance of the previous bacterium was due to antibiotic expulsion via an efflux pump.

Quantitative data collection was considered during the investigation; however, the necessary techniques were not available at the time.

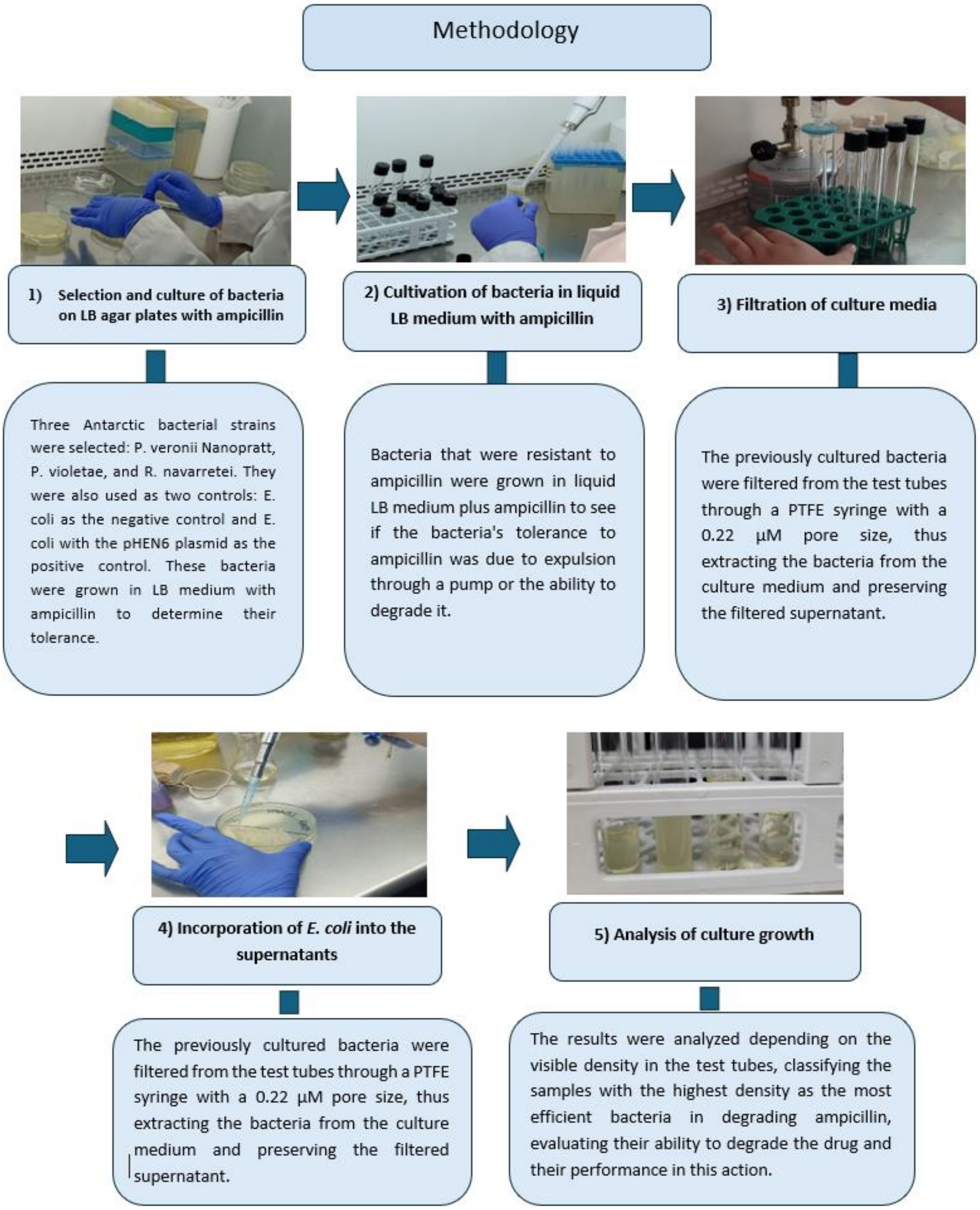

Image N°2: Methodology diagram

Source: Prepared by the authors

3. RESULTS

The following images show the test tubes used in the experiment:

- **Test Tube No. 1**: Culture medium with ampicillin, used as an untreated control.

- **Test Tube No. 2**: Filtered culture medium plus *E. coli* colony. Previously contained our *E. coli* strain; used as the negative control.
- **Test Tube No. 3**: Filtered culture medium plus *E. coli* colony. Previously contained our *E. coli* strain with the pHEN6 plasmid; used as the positive control.
- **Test Tube No. 4**: Filtered culture medium plus *E. coli* colony. Previously contained our *P. violetae* bacterium.

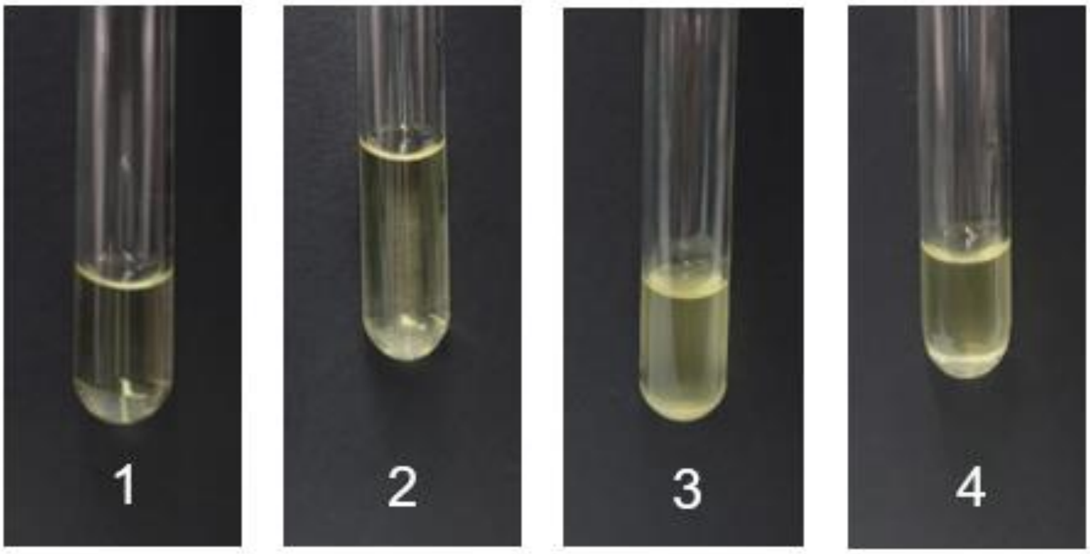

Figure No. 3: Results of *E. coli* growth in different supernatants

1 – medium without bacteria, 2 – sensitive bacterium, 3 – resistant bacterium, 4 – *P. violetae*

Source: Prepared by the authors

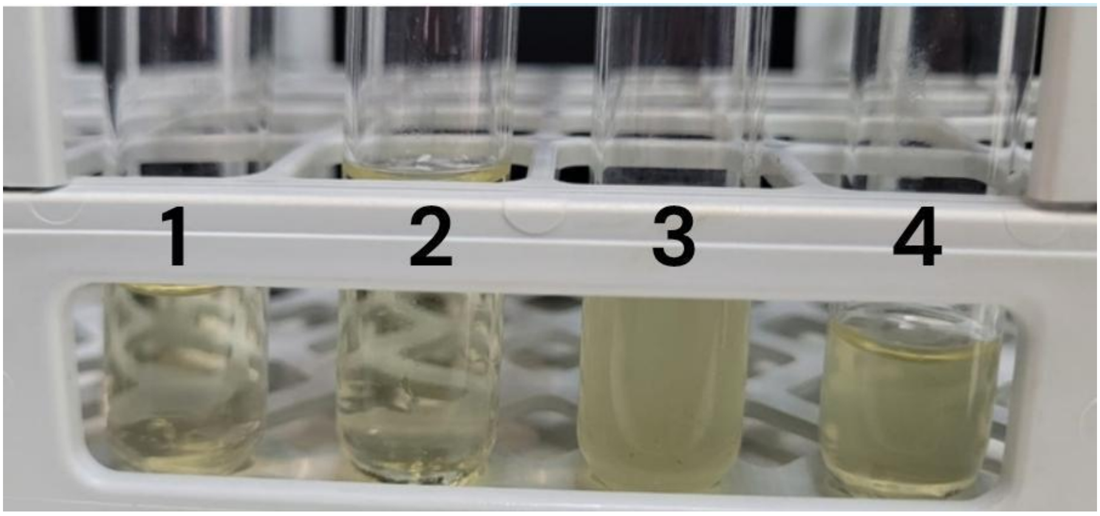

Figure No. 4: Results of *E. coli* growth in different supernatants

1 – medium without bacteria, 2 – sensitive bacterium, 3 – resistant bacterium, 4 – *P. violetae*

Source: Prepared by the authors

The bacteria *R. navarretei* and *P. veronii Nanopratt* did not show growth when exposed to the antibiotic, indicating that they are not resistant to these drugs. For this reason, they were not selected to continue in the next phases of the investigation.

● **Analysis of Results:**

In the analysis of photographs (Figures 3–4), we present the qualitative results of our experiment. Results from Test Tube No. 1 (culture medium with ampicillin) are compared with those of the remaining tubes.

Test Tube No. 2, the negative control, shows high transparency, indicating that the *E. coli* bacterium, which is sensitive to ampicillin, was unable to grow. This suggests that the ampicillin was not degraded.

By contrast, Test Tube No. 3, the positive control, presents high turbidity, indicating that the *E. coli* strain grew, suggesting that it was able to degrade the ampicillin.

Finally, in Test Tube No. 4, which previously contained the Antarctic bacterium *P. violetae*, moderate turbidity is observed. Although it is not as pronounced as in Test Tube No. 3, compared to Tube No. 1, the turbidity suggests that *E. coli* was able to grow—indicating that *P. violetae* was capable of degrading ampicillin.

4. DISCUSSION

It is evident that the bacterium *P. violetae* can degrade ampicillin, as it allowed the proliferation of *E. coli* (a bacterium sensitive to the antibiotic) in a medium that initially contained ampicillin. This confirms the hypothesis that *P. violetae* is capable of degrading ampicillin.

The qualitative results indicated that the bacterium achieved significant degradation in LB + ampicillin culture media, demonstrating its efficiency.

By degrading ampicillin, this bacterium can be used as an effective method to clean environments contaminated with antibiotics similar to ampicillin. These bacteria could be implemented in systems for treating wastewater and reducing the presence of emerging contaminants in the environment.

Bioremediation using bacteria has the advantage of being more accessible and environmentally friendly compared to chemical treatments such as ozonation. This is illustrated in the following comparative table:

- **Comparative Table:**

The table below compares two methods used to treat wastewater contaminated with various antibiotics:

Criteria	Bioremediation Method for Wastewater Treatment	Ozonation Method for Wastewater Treatment
Effectiveness	High for contaminants	High for contaminants
Costs	Low to moderate	Moderate to high
Treatment time	Prolonged	Fast
Infrastructure requirements	Minimal	Moderate to high
Environmental impact	Low	Low to moderate
Limitations	Depends on environmental conditions	Requires ozone generation, potential health risks
Advantages	Cost-effective, sustainable, minimal waste	Effective against a wide range of contaminants

Table No. 3: Comparative table of wastewater treatment methods

Source: Prepared by the authors based on Urbina (2020) and Rodríguez (2022)

5. LIMITATIONS

A major limitation of the research is the uncertainty about the behavior of *P. violetae* under real-world field conditions. Since the studied bacteria were developed in controlled and ideal laboratory conditions, their behavior outside that environment cannot be guaranteed. This is because environmental or industrial variables such as temperature, pH, pressure, and the presence of other bacteria or organisms can influence the outcome. Therefore, it is crucial to conduct tests under uncontrolled conditions to assess the viability, effectiveness, and coexistence of *P. violetae* with other bacteria in real-world scenarios and to determine its true potential.

6. CONCLUSIONS

- It has been successfully demonstrated that Specific Objectives 1 and 2 were achieved. It was verified that the bacterium *P. violetae* is effective in degrading the antibiotic ampicillin.

- *P. violetae* stood out for its ability to degrade ampicillin more efficiently, allowing *E. coli* to grow to higher density compared to other culture media.

- This bacterium could potentially be used in the development of a bacterial filter to improve the efficiency of eliminating emerging contaminants such as antibiotics.

- This process could represent an innovation in the control and reduction of emerging contaminants in wastewater, benefiting both ecosystems and human health.

7. PROJECTIONS

- To use ***P. violetae*** in the creation of a **biofilter** for implementation in **bioreactors** used in wastewater treatment plants. This would improve water quality in lower-income countries, as it represents a more economically feasible option for removing antibiotics.

- To investigate quantitative data using inhibition halo tests.

- Due to the remarkable potential of this new bacterial species, we aim to evaluate its ability to metabolize the antibiotic and use it as a carbon source.

- To begin testing for the degradation of other emerging contaminants.

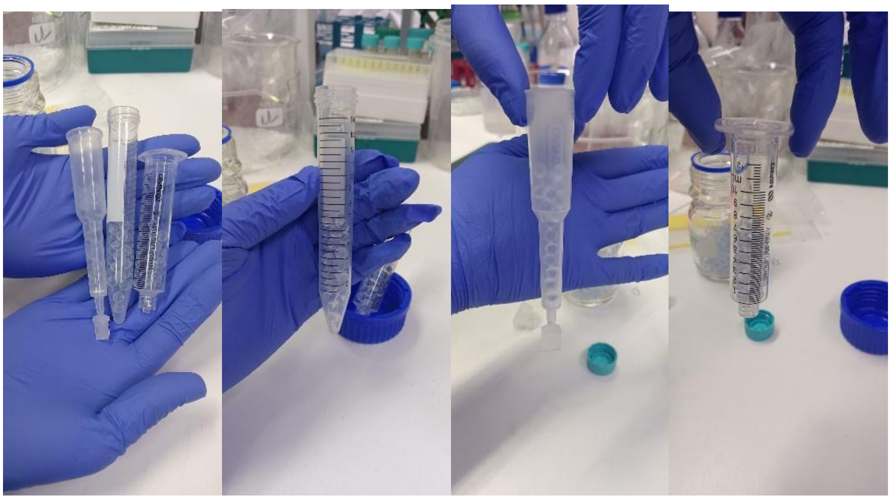

Figure No. 5: Projection of the first filter prototypes

Source: Prepared by the authors

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