Isolation and Identification of Gram-Negative Bacteria in the Soil Samples of Koronadal City Sanitary Landfill

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Abstract
Sanitary landfills, designed to manage waste and minimize environmental impact, can inadvertently become reservoirs for pathogenic microorganisms, posing health risks. This study aimed to isolate and identify gram-negative bacteria in soil samples from the Koronadal City sanitary landfill, which processes 4 of its 15-ton daily capacity of residual and biodegradable waste through composting and plastic recycling. Sterile techniques, including the use of a Class II Biosafety Cabinet, ensured the accuracy of bacterial isolation using nutrient, tryptic soy, and MacConkey agar, with identification performed via the API 20E kit.

The study revealed diverse bacterial colonies with varied morphological characteristics, including forms such as circular, convex, irregular, and spreading, and colors like white, off-white, yellow, and pink. There are eleven (11) gram-negative bacteria were identified such as *Enterobacter cloacae, Serratia liquefaciens, Enterobacter aerogenes, Serratia plymuthica, Klebsiella pneumoniae ssp. rhinoscleromatis, Erwinia spp., and Pantoea spp., Stenotrophomonas maltophilia, Vibrio fluvialis, Pseudomonas fluorescens/putida, and Burkholderia cepacia,* were isolated.

Soil analysis showed an alkaline pH of 7.87, high phosphorus, sufficient potassium, and moderate salinity (158.27 µS/cm), creating conditions favorable for gram-negative bacteria. These bacteria possess adaptive traits such as metabolic versatility, stress responses, and a protective outer membrane, enabling survival. The presence of known human pathogens raises health concerns due to waste pollution. However, these bacteria also hold potential for genetic engineering to mitigate pathogenicity while enhancing plastic waste degradation.

Keywords: *Gram-negative bacteria, Sanitary landfill, API 20E, pathogenic microorganism*

Introduction

Sanitary landfills are designed to contain waste and minimize environmental impact, but they are added can inadvertently become reservoirs for pathogenic microorganisms (Imron et al.; 2021). Community waste disposal in public landfills often presents significant health risks due to the diverse and potentially hazardous materials they contain. The public landfill, which receives solid, liquid, and pollutant waste from various sources, poses a direct threat to human health due to the potential for contamination by pathogenic bacteria. Rainwater runoff from the landfill can infiltrate the soil and contaminate both

groundwater and nearby bodies of water used by local residents, exacerbating the public health concerns (Schiopu & Gavrilescu, 2010).
Microorganisms can thrive in landfills such as gram-negative species can act as opportunistic pathogens that may affect immunocompromised individuals among other people (Venezia et al., 2017). Gram-negative bacteria were particularly isolated from landfill leachates in Indonesia (insert citation) and in Colombia, with Klebseilla pneumoniae being the most frequent isolates recorded (Mondragon-Quiguanas et al., 2022). Gram-negative anaerobic bacteria found in the human upper respiratory, digestive, and urogenital systems include genera such as Bacteroides, Fusobacterium, Porphyromonas, Prevotella, and Veillonella, and most of these bacteria have a rod shape, except Veillonella, which is spherical. Bacteroides fragilis is frequently implicated in polymicrobial intra- abdominal infections, while other species are related to infections in the upper respiratory tract and the female reproductive system. These bacteria often work together in infections and possess a variety of virulence factors, such as adhesins and capsules (Oliveira & Reygaert, 2023).
Isolating and monitoring microorganisms in landfills is also essential for environmental studies, as well as gram-negative bacteria as indicators of leachate contamination with organic pollutants and heavy metals, as indicators of landfill functionality and condition. Given the known pathogenicity and increasing antibiotic resistance of these bacteria, understanding their presence and behavior in landfill leachate is crucial for developing effective strategies for leachate management and environmental protection (Antic, Cepic, Stosic, & Radonic, 2021).
This study was conducted to determine the gram-negative bacteria present in the sanitary landfill of Koronadal City, South Cotabato, Philippines. This study seeks to provide data that may be
of use in the improvement of waste disposal practices in the city. This study may also render data on the proper assessment of public health and environmental sustainability in Koronadal City.

