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Bioremediation of Bisphenol A Using Different Types of Bacteria

A thesis Submitted to the Council of the Faculty of Veterinary Medicine / University of Kerbala in Partial Fulfillment of the Requirement for the Master Degree of Science in Veterinary Medicine /Veterinary Public Health.

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بسم الله الرحمن الرحيم

(*من عملَ صَالِحًا مِنْ ذَكَرٍ أَوْ أُنْثَى وَهُوَ مُؤْمِنٌ فَلَنُحْيِيَنَّهُ حَيَاةً طَيِّبَة ولنجزينهم اجرهم باحسن ماكانوا يعملون*)

صدق الله العلي العظيم

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I certify this thesis entitled (**Biodegradation of Bisphenol A by Different Types of Bacteria Isolated from Polluted Soils**) was carried out by (Fatima Shehab Hamad) has been prepared under our supervision at the College of Veterinary Medicine/University of Kerbala as a Partial Fulfilment of the requirements for the degree of Master in Science of Veterinary Medicine/ Veterinary Public Health .

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We, the members of the examining committee, certify that we have read this thesis entitled: **(Bioremediation of Bisphenol A Using Different Types of Bacteria)** presented by the (Fatima Shehab Hamad) from the Department of (Veterinary Public health) and we have examined the student in its contents. We have found that it is adequate for the award of the **Degree** of **Master** in **Science of Veterinary Medicine/ Public Health.**

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Dedication

To my dear husband:

I dedicate this research as an expression of my sincere love and thanks. Because of the moral and material support he provided me throughout my studies, he was a wonderful husband and friend.

Thank you....

My dictionaries do not contain a word of gratitude or an expression of gratitude that expresses what is in my heart, but perhaps my prayers knock on the doors of heaven and will be more eloquent than our fading letters at this moment. To my dear teacher, ***Dr. Khadim Saleh Khadim and, Dr. Juman Khaleel Al-Sabbagh.***

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Summary

Bisphenol A (BPA), also known as 2,2-bis(hydroxyphenyl) propane, is a synthetic substance extensively employed in the manufacturing of plastics, specifically polycarbonate and epoxy resins; over numerous years, BPA has been extensively distributed into soils, sediments, as well as ground and surface waters. This study aimed to gain a greater insight into BPA removal by studying the biodegradation ability of polluted soil's bacteria toward BPA.

In this study fifty samples were taken from polluted soils with plastic wastes of different sites of Holy Karbala province from (Center, Al-Hussainia and Aoun) regions and Babylon province from (Center and The Nile) regions; ten samples were taken from each region.

Minimal salt media (MSM) were used to identify the ability of these isolates to degrade BPA by using HPLC technique. After inoculation the bacterial isolates to the MSM with BPA as sole carbon source. The results obtained revealed that, only 31 isolates showed the ability to grow in this media

The results found that, there was no significant differences among the number of bacteria with BPA-biodegradability isolated from the regiones. In spite of that, the urban regiones (the center of Kerbala and Babylon) had a greater number of isolates with BPA-degradation compared with rural regiones (Al-Hussainia, Aoun, and Nile).

The results found that, the bacterial isolates could degrade the BPA in different ranges started from 18.7% to 99.9%. And there were significant differences of the ability of bacteria to remediate BPA such as *Serratia plymuthica*, *Pantoea spp*, *Shingomonas paucimobilis*, *Pseudomonas*

aeruginosa and *Bacillus spp*., comparing with other isolates of the recent study such as, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Escherichia coli* and *Proteus spp.,* according to the BPA biodegradation capacity.

After DNA purification, *bisdAB* operon were identified in BPA-degrading bacteria, and the results found that this gene found in the most isolates under this study. Some bacterial isolates yielded negative results for the *bisdAB* gene, indicating a potential absence of this specific metabolic pathway for BPA degradation in those bacteria; this observation suggested the possibility of an alternative BPA metabolic pathway existing in these bacterial strains.

Ten of bacterial isolates were subjected to identification by using sequencing for 16rRNA gene, and the results gave more precise identification for these bacterial isolates.

We conclude that, many bacterial isolates could convert the toxic organic compound Bisphenol A to other metabolite in the media-containing it as only carbon source. And according to recent study we could employee this activity to eliminate these materials safely from the polluted soils and water efficiently in comprainal with chemicals that might be toxic to environment, human being and animals. And finally, the operon of *bisAB* gene detected in most isolates and this gene suggested to involved in BPA degradation.

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List of abbreviations

Abbreviation	Meaning
BPA	Bisphenol A
EDCs	endocrine-disrupting chemicals
CYP	Cytochrome P450
HPLC	High Performance Liquid Chromatography
LOAEL	Lowest Observed Adverse Effect Level
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
ADI	acceptable daily intake
µg	Micro gram
kg	Kilo gram
MPs	microplastics
PE	polyethylene
PP	polypropylene
EDTA	Ethylene diamine tetra acetic acid
KD	Killodalton
^{o}C	Degrees Celsius
PCR	Polymerase chain reaction
TBE buffer	Tris Borate- EDTA- buffer

TCA	Tri-chloro acetic acid
µl	Micro liter
ml	Milliliter
TE buffer	Tris - EDTA buffer
dNTP	Deoxy nucleotide triphosphate
PCR	Poly Mares Chain Reaction
NPs	Non Plastic Substance
CD	Compact Disc
DVD	Digital Versatile Disc
ABT	Amino Benzo Triazole
Bis AB	Bisphenol Operon
Bis dfd	Bisphenol Ferredoxin
MSM	Minimal Salt Medium
DNA	Di Nucleic Acid

Chapter One

Introduction

1. Introduction:

Bisphenol A (BPA); 2,2-bis (hydroxyphenyl) propane is a man-made compound commonly used in the manufacture of plastics matrerials, in particular, epoxy resins and polycarbonate with an annual production exceeding 5.0 million tons in 2010 **(Huang *et al*., 2014).** For many decades, BPA has been intensively introduced into soils, sediments, ground and surface waters **(Careghini *et al.*, 2015).** Industrial and municipal wastewaters are the most common sources of BPA contamination in aquatic environments **(Gorga *et al.* 2015).**

In addition to natural environments, human and animals' exposure to BPA for adults and young was confirmed **(Lehmler *et al*. 2018).** In fact, BPA has been detected in serum, urine, tissue and blood of both occupationally and environmentally exposed individuals **(Asadgol *et al*. 2014; Ribeiro *et al.* 2017).**

It is noteworthy that COVID-19 pandemic has lead to increased the plastic use and environmental contamination by plastic as a result of the common use of masks, gloves and other plastic consumables; this has enormous effects on daily life not only regarding humans but also other animals **(Rochman *et al.,* 2019).** The distribution of plastics is ubiquitous in the environment and includes atmosphere, soil and water; this likely represents a potential entry of microplastics into the food chain and, therefore, a concern for human and animal health (Yee *et al*., 2021).

So, BPA had an acute toxicity toward aquatic organisms **(Canesi *et al.*, 2015).** As well as, it had been confirmed to be an endocrine-disrupting chemical (EDC) The estrogenic activity of BPA was first reported by Dodds and Lawson in (1936) and further supported by several studies **(Segner *et al*. 2003).** In fact, BPA influences growth, reproduction and biota development through interfering with the activities of several

endocrine hormones, specific intracellular signaling pathways, and epigenetic regulators **(Booth *et al.* 2016).**

Recent data also suggested that, the exposure to BPA causes hepatotoxic, immune-toxic, mutagenic and carcinogenic effects **(Michalowicz 2014).** The BPA significantly affected keratinocytes viability, induced apoptotic fraction and increased activation of DNA-damage marker protein in human and animals **(Son *et al.*, 2018).**

More and more BPA-degrading bacteria have been isolated from water, sediment, soil and wastewater treatment plants; these bacteria included both Gram-negative and Gram-positive strains **(Fouda 2015; Ebenezer and Ki 2016; Das *et al.* 2018).** Different BPA degradation pathways have been proposed in the literature according to the intermediates detected during the degradation process for specific bacterial strains **(Vijayalakshmi *et al.* 2018).**

Moreover, mammals' exposure to BPA may increase obesity, diabetes and heart diseases risks **(Shankar *et al.* 2012; Liu *et al.* 2017).** Thus, BPA removal from the natural environment is an increasing worldwide concern; several BPA transforming or/and degrading organisms such as bacteria, fungi, algae and plants were effectively used to remove BPA from the environment **(Michalowicz 2014; Caretto *et al.* 2015; Vijayalakshmi *et al.* 2018).**

Until now, most BPA biodegradation studies were conducted by single-strain or bacterial consortia isolated from sediments or aquatic environments but no studies were made for deserts and arid area's bacteria **(Kamaraj *et al.* 2014; Peng and Tarleton, 2015; Moussavi and Haddad 2019).**

Biodegradation of BPA by microorganisms has been proven to be a safe and economical approach to remove BPA from the environment **(Fu *et al*. 2019).** Cytochrome P450 (CYP) monooxygenase gene involved in

BPA degradation **(Jia *et al.* 2020).** Although BPA degradation pathways have been extensively studied, the knowledge of metabolic mechanisms, including catalysts and genes, was still limited **(Cajthaml, 2015).**

2. The Aim of This Study:

The aim of this study, was to gain a greater insight into BPA removal by studying the biodegradation ability of polluted soil's bacteria toward BPA. And this conducted through the following ways:

1- Isolation and identification of different types of bacteria from polluted soil with plastic residules.
2- Study the biodegradation activity of BPA by these bacteria using HPLC technique
3- Molecular detection of specific gene (*bisAB*) that may be responsible of biodegradation of BPA.

Chapter Two

Review of Related Literature

2. Review of Related Literature:

2.1. Environmental Pollution:

Pollution defined as, the introduction of the harmful materials into the environment; these harmful materials are called pollutants. So, pollutants could be natural, such as volcanic ash or, they could also be created by human activity, such as trash or runoff produced by factories **(Shetty *et al.*, 2023).** Pollutants damage the quality of air, water, and soils; many things that are useful to people produce pollution, as the cars spew pollutants from their exhaust pipes **(Mehmood *et al.*, 2023).**

Burning oil to create electricity pollutes the air; while, industries and homes generate garbage and sewage that could pollute the soils and water; pesticide chemical poisons which used to kill weeds and insects seep into waterways and harm wildlife; and when the resources were polluted, all forms of life are threatened to be affected **(Pathak *et al.*, 2022).** Pollution is a global problem; although urban areas are usually more polluted than the countryside, pollution can spread to remote places where no people live; for example, pesticides and other chemicals had been found in the Antarctic ice sheet; in the middle of the northern Pacific Ocean, a huge collection of microscopic plastic particles forms what is known as the Great Pacific Garbage Patch **(Manisalidis *et al.*, 2020).**

Air and water currents carry pollution; such as Ocean currents and migrating fish carry marine pollutants far and wide, winds could pick up radioactive material accidentally released from a nuclear reactor and scatter it around the world, smoke from a factory in one country drifts into another country; as well as, the three major types of pollution are air pollution, water pollution, and also, soil pollution **(Manucci and Franchini, 2017).**

2.2. The Soil Pollution:

Many of the same pollutants that foul the water also harm the soils; mining sometimes leaves the soil contaminated with dangerous chemicals **(Münzel *et al.*, 2023).** Pesticides and fertilizers from agricultural fields are blown by the wind; they could harm plants, animals, and sometimes people; also, some fruits and vegetables absorb the pesticides that help them grow; and when people and animals consume the fruits and vegetables, the pesticides enter their bodies; some pesticides can cause cancer and other diseases **(Costa *et al.*, 2019).**

Trash is another form of soil pollution; around the world, paper, cans, glass jars, plastic products, and junked cars and appliances mar the landscape; litter makes it difficult for plants and other producers in the food web to create nutrients; and animals can die if they mistakenly eat plastic; besides, garbage often contains dangerous pollutants such as plastic products, these pollutants can leech into the soil and harm plants, animals, and people **(Ferronato and Torretta, 2019).**

Sometimes, landfills are not completely sealed off from the land around them, pollutants from the landfill leak into the earth in which they are buried, plants that grow in the earth may be contaminated, and the herbivores that eat the plants also become contaminated **(Aryan *et al.*, 2019).** So do the predators that consume the herbivores; the process, where a chemical builds up in each level of the food web, is called bioaccumulation **(Tison *et al.*, 2024).**

Plastic is one of the major inorganic solid waste fractions in our daily municipal solid waste (MSW) production; ever-increasing population, rapid urbanization, and industrial advancement are some of the key driving factors for creating critical man-made problems that eventually threaten the existence of living beings on this earth **(Mazhandu *et al.*, 2020).**

Soil contamination with solid materials were considered one of the most important sources of Bisphenol A(BPA), contamination because high product from plastic materials in world **(Kirstein *et al.*, 2019).** Plastic bags, when they decompose, a major threat to soil quality and the wider ecosystem; when plastic deteriorates, it turns into microplastics, small particles that can remain in the soil for up to 1,000 years; these microplastics leach toxic substances such BPA, and phthalates into the soil **(Kasar *et al.*, 2020).** These chemicals have hormonal effects on both vertebrates and invertebrates, can cause inflammation, may cross biological barriers such as the blood-brain barrier; so, the presence of microplastics in soil can change the physical and chemical properties of soil, affecting plant growth and the health of soil fauna **(Antelava *et al.*, 2019).**

2.3. Bisphenol A (BPA):

Endocrine disruptors, identified as certain compounds, present dual hazards to public health and the environment; they disrupted the endocrine system's regulation of hormone release, consequently causing adverse effects on different types of the organisms **(Roncaglioni *et al.*, 2008).** A multitude of chemicals are encompassed within the realm of endocrine-disrupting compounds (EDCs) due to their ability to affect the endocrine system; foremost among these are plastics and plasticizers, serving as the principal disruptors encountered by organisms; their notably extended half-lives in natural settings promote accumulation and pollution of the soils, consequently posing adverse effects on ecosystems and endangering various life forms **(Morales *et al.*, 2015).**

Endocrine-disrupting compounds, as exogenous substances, which had the capacity to either stimulate or suppress endogenous hormone responses, thereby disrupting the reproductive and developmental processes of living organisms **(Czarny-Krzymińska *et al.*, 2023).**

Bisphenol A (BPA) serves as an endocrine-disrupting compound (EDC) by mimicking estrogen's function, resulting in disoerder of numerous vital organs; structurally, BPA comprises hydroxyphenyl propane, featuring two phenol groups substituted by hydroxyl groups in the para position, granting the chemical notable reactivity conducive to electrophilic substitution or conversion into ethers, esters, and salts **(Gingrich *et al.*, 2021).**

Consequently, over the past decade, the global production of BPA had steadily risen to satisfy increasing worldwide demand, coinciding with the surge in synthetic plastics production, which surpassed 368 million tons in 2019 **(Filho *et al.*, 2021).** By 2015, the annual production of BPA was approximated at 4.85 million tons **(Eladak *et al.*, 2015).**

Bisphenols constitute a class of compounds characterized by two phenyl rings linked together by a small connecting group **(Zühlke *et al.*, 2020),** as depicted in (Figure 2-1). Among these, the most extensively utilized in industrial applications is BPA, which was first identified in 1891 **(Tumu *et al.*, 2023).**

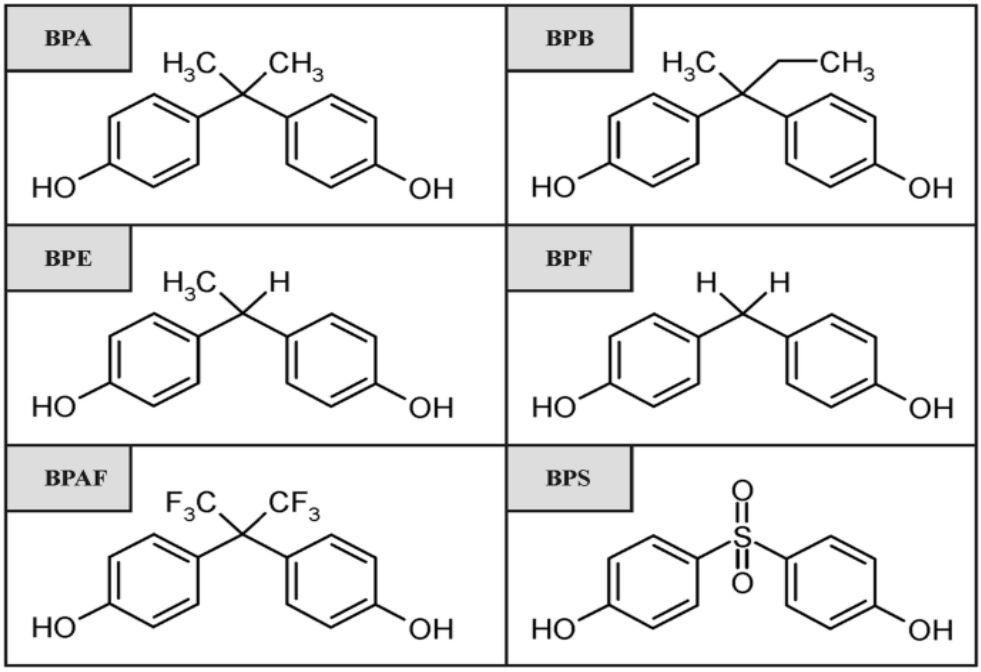


Figure (2-1): The types and structure of Bisphenols, (Zühlke *et al.*, 2020)

Bisphenol A (BPA) is an industrial chemical recognized as an environmental endocrine disruptor with estrogenic properties; it has extensive application in the production of consumer goods, including various plastics like polycarbonate and epoxy resin, as well as thermal paper; additionally, BPA can potentially leach from dental sealants **(Ramakrishna *et al.*, 2021).** Moreover, it is identified as a fundamental material utilized in the manufacturing of different types of plastics; discovered in 1891, BPA gained widespread use from around 1930 when scientists pioneered the creation of polycarbonate plastic using this compound **(Metz, 2016).**

Polycarbonate plastics and resins find applications in various everyday items like water bottles, milk bottles, baby bottles, plastic plates, paints, cups, coatings, primers, and packaging for dairy products; over the time, the BPA contained in these plastics can migrate from the packaging into the dairy products **(Yang *et al.*, 2018).**

Exposure to food occurs when these plastics are heated, used extensively, come into contact with acidic or alkaline substances, or are exposed to microwave radiation; this can result in the transfer of BPA into the food, leading to its ingestion; several studies have indicated that, such food contact can lead to the presence of non-conjugated BPA in the serum of adults and fetuses **(Almeida *et al.*, 2018).**

The consumption of animal and plant products containing BPA can potentially impact human health, as noted by **(Gorecki *et al.*, 2017).** However, there has been significant growth in global environmentally friendly technologies, which have shown promise in effectively mitigating pollutants and aiding in the preservation of the environment **(Li *et al.*, 2023, A).** Pollution

prevention and mitigation strategies, as outlined by **Lei *et al.*, (2021),** can be employed to address this issue; though, BPA, an organic pollutant, continues to be a significant environmental worry due to its extensive use in manufacturing processes; more than 90% of BPA is utilized as a monomer in the production of polycarbonate plastic and flame retardants **(Liu *et al.*, 2017).** Bisphenol A (BPA) serves as an intermediate chemical in numerous applications, finding wide usage across various industries **(Li *et al.*, 2021).**

2.3.1. The Effect of Bisphenol A On Enviroment:

Bisphenol A (BPA) has the potential to function as an environmental endocrine disruptor, which can lead to metabolic, reproductive, and/or developmental issues and diseases in both humans and animals **(Oh and Choi, 2019).** The global distribution of BPA in diverse environments has been recognized as a significant source of exposure for living organisms **(Matuszczak *et al.*, 2019).** The primary route of exposure to BPA is through ingestion, with subsequent accumulation in fatty tissues being a significant mechanism **(Manzoor *et al.*, 2022).**

Bisphenol A (BPA) exhibits acute toxicity in the animal kingdom and has mutagenic and estrogenic implications for their health at all **(Badiefar *et al.*, 2015).** Furthermore, BPA exerts estrogenic and genotoxic effects on the mammalians; given that, hormone levels in their bodies are maintained at biologically active concentrations; so, exposure to such exogenous chemicals, even in low doses, can disrupt the proper functioning of the body's endocrine system; the production of BPA has surged in recent decades due to its extensive use across various industries to meet the high global demand, which reached approximately 6.5 million tons in 2012 **(Larsson *et al.*, 2017).**

Animal-derived foods are significant contributors to human dietary exposure to Bisphenols (BPs) due to the high levels present in these food sources

(Liao and Kannan, 2014). While the migration of BPA from food-contact materials is a known pathway for BPA accumulation in animal-derived foods, it is not the sole mechanism; for instance, the presence of BPs in milk can be attributed in part to the feeding practices of dairy cows **(Russo *et al.*, 2019).** Figure (2-2).

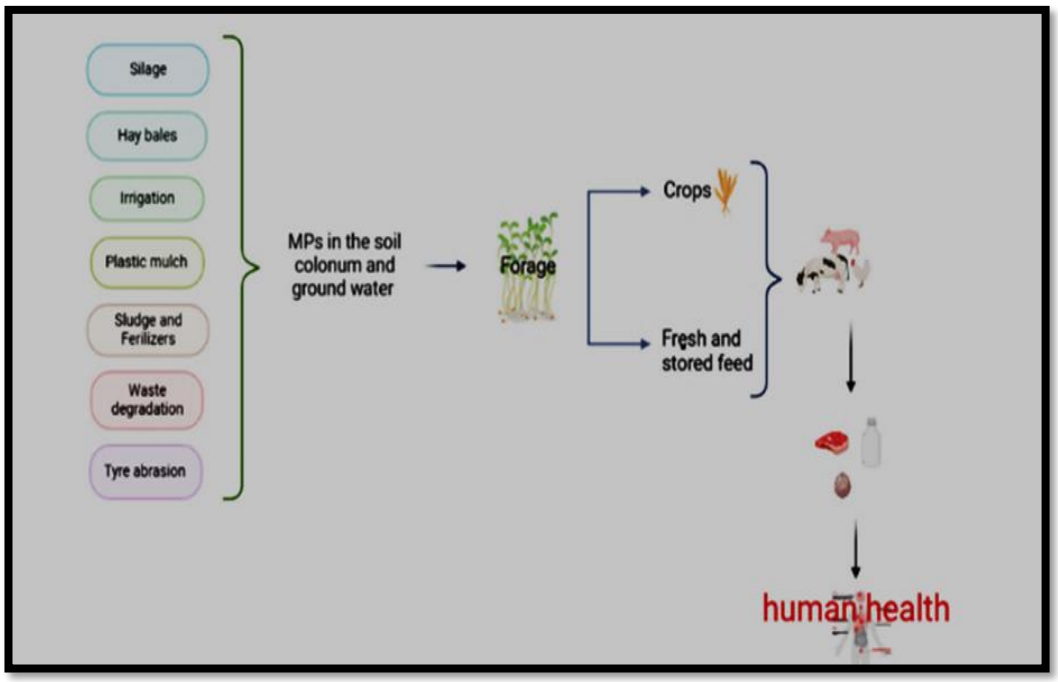


Figure (2-2): A simplified schematic representation of the sources and fate of plastic particles in soil (Urli *et al.*, 2023).

A study done by**(Lee, *2018*)**, found that, BPA consumed by lactating mothers has been detected in breast milk at concentrations as high as 43.2 ng g−1. Additionally, a separate study on food items discovered BPA contamination in non-packaged fresh meats like chicken, beef, and fish; this suggests the existence of potential contamination pathways beyond migration solely from food packaging **(Zhou *et al.*, 2019).**

Bisphenols have been identified in blood, urine, amniotic fluid, breast milk, and cord blood, underlining the widespread exposure animals experience to these substances; consequently, numerous studies have indicated that even at low concentrations, exposure to BPA during gestation can lead to adverse effects on

fetal development **(Gingrich *et al.*, 2021).** "Low doses" of BPA encompass any doses below the previously determined Lowest Observed Adverse Effect Level (LOAEL) by regulatory bodies such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA); currently, the LOAEL for orally administered BPA is set at 50,000 µg/kg/day; if any significant adverse effects occur at doses below this threshold, it would necessitate a revision of the "safe" daily exposure level for humans, known as the acceptable daily intake (ADI) dose by the FDA and reference dose by the EPA; presently, this assumed "safe" dose is set at 50 µg/kg/day; the calculation of this estimated "safe" dose is based on the assumption that a dose 1000-fold lower than the LOAEL should not produce adverse effects if oral exposure occurs daily over the course of a lifetime **(Leung *et al.*, 2020).**

Estrogens and their mimics not only increase the expression of estrogen receptors but also stimulate the upregulation of related receptors and genes, along with various biochemical entities like cytokines, peptides, and lipids found in the endometrial environment **(Landahur *et al.*, 2023; Mari *et al.*, 2023).** However, these harmful substances were polluting both surface soils and subsurface water sources **(Kareem *et al.*, 2022).**

Besides, **Urli *et al.*, (2023)**, silage covers, baling hay with plastic strings, irrigation using water contaminated by microplastics (MPs), plastic mulching, application of sludge and plastic-laden fertilizers, breakdown of municipal waste, tire wear and tear, and roadside litter significantly affect soil structure, fertility, nutrient balance, and microbial communities, since land serves as a crucial resource for producing feed for livestock, which may ultimately be consumed either fresh or preserved, these various sources collectively increase the risk to human health through the ingestion of MPs present in eggs, dairy products and meat.

2.3.2. Effect Of BPA on The Soil:

Numerous toxicology studies have concluded that BPA has the capacity to disturb hormonal balance, affecting the growth, development, and reproductive capacities of animals, and potentially humans as well **(Zhenkun *et al.*, 2017).** Indeed, BPA is categorized as a substance that exerts toxic effects on reproductive abilities. As a widely produced chemical monomer, its release into the soil can lead to adverse effects on both ecology and public health **(Chouhan *et al.*, 2014).**

Improper management of BPA and its derivatives frequently culminates in soil contamination, raising concerns about the migration of BPA from soil to water; this emerging issue presents a potential peril to other ecosystems **(Abu Hasan *et al.*, 2023).** Sewage sludge stands as the primary origins of soil pollution attributed to BPA; also, landfill leachate discharge and wastewater irrigation represent significant contributors to soil contamination with BPA **(Morin *et al.*, 2015).** Furthermore, equally significant of this contamination is the recycling and disposal of electronic waste **(Huang *et al.*, 2014).**

Nano-plastics (NPs) are generated through the exposure of plastic fragments to ultraviolet radiation, natural weathering processes, and biodegradation **(Haldar *et al.*, 2023).** The extensive use of BPA has resulted in its accumulation in the soils and to the environment; numerous environmental studies have demonstrated that BPA is frequently present in significant quantities in various environmental compartments, including soil, surface water, groundwater, wastewater resulted from sewage treatment plants, and landfill leachate; consequently, soil is exposed to this chemical through sources like sludge and other effluents, adversely impacting not only soil microfauna but also other important organisms **(Ouada *et al.*, 2018).** Bisphenol A (BPA) is not

naturally found in the soils, but its extensive use in plastic materials as an industrial component has made it pervasive globally; soil contamination by BPA occurs through multiple pathways, including discharge from BPA manufacturing facilities and the introduction of products containing BPA into the soil; the deposition of these plastic products into rivers and marine waters acts as a major source of BPA pollution, thereby exacerbating environmental contamination **(Corrales *et al.*, 2015).** As shown in figure (2-3).

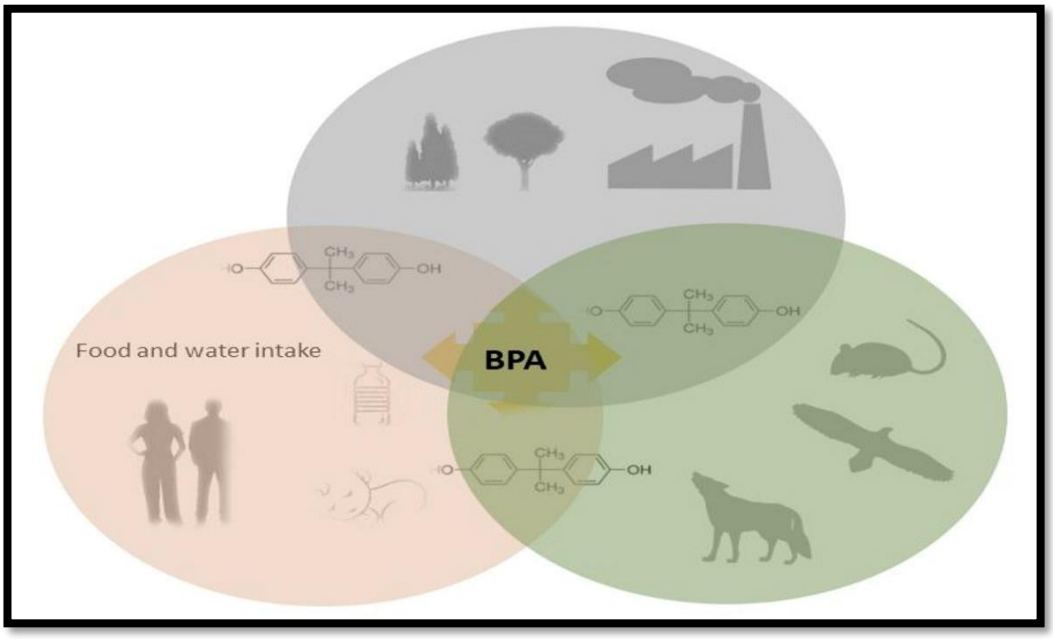


Figure (2-3): The Effect of BPA on different types of environments (Molina-López *et al.*, 2023).

Human activities contribute to the widespread presence of BPA due to the extensive use of various plastic products; once these products are consumed and disposed of, BPA is often released into soils and sewage water during the digestion process; as a result, sewage becomes a significant source of BPA in the environment; interestingly, research indicates that wastewater sludge tends to contain considerably higher concentrations of BPA compared to the wastewater itself **(Ramakrishna *et al.*, 2021).**

Bisphenol A (BPA) is readily absorbed through oral ingestion due to its chemical properties, which enable easy release from the polymer products in

which it is contained; this facilitates its migration into the environment, particularly into the soil; the ester bond connecting BPA molecules in materials like polycarbonates or epoxy resins is susceptible to hydrolysis under conditions such as heating or exposure to acidic or alkaline environments **(Mikolajewska *et al.*, 2015).** The breakdown of these materials releases free BPA, which can then migrate into food, beverages, and the surrounding environment; factors such as repeated washing with detergents, friction, and sterilization further facilitate this migration process **(Zaborowska *et al.*, 2021).**

A study examining food storage cans coated with epoxy resin, simulating sterilization processes such as canning, found substantial BