ISOLATION, SCREENING AND BIOSAFETY EVALUATION OF *Escherichia coli* ISOLATED FROM ABATTOIR SOIL FOR BIOPLASTIC PRODUCTION AND PLANT GROWTH PROMO TING POTENTIAL

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GOU/U22/MCB/530

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE S, FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES, GODFREY OKOY E UNIVERSITY, UGWUOMU NIKE, ENUGU. ENUGU STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHEL OR OF SCIENCE (B.Sc), DEGREE IN MICROBIOLOGY

SUPERVISOR: DR FRANCES .N. OLISAKA

JULY, 2026

APPROVAL

This research titled "**Isolation, Screening and Biosafety Evaluation of *Escherichia coli* Isolated from Abattoir Soil for Bioplastic Production and Plant Growth Promoting Potential**" has been assessed and approved by the committees of Department of Microbiology and Faculty of Natural sciences and Environmental Studies, Godfrey Okoye University, Thinkers Corner, Enugu Nigeria.

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CERTIFICATION

This attests to the fact that the study named "**Isolation, Screening and Biosafety Evaluation of *Escherichia coli* Isolated from Abattoir Soil for Bioplastic Production and Plant Growth Promoting Potential**" was carried out by Detso, Doochivir Joy with the Registration Number GOU/U22/MCB/530 under the supervision in the Department of Biological Sciences Godfrey Okoye University Enugu Nigeria.

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GOU/U22/MCB/530

DEDICATION

I give all glory to God Almighty in this work, for giving me the grace and strength to overc ome all the challenges that were encountered during the study.

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ABSTRACT

The demand for sustainable agricultural practices and the growing problem associated with non-biodegradable waste has gained attention globally and has made the search for microbes possessing dual biotechnological applications necessary. This study was aimed to isolate and screen potential bioplastic producing bacteria possessing plant growth promoting potentials from abattoir soils. Soil samples were collected aseptically and then microorganisms were isolated using standard cultural and biochemical techniques. The isolates were screened for intra cellular bioplastic production detecting Polyhydroxyl alkanoates accumulation using Sudan black B stain. Phosphate solubilization ability was assessed using Pikovaskaya agar, while antibiotic testing was carried out using the Kirby-Bauer disc diffusion method. The bacterial counts ranged from 2.12×10^4 to 18.6×10^4 CFU/g. *E.coli* was detected in all samples. Multidrug Antibiotic index ranged from 0.25 to 0.83. All isolates were 100% resistant to Cloxacillin (30µg), Erythromycine (30µg), Ciproflaxacin (30µg), Ampicillin (30µg) and non were resistant to Gentamicin (15µg). The findings showed that some *E. coli* isolates had intraclular bioplastics abilities while exhibiting plant growth promoting traits. This study provides valuable information that abattoir soils serve as reservoirs of bacteria strains with industrial agricultural applications.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plastics, whether synthetic or semisynthetic are widely utilized in everyday life owing to t he unique physical as well as thermal properties along with remarkable stability which al lows for a vast application. Extensive application of plastic waste and products in our liv es and the environment have raised major problems on health and also the environment. Plastics have adversely impacted about 700 endangered and other species (Lokesh *et a l.*, 2023).

Plastic pollution is now a prominent and growing environmental hazard with countless t ons of plastic waste accumulating in landfills, oceans and rivers.

Traditional plastic, sourced from petroleum, do not biodegrade in nature and can last for hundreds of years in the ecosystem posing potential hazards. Bio plastics are alternative biodegradable materials that come from renewable resources and have ecofriendly appli cations (Zhou *et al.*, 2023). Among several types of bio plastics, PHAs (Polyhydroxyalka noates), which accumulate in intracellular materials as storage compounds within vario us microorganisms are more promising as their physical properties resembles those of p etroleum derived plastics (Borrelle *et al.*, 2020). Current commercial Polyhydroxylalkanoa tes production suffers due to high cost of raw materials and the necessity of highly spec ialized strains of bacteria.

Thus, efforts to discover an effective Polyhydroxylalkanoates producing strains are need ed.