Objectives

This study aimed to determine the gram- negative bacteria present in the sanitary landfill of Koronadal City, South Cotabato, Philippines Specifically, it aims to:

- isolate gram-negative bacteria from various sampling points within the sanitary landfill in Barangay Paraiso, Koronadal City;
- identify the isolated gram-negative bacteria in the sanitary landfill of Koronadal City using morphological analysis and API 20E Analysis Kit; and
- determine the physico-chemical characteristics of the soil, including pH, phosphorus, potassium, and electrical conductivity, within the sanitary landfill in Barangay Paraiso, Koronadal City.

Methods

Soil Sample Collection and Preparation

The sampling site was initially surveyed for the parameter with waste products that are visibly degraded. The area was divided into triangular to obtain enough comparable samples from areas of consistent size and shape. Using a sterile spatula, ten (10) grams of soil was collected from the upper ten (10) centimeters of the ground surface. Four (4) samples of ten (10) grams each was collected from each of the quadrants. The samples were placed in plastic resealable bags (Ziploc, 8" x 3"x 19") and placed in a cooler with ice packs. The low-temperature environment preserved the soil samples by minimizing biological, chemical, or physical changes that could occur between the time of collection and analysis. Forty (40) grams of soil samples were acquired from the sampling site. These were brought to the St. Alexius College-Microbiology Laboratory working laboratory within 4 hours, where it was stored at 4°C in a low-cool refrigerator until the samples were subjected to further tests and analysis.

Physicochemical analysis of soil in the sanitary landfill

Soil samples were collected from 3 sampling sites (triangular) within the Cell 2 landfill at a depth of 10 cm, ensuring a representative distribution. The parameters determined were pH, organic materials (NPK), and electrical conductivity. The location, date, and time of sampling were also noted and the soil samples where be sent to Provincial Soils and Water Laboratory of Sultan Kudarat.

Isolation of Gram-Negative Bacteria

A serial dilution technique was used to obtain viable colonies. Ninety (90) milliliters of 0.9% physiological saline diluent was placed in dilution bottles (160 mL, Corning) and sterilized at 121 ºC for 15 minutes. The soil samples from each quadrant were mixed homogeneously in a plastic resealable bag (Ziploc, 8" x 3" x 19"), yielding a total of fifty (40) grams. Ten (10) grams of soil sample was added into the ninety (90) mL 0.9% physiological saline diluent and was labeled A, B, C, and D, corresponding to the specific quadrant of the sampling site. This constituted the 1:10 dilution ratio. The dilution bottles were shaken fifteen (15) times in an up- and-down motion to attain a good distribution and break bacterial clumps. One (1) mL of aliquot from the 1:10 dilution was transferred to a nine (9) mL diluent (Pyrex, 18x150 mm) using a micropipette to make a 1:10 dilution. The same procedure was repeated until a dilution of 10-7 is reached (Harley et al., 2002). The dilution tubes were shaken using a Vortex Mixer to ensure a homogeneous distribution. From the test tube containing 1:10 dilution, one (1) mL of the aliquot sample was transferred into a sterile Petri dish (Pyrex 90x15mm). The same procedures were carried out for the succeeding dilutions. When the solution was made, a sterile inoculation loop was used to do a streak plate method to avoid overgrowth of the bacteria on the media plates. The plates were allowed to rest and to be placed inside the incubator at 36 ± 1 °C for 24 to 72 hours and, after, ready for colony spotting.

Morphological Identification of Gram-Negative Bacteria

The following procedures were based on the identification schemes presented in Bailey's and Scotts Diagnostic in Microbiology (2021, 16th Edition).

Biochemical Identification of Gram-Negative Bacteria

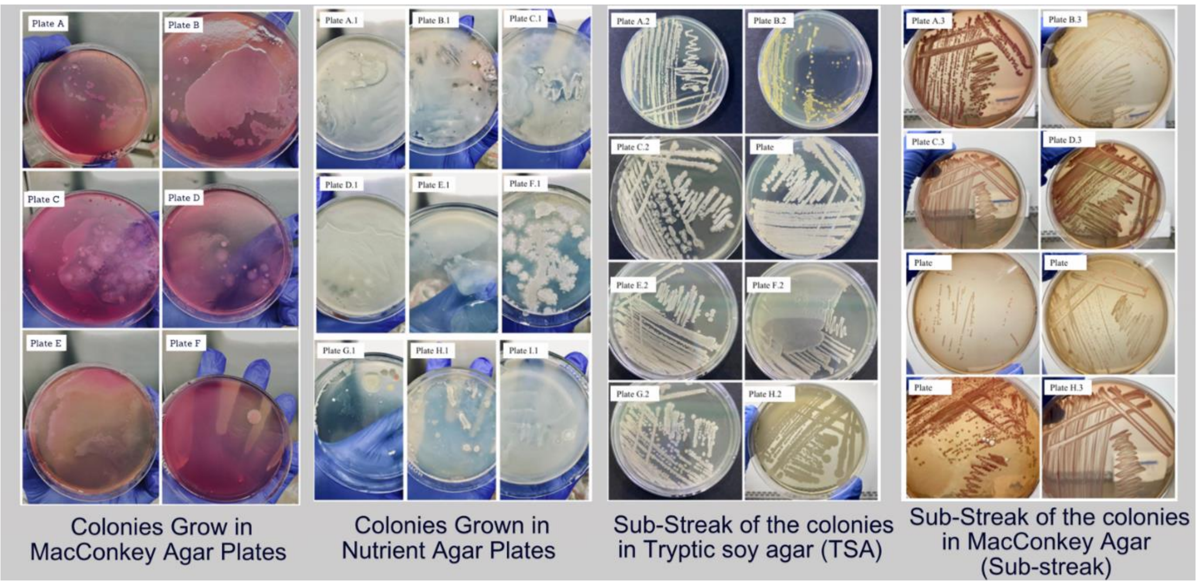
API 20E kit was used to identify the Gram-Negative bacteria isolated from the soils of the sanitary landfill. The oxidase test is a crucial component of bacterial identification, requiring adherence to the manufacturer's guidelines. The test result is essential for the final profile, serving as the 21st identification test.

To prepare, an incubation box with humidified wells is set up, and the strain reference is recorded on the tray's flap. A sterile saline or distilled water suspension of a single, well-isolated bacterial colony is created. This suspension is then distributed into the strip's tubes, with specific tests requiring mineral oil overlay for anaerobic conditions.

After incubation, the strip is read, and tests requiring reagents (TDA, IND, VP) are revealed. If fewer than three tests are positive initially, the strip is reincubated for an additional 24 hours. The numerical profile is determined by assigning values to positive reactions within groups of three tests. This profile, along with the oxidase test result, is used for identification through software like APIWEB™ or ATB™ NEW. If the identification is inconclusive, supplementary tests or reference materials may be necessary.

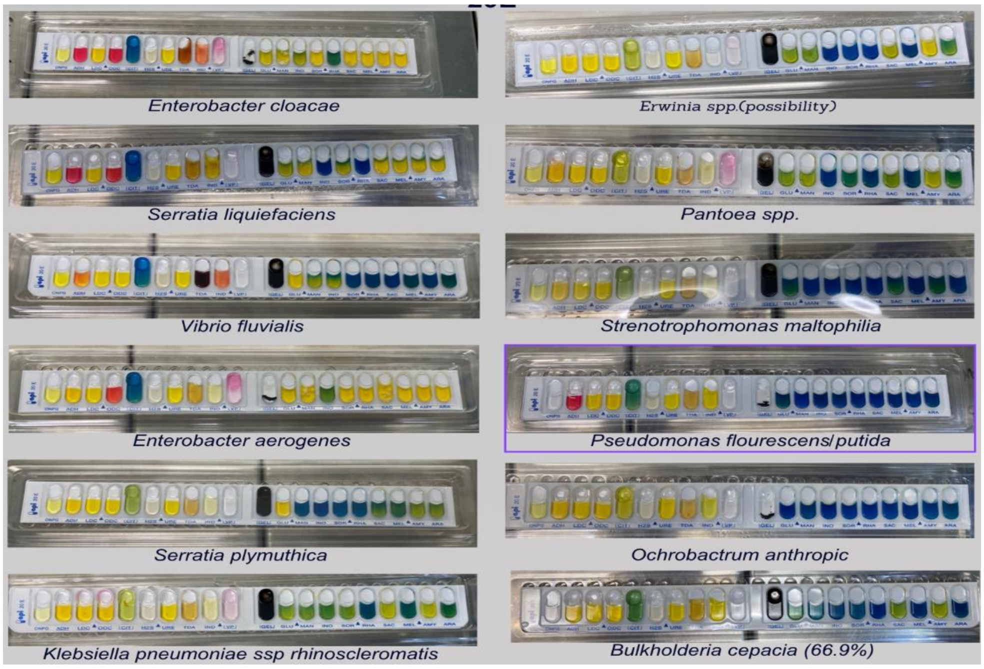
Results and Discussions

A. Morphological characteristics of every colony



A total of 15 colonies were isolated from the different culture media used in the study. The colonies were morphologically identified based on size, shape, color, texture, elevation, margin, and opacity. it shows a great colony of every media and they were examined according to their morphological characteristics based on their form, elevation, margin, and color. It seems that most of them are single, irregular, spreading, circular, and convex, and small. In terms of color pigmentation are white, off-white, yellow, and pink.

B. Identification of Bacteria using Analytical Profile Index 20E



Analytical profile index for the gram-negative bacteria test was used to confirm the identification of all the bacteria that were isolated. Gram-negative bacteria possess a periplasmic space between the inner and outer membranes, filled with enzymes and proteins involved in nutrient transport and defense mechanisms. The outer membrane also contains porins, which regulate the passage of molecules. Unlike gram-positive bacteria, gram-negative species lack teichoic acids but may have pili or fimbriae for attachment and genetic exchange via conjugation. These features enhance their adaptability in various environments, including oxygen-rich and oxygen-deprived conditions (Rogers, 2025).
According to Nakazato et al; (2022), the molecular characterization of gram- negative bacteria involves understanding their genetic makeup and how it contributes to antimicrobial resistance and virulence. This includes studying the mechanisms of resistance, such as efflux pumps and beta-lactamase enzymes, which are common in many genera of gram-negative bacteria like Escherichia coli and Pseudomonas aeruginosa. The diversity of gram-negative bacteria is vast, with various genera exhibiting different levels of resistance and virulence such as E. coli is a well-studied model organism, while P. aeruginosa is notorious for its resistance in hospital settings. Soil samples were collected from the sanitary landfill of the City of Koronadal. Using the API 20E kit, a diverse species of gram-negative bacteria was isolated from MacConkey, Tryptic Soy, and Nutrient Agar. There are 12 gram-negative bacteria from Soil samples were collected from the sanitary landfill of the City of Koronadal using the API 20E kit, a diverse species of gram-negative bacteria was isolated.

C. Soil Sample Analysis

Soil Test Report of Sanitary Landfill of Koronadal City

	MACRONUTRIENT					
FIELD NAME	**pH**		**N (as OM)**	**P**	**K**	**ELECTRICAL CONDUCTIVITY**
	value	**classification**	**%**	**ppm**	**ppm**	**uS/cm**
T1	7.6	slightly alkaline	L	H	S	233.0
T2	8.0	moderately alkaline	L	H	S	121.6
T3	8.0	moderately alkaline	L	H	S	120.2
L-Low ML-Moderately Low M-Medium MH-Moderately High H-High VH-Very High S-Sufficient						

In this table discusses the soil quality analysis of Sanitary Landfill in Brgy. Paraiso, Koronadal City, South Cotabato about their physical and chemical properties. The results from the three soil samples collected on November 11, 2024, revealed significant findings regarding pH levels, macronutrient availability, and electrical conductivity (EC), all of which can influence the microbial community structure and function in the soil. The alkaline pH, high phosphorus, sufficient potassium, and moderate salinity create an environment that is relatively favorable for Gram-negative bacteria. Their adaptive traits, including a protective outer membrane, metabolic versatility, and stress response mechanisms, enable them to survive and potentially thrive under these conditions. Moreover, their ability to degrade organic matter and recycle nutrients supports their growth and contributes to the dynamic soil ecosystem of the landfill.

Conclusions

This study aims to isolate and identify of gram-negative bacteria in the soil samples of the Koronadal City sanitary landfill. From the findings of the study, it shows a great colony of every media and they were examined according to their morphological characteristics based on their form, elevation, margin, and color. It seems that most of them are single, irregular, spreading, circular, and convex, and small. In terms of color pigmentation are white, off-white, yellow, and pink. The isolated bacteria were identified using the Analytical Profile Index 20E and it was found out that there are eleven (11) gram-negative bacterias' namely *Enterobacter cloaceae, Serratia liquiefaciens, Enterobacter aerogenes, Serratia plymuthica, Klebsiella pneumoniae ssp rhinoscleromatis, Erwinia spp., Strenotrophomonas maltophilia, Vibrio fluvialis, Pseudomonas flourescens/putida, Pasteurella pnuemotropica/Mannheimia, Bulkholderia cepacia, and Pseudomonas luteola.* For soil analysis, the alkaline pH of 7.87, high phosphorus, sufficient potassium, and moderate salinity of 158.27 µS/cm can create an environment that is relatively favorable for Gram-negative bacteria. Their adaptive traits, including a protective outer membrane, metabolic versatility, and stress response mechanisms, enable them to survive and potentially thrive under these conditions. Some of these bacteria are known human pathogens, and this raises concerns about the potential health implications of increased waste pollution, as it may lead to an increase in the abundance of these pathogenic bacteria. However, there is potential to genetically engineer these bacteria to eliminate their pathogenic properties while enhancing their plastic-waste degrading capabilities.

Recommendations

Based on the findings of the study that isolated and identified gram-negative bacteria from soil samples at the Koronadal City sanitary landfill, several recommendations can be made for various stakeholders, including CENRO (Community Environment and Natural Resources Office), local communities, and future researchers.

A. For CENRO (CENRO (Community Environment and Natural Resources Office)

1. Implement regular monitoring of microbial populations in landfills to assess the presence of pathogenic bacteria. Intensify the guidelines for waste management practices that minimize the risk of microbial contamination as anchored to RA 9003 (Solid Waste Management Act of 2000).
2. Educate the community about the potential health risks associated with waste pollution and the presence of pathogenic bacteria in landfills by conducting workshops, informational brochures, and community meetings.
3. Allocate resources for further research into microbial ecology in waste environments, focusing on both pathogenic and beneficial microorganisms and will help in understanding their roles in waste degradation and public health.

B. For the Community

1. Encourage community members to practice proper waste segregation to reduce organic waste that can foster bacterial growth.
2. Promote composting and recycling initiatives to manage waste effectively.
3. Advise residents living near landfills to take precautions, such as avoiding direct contact with soil or any bodies of water around the landfill and ensuring proper sanitation practices to minimize health risks.

C. For Future Researchers

1. Investigate the genetic mechanisms of the identified bacteria, particularly those with potential for bioremediation. Research could focus on genetically engineering these bacteria to enhance their plastic-degrading capabilities while reducing their pathogenic properties.

2. Further explore the pathogenic potential of isolated strains, particularly those known to cause human infections. Understanding their resistance mechanisms can inform treatment strategies and public health policies.
3. Future researchers can also utilize the gram staining, antimicrobial test, and DNA extraction for further validation of identified bacteria.

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