Abattoirs soil are polluted with variety of organic compounds and a heterogeneous popu lation of micro organisms that could be used as a source of plastic like material produce r microbes(Geyer *et al.*, 2017). Soil ecosystems in such sites contains microbes that are able to cope with pollutants and nutrient stress which in turn could improve efficiency of biopolymer production. Studies in wastewater, sludge and polluted soils have revealed th e presence of polyhydroxyalkanoate producing bacteria (Kane *et al*, Larue *et al.*, 2021).

The global agricultural sector is under increasing pressure to meet the demands of a gro wing population while simultaneously minimizing its environmental footprint. Traditiona l farming practices, heavily reliant on synthetic fertilizers and pesticides, have led to conc erns about soil health degradation, water pollution, and the development of pest resistan ce. In this context, there's a significant and growing interest in developing sustainable an d eco-friendly alternatives to enhance crop productivity and resilience. One promising av enue lies in harnessing the power of beneficial microorganisms, naturally occurring bact eria and fungi that can form symbiotic relationships with plants, offering a range of adv antages from improved nutrient acquisition to enhanced disease resistance.

1.2 Aim and Objectives of the Study

1.2.1 Aim

The aim of this research is to isolate and screen bioplastic producing and phosphate sol ubilizing bacteria from abbatoir soil sample.

1.2.2 Special objectives

1. Isolate of bacteria from abbatoir soil samples using conventional microbiological methods.

2. Screen of bacteria for bioplastic (polyhydroxyalkanoate, PHA) production using st aining Sudan black B.
3. Screen the bacteria isolates for ability to solubilize phosphate
4. Determination of the pattern of antibiotics susceptibility for isolates of the bacteri a.

CHAPTER TWO

LITERATURE REVIEW

2.1 Polyhydroxylbutyrate producing bacteria in soil

Soil, the uppermost layer of the Earth's crust, hosts living and non-living organisms and p lays many essential and natural functions (Nwaugo, 2008). Microbes are incredibly tiny units of life, appearing as single cells or colonies. Many reside in the top few centimeters of soil where food is readily available, as opposed to the deeper subsoil.

Microbes are particularly abundant near plant roots, a zone known as the rhizosphere, w here root cells and chemicals sloughed off provide a consistent food source (Panigrahi a nd Badveli, 2013).

Though often perceived as pathogens, microbes have been integral to soil since its gene sis. Despite making up less than 1% of soil organisms, a small sample of soil teems with microbes that enhance fertility and stimulate plant growth. The number of specific micro bial species present is influenced by soil conditions that can be either favorable or unfav

orable to them (Yasser *et al.*, 2020). For example, Polyhydroxylbutyrate-producing bacteri a found in mangrove rhizospheres differ significantly from those in marine environment s.

A wide variety of bacterial strains were linked to the accumulation of Polyhydroxylbutyra te among both gram-positive and gram-negative bacteria, as well as photosynthetic bact eria such as Cyanobacteria (Sim-Cabrera *et al.*, 2021).

Examples of Polyhydroxylbutyrate-synthesizing bacteria include *Pseudomonas, Bacillus, Citrobacter, Enterobacter, Klebsiella,* and *Escherichia* (Pradeep *et al.*, 2021).

2.2 Properties of Polyhydroxylbutyrate

Polyhydroxylbutyrate (PHB) is a type of bioplastic that is environmentally friendly due to its decreased persistence in the environment when disposed of (Carpine *et al.*, 2020). Th ese bioplastics are also non-toxic and compostable, meaning they pose no threat to livin g beings (Boura *et al.*, 2021). Bioplastic packaging materials effectively shield products f rom environmental damage and maintain their quality (Ibrahim *et al.*, 2021).

The food industry is the largest consumer of bioplastic packaging for a variety of applic ations, including food and beverage packaging, healthcare, and cosmetics.

Food packaging is the art and science of containing food products for their safe delivery to consumers in pristine condition (Ibrahim *et al.*, 2021). Polyhydroxylbutyrate is frequent ly utilized in cosmetic packaging because it is harmless upon contact with the skin, gene rates fewer greenhouse gases, exhibits high biodegradability, and is highly compatible wi th diverse environments (Ibrahim *et al.*, 2021).

2.3 Production of polyhydroxylbutyrate commercially

Polyhydroxylbutyrate can be commercially produced through the extraction and purification of polyester from bacteria by optimizing microbial fermentation of sugars, such as glucose (Carpine *et al*., 2020).

2.3.1 Techniques used for detecting polyhydroxylbutyrate

I. Sudan black B staining method of screening

According to Amish *et al*. (2013) and Wala'a *et al*. (2020), bacterial isolates were cultured on nutrient agar media for three days at 70°C. The Sudan black B solution was applied to stain the colonies on the plates, and the plates were left undisturbed for 30 minutes. After this period, excess dye was removed, and the plates were gently rinsed with 100% ethanol. White colonies indicate that they cannot incorporate Sudan black B, while bluish-black coloration suggests the presence of Polyhydroxylbutyrate; this is considered a presumptive test (Yasser *et al*., 2020).

2.3.2 Methods of polyhydroxylbutyrate extraction

Extraction procedures typically involve cell wall lysis, solubilization, purification of Polyhydroxylbutyrate, and precipitation of the polymer. Common techniques for extracting Polyhydroxylbutyrate from microbial biomass include solvent extraction, and chemical and enzymatic digestion. Solvent extraction is the most widely adopted and established method for obtaining high-purity Polyhydroxylbutyrate from biomass.

In a study by Folino *et al*. (2020), methylene chloride, chloroform, and 1,2-dichloroethane solvents were evaluated for Polyhydroxylbutyrate extraction.

Following solvent extraction, filtration was performed, and rotary evaporation was used to concentrate the Polyhydroxylbutyrate polymer solution, which was then precipitated by

the gradual addition of ice-cold ethanol. The main advantages of solvent extraction are t he high recovery of pure Polyhydroxylbutyrate; however, the disadvantages include signif icant drawbacks, high costs, and environmental impact due to toxic waste generation. One of the approaches to get this done is using of waste solvent in extraction of polyhyd roxybutyrate. Sodium hydroxide is applied in chemical extraction in order to make solubil ity of the polyhydroxybutyrate content which will lead to Separation of polyhydroxybutyr ate from other biomass after extraction (Zhou *et al.*, 2021). While simple and effective it does not guarantee high molecular mass of polyhydroxybutyrate as there may be severe destruction due to sodium hypochlorite (Zhou *et al.*, 2021). A hybrid strategy where there is distribution of sodium hypochlorite with chloroform has overcome this problem by for ming a composite which not only solves problem of hypochlorite treatment of cell but at the same time gives a combined advantage of protecting it from degradation after the re leased polyhydroxybutyrate.

While chemical extraction method using NaOCl leads to severe damage, the enzyme trea tment for digestion results in negligible loss or destruction of polymers and provides very mild operating conditions (Abate *et al.*, 2022). A biomass sample is subjected to a speci alized medium and heated to the optimum temperatures for enzyme to act and thereafte r polyhydroxybutyrate content is separated (Abate *et al.*, 2022).

2.4. Disadvantages of bioplastics

Some major limitations of using bioplastics are

i) High cost: they are often twice as expensive as petroleum plastics although this is exp

ected to change with greater industrial production of plastics on large scale as new cost-effective production methods will come up.

ii) Contamination of recycling streams: they need to be separated to avoid contaminatio n of the standard plastic recycling stream as contamination would impact negatively on recycling of plastics.

iii) Raw material scarcity: production from renewable resources could lead to depletion o f raw material and increased pressure on agricultural land for food. It might increase ene rgy demand during the production process of plastics, competing for raw materials used for production of food and other raw material needs.

iv) Misleading claims: The term "compostable" for bioplastics is misleading as they do n ot degrade readily at household conditions unlike organic food wastes and often require industrial composting. In some instances, manufacturers misuse bioplastic terms in thei r labels, claiming their products as 'environment friendly,' 'non-toxic', or 'fully degradable', which are all lies designed to deceive an unwary consumer (Filho *et al.*, 2021; Bandini *et al.*, 2022).

2.5 Applications of bioplastics

The application of bioplastics in food packaging has a wide array of usages ranging fro m wrap for snacks and confectionaries to films for covering fresh produce and sandwich es, for food packaging for bakery and pasta products (Sidek *et al.*, 2019). The usage of b ioplastics is not only restricted to the food packaging sector but extends to numerous ot her applications. These other applications include usage in construction sector as altern ative for timber and for household use as decking and fencing, as well as for constructio

n of a wide range of building applications (Bandopadhay *et al.*, 2020). Electro acoustic d evices producers use bioplastics in for creating high quality sound components and me mbranes (Bauer *et al.*, 2021). A study demonstrated that there are biopolymers for almos t all packaging and packaging applications except for items that requires significant gas barrier properties and applications that may demand excellent heat and chemical resista nce. Critical properties of food packaging include oxygen barrier properties (OBP), moist ure barrier properties (MBP), mechanical properties. Raw meat degradation is commonly initiated by pathogenic bacteria, necessitating excellent OBP, therefore vacuum packagin g and active packaging incorporating oxygen scavengers are ideal to maintain raw meat colour, but for fat and oil oxidation and microbes growth, materials with lower OBP coupl ed with light barrier and optimum water release rates to prevent food flavour from fruits and vegetable absorption and potential safety issues should be applied (Coppola *et al.*, 2021). PHB is a body-compatible biopolymer making it possible to apply them for medic al applications including in ocular devices, surgical sutures and in wound dressing (Kavit ha *et al.*, 2018). PHB biopolymer has potential for application in heteropolymers producti on; by extrusion moulding spinning and into food packaging films, and this is distinct fro m plastic films because these have good oxygen permeability, excellent tensile strength and significant antimicrobial activity as well as water vapour transmission rate (WVTR) and high antioxidant activity (Varghese *et al.*, 2022). However, bioplastic application is ra ther confined for agriculture purposes so far. The main areas investigated for application in agriculture is the production of mulch films for managing nuisance weeds and retain moisture in the soil (Varghese *et al.*, 2022). However, 40% of mulch films applied

for agricultural practice in the world is from plastic mulch (Varghese *et al.*, 2022). Agricul tural applications also includes the manufacture of grow bags for water treatment which help in managing water pollution by filtering nutrients and the prevention of plant root re formation by controlling overgrowth of plant roots. Farm nets are made up of the biodegr adable polyhydroxylalkanoates, since polyhydroxyalkanoates is compostable to a greate r extent, their use makes easier direct deposit of the farm net into the soil, this is unlike of other traditional plastic materials that contribute to solid waste (Varghese *et al.*, 2022). P olyhydroxyalkanoate and Polyhydroxylbutyrate based blend of plastics has a better perf ormance and can be widely applied in manufacturing of nets. High mechanical strength of polyhydroxyalkanoate/ Polyhydroxylbutyrate has been proven to increase tensile prop erties when they are made as an agricultural net (Varghese *et al.*, 2022). The high strengt h of these plastics, coupled with their flexibility makes it possible to form them into desir ed shapes and structures required for various agricultural applications (Varghese *et al.*, 2 022).

2.6 Challenges to Industrial sale

Production of Polyhydroxylbutyrate Polyhydroxylbutyrate is a class of biopolymer which i s unique, thus the production of polyhydroxylbutyrate poses industrial opportunities. Ther e are several issues of commercializing the polyhydroxylbutyrate biopolymer. One is prod ucing the material without harming the human food supply, although this issue can be ad dressed by utilizing other sources (Bauer *et al.*, 2021). The commercial viability of polyhy droxylalkanates in its application as replacement for conventional plastic is hindered by t

he relatively high cost of production (Gonzalez *et al*., 2020). Although research efforts are ongoing to minimize this constraint there are two bottleneck challenges. Firstly, substrate source can have a significant impact on the composition and quality of the biopolym er. Various substrates like xylene, acetate or glycerol have been used, which are costly (V arghese *et al*., 2022). However, the cost of carbon source could be

greatly reduced if waste water or low cost materials are utilized (Varghese *et al*., 2022). S econdly, bacterial selection is another key factor contributing to the cost of polyhydroxylb utyrate biopolymer. This can also be lowered if mixed microbial populations like the ones found in active sludge from wastewater treatment facilities are utilized (Mesquita *et al*., 2 015). This approach may lead to the saving of energy and minimize expenditure required in microbiological research and analysis (Mesquita *et al*, 2015). Secondly, the recovery m ethod for polyhydroxylbutyrate is presently considered the biggest challenge in order to re cover the biopolymer efficiently with desired purity, while maintaining its thermal, mechan ical, and molecular weight (Moshood *et al*, 2021). There are other constraints limiting scal ability. Key issues such as lowering of melting and glass transition temperatures, reductio n of elastic modulus, tensile Strength, and elongation are of concern. This could be overc ome by using polyhydroxylbutyrate copolymers with improved characteristics and also by controlling feeding parameters and nutritional imbalance to ensure better microbial growt h, and also expression of polymerase gene to guarantee efficient synchronization of poly hydroxylbutyrate production to cell lysis (Sakthiselvan *et al*, 2022).

2.7 Concept of Bioplastics

Bioplastics refer to polymer materials manufactured from renewable, biological sources

such as starch, sugar, cellulose, and microbial biomass. These polymers can be either bi odegradable, compostable, or both, or they can be biobased but not necessarily biodegra dable, depending on their molecular structure. Research highlights bioplastics as signific ant materials for promoting sustainable and circular economies, as they decrease relianc e on fossil fuels and mitigate carbon emissions (Huang *et al.*, 2025; Rosenboom *et al.*, 2 022). However, the biodegradability of all bioplastics is a crucial and often misinterprete d aspect within policy-making and environmental discussions.

2.7.1 Classification of bioplastics

There are typically three main classes of bioplastics

1. Biodegradable and bio-based
2. Bio-based but non-biodegradable
3. Non-biodegradable and fossil-based

A study by Chebet (2026) further subdivided bioplastics into microbial-based, plant-base d, synthetic biopolymers and agro-waste-derived materials, illustrating the variety of raw material and production methods. This classification is crucial, as a polymer's ability to biodegrade is dictated more by its molecular structure rather than its biological source.

2.7.2 Raw materials for bioplastic production

Bioplastics are made from renewable feedstock such as, but not limited to:

1. Corn starch
2. Cassava
3. Sugarcane

4. Lignocellulosic biomass
5. Agricultural waste

Microorganisms can also be used in the production of polyhydroxyalkanoates (PHA) via fermentation. Lignocellulosic biomass has been cited as a promising feedstock for biop olymer and bio-based chemicals production due to its abundance and cost-effectiveness (Isikgor and Becer, 2016). Emerging research also looks at using organic waste for biopo lymer production, thereby contributing to better waste management and sustainability.

2.8 Production Methods of Bioplastics

Several techniques exist for the production of bioplastics, including

1. Microbial fermentation for PHA production
2. Chemical polymerization of bio-based monomers (like PLA)
3. Modification of natural polymers (such as starch)
4. Blending of biodegradable and non-biodegradable polymers

More advanced techniques are being explored to increase efficiency and the utilization o f waste-based materials through biotechnology (Huang *et al.*, 2025).

2.9 Types of Bioplastics

The most commonly found types are:

1. Polylactic Acid (PLA)
2. Polyhydroxyalkanoates (PHA)
3. Starch-based bioplastics
4. Polybutylene succinate (PBS)

Polylactic Acid is a popular choice for food packaging and biomedical applications, whil

e PHA is noted for its excellent biodegradability in the environment. However, the two ma terials behave differently when it comes to degradation rates under varying conditions (A li *et al.*, 2023).

2.9.1 Properties of Bioplastics

The performance characteristics of bioplastics differ greatly depending on the blend, pro cess and feedstock. The main properties include:

1. Biodegradability under certain conditions
2. Reduced greenhouse gas emissions relative to petroleum plastics
3. Moderate mechanical performance
4. Sensitivity to heat (especially PLA)
5. Potential for compostability (usually in industrial facilities)

The degree to which a bioplastic will degrade is strongly tied to the environmental setting it is exposed to (temperature, moisture, microorganisms, polymer structure), according to Shanmugam *et al* (2026).

2.10 Future Prospects

Growing awareness of the detrimental environmental effects of the mass production an d use of non-degradable materials is driving research into new types of biodegradable pl astics that can be extracted from bacteria and fungi. Innovation in this field has the pote ntial to significantly improve production efficiency, create new applications, and expand market opportunities for bioplastics (Varghese *et al.*, 2022). Increased global demand for sustainable solutions will also fuel the future bioplastic market.

Moreover, developments in microbial biotechnology offer significant avenues for comme

rcializing bioplastics across a range of sectors, including agriculture, medicine, pharmac euticals, and food (Bauer *et al.*, 2021).

Therefore, global guidelines and standards for the production, use, and disposal of biopl astics should be developed, and labeling should accurately reflect a product's source ma terial, energy inputs, and associated emissions. Technological advances, ongoing innova tion, and global collaboration are crucial to achieving successful commercialization and demonstration of bioplastics. Fundamentally, however, bioplastics must be developed us ing integrated environmentally friendly processes throughout their lifecycle to enhance o verall sustainability (Brizga *et al.*, 2022). Many countries are already incorporating biopla stics into their technologies (Ojha and Das, 2022); for example, McDonald's is utilizing bi odegradable packaging in its fast-food restaurants, and other large companies such as Bayer, DuPont, Dow Cargill, Nike, and Danone are also producing them (Arikan and Ozso y, 2015).

Given the benefits of bioplastics, vast amounts of materials and resources exist for creat ing and discovering additional uses.

For sustainability, however, the challenges and drawbacks associated with bioplastics, e specially concerning raw material sourcing, need careful consideration.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Soil samples were collected from two major abattoirs in Enugu State, Nigeria: Abakpa Market Abattoir and Old Artisan Market Abattoir. These locations were selected because they receive large quantities of animal wastes, blood, tissues, fats, and wastewater, creating a nutrient-rich environment that supports diverse microbial populations capable of producing valuable metabolites such as bioplastics and plant growth-promoting substances.

3.2 Sample Collection

Composite soil samples were collected aseptically from different points within each abattoir at a depth of approximately 5–10 cm using a sterile hand trowel. About 200 g of soil was collected from each site and transferred into sterile polyethylene bags. The samples were properly labelled, transported to the Microbiology Laboratory in an ice box, and processed within 24 hours of collection (Cappuccino and Welsh, 2017).

3.3 Isolation and Enumeration of Bacteria

One gram (1 g) of each soil sample was suspended in 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Serial ten-fold dilutions were prepared up to 10^{-6}.

From appropriate dilutions, 0.1 mL aliquots were inoculated onto Nutrient Agar (NA) plates using the spread plate technique. The plates were incubated at 37°C for 24 hours. After incubation, colonies appearing on plates containing 30–300 colonies were counted and expressed as colony-forming units per gram (CFU/g) of soil using the standard plate c

ount method (Cheesbrough, 2018).

Distinct colonies differing in colour, shape, elevation and margin were subcultured repeat edly on fresh Nutrient Agar plates until pure cultures were obtained. Pure isolates were m aintained on nutrient agar slants at 4°C for further analyses.

3.4 Identification of Bacterial Isolates

Pure bacterial isolates were identified based on their cultural characteristics, colony mor phology, Gram staining reaction and biochemical characteristics according to standard microbiological procedures (Cheesbrough, 2018; Cappuccino and Welsh, 2017).

The characteristics examined included:

Colony size

Colour

Margin

Elevation

Surface appearance

Gram reaction

Cell shape

Catalase test

Oxidase test

The results obtained were compared with standard identification keys for bacterial identi fication.

3.5 Catalase Test

Catalase activity was determined using the slide method. A drop of 3% hydrogen peroxid

e (H_2O_2) was placed on a clean grease-free glass slide. A sterile inoculating loop was use d to transfer a portion of a fresh bacterial colony into the hydrogen peroxide.

The immediate production of vigorous oxygen bubbles indicated a positive catalase reac tion, while the absence of bubble formation indicated a negative reaction (Cheesbrough, 2018).

3.6 Oxidase Test

The oxidase test was performed using oxidase reagent containing 1% tetramethyl-p-phe nylenediamine dihydrochloride.

A portion of a fresh bacterial colony was smeared onto filter paper impregnated with the oxidase reagent using a sterile wooden applicator stick. Development of a dark purple co lour within 10–30 seconds indicated a positive oxidase reaction, while no colour change after 30 seconds was regarded as a negative reaction (Cappuccino and Welsh, 2017).

3.7 Screening for Phosphate-Solubilizing Ability

The phosphate-solubilizing ability of the bacterial isolates was determined using Pikovs kaya's agar containing tricalcium phosphate (TCP) as the insoluble phosphate source.

Pure bacterial isolates were spot inoculated onto the centre of sterile Pikovskaya agar pl ates and incubated at 30°C for 5–7 days. Following incubation, the plates were examine d for the formation of clear halo zones surrounding the bacterial colonies, indicating pho sphate solubilization.

The diameter of the colony and the diameter of the clear halo zone were measured in mil limetres (mm) using a transparent ruler or Vernier caliper.

The Phosphate Solubilization Index (PSI) was calculated as:

PSI = (Colony diameter + Halo zone diameter) ÷ Colony diameter

Isolates that produced visible clear halo zones were regarded as phosphate-solubilizing (positive), while isolates without halo zones were regarded as negative (Kalayu, 2019).

CHAPTER FOUR

RESULTS

4.1 Cultural and biochemical test of *E. coli*

Table 4.1 presents the cultural characteristics, Gram reaction and biochemical test results of the bacterial isolates recovered from the abattoir soil samples

Table 4.1: Morphology and appearance results of isolates from both abattoirs.

Cultural Characteristics	Morphology	Motility	Gram Stain	Glucose	Fructose	Galactose	Lactose	Maltose	Catalase	Oxidase	Coagulase	Citrate	Indole	Methyl Red	Vp	Probable Organism
Irregular	Single Rods	-ve	+ve	+ Ve	A	A	+ve	A	+ve	+ve	-ve	+ve	-ve	-ve	+ve	*E. coli*
Entire Whitish	Cocci In Bunch	-ve	+ve	+ve	A	A	+ve	A	+ve	-ve	+ve	+ve	-ve	-ve	+ve	*E. coli*
Smooth Reddish	Paired Rods	+ve	-ve	+ve	A	A	A	A	+ve	-ve	-ve	-ve	+ve	+ve	+ve	*E. coli*
Creamy Reddish	Single Rods	+ve	-ve	-ve	A	A	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	*E. coli*
Irregular	Singles Rod	-ve	-ve	-ve	A	A	-ve	A	+ve	-ve	-ve	-ve	-ve	-ve	+ve	*E. coli*
Irregular	Singles Rod	-ve	-ve	-ve	A	A	A	A	-ve	-ve	-ve	-ve	+ve	-ve	+ve	*E. coli*
Dull Black	Singles Rod	-ve	-ve	A	A	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	*E. coli*
Pink	Singles Rod	+ve	-ve	-ve	A	A	-ve	A	-ve	-ve	-ve	+ve	+ve	-ve	+ve	*E. coli*
Entire Whitish	Single Rod	-ve	-ve	A	A	-ve	A	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	*E. coli*
Irregular	Singles Rod	-ve	-ve	-ve	A	A	A	A	-ve	-ve	-ve	-ve	+ve	-ve	+ve	*E. coli*
Dull Black	Singles Rod	-ve	-ve	A	A	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	*E. coli*

Legend: A = acid, +ve = positive, -ve = Negative

4.2 Microbial count of organisms from both abattoir samples on Nutrient agar

Table 4.2 shows the total viable bacterial counts of bacteria isolated from soil samples c ollected from Abakpa Market and Old Artisan Market abattoirs. The bacterial counts obt ained from the two sampling sites are presented in colony-forming units per gram (CFU/ g)

Table 4.2: Microbial table count on nutrient agar

Week 1	Samples	Nutrient Agar Count	Week 2	Samples	Nutrient Agar Count	Week 3	Samples	Nutrient Agar Count
	ArA1	15.1 x 10^4		ArA5	4.2 x 10^4		AbA9	18.6 x 10^4
	ArA2	13.3 x 10^4		ArA6	12.9 x 10^4		AbA10	7.8 x 10^4
	ArA3	8.7 x 10^4		ArA7	17.2 x 10^4		AbA11	14.6 x 10^4
	ArA4	2.12 x 10^4		ArA8	15.8 x 10^4		AbA12	8.8 x 10^4

Keyword: ArA= Artisan Abatooir, AbA=Abakpa Abbatoir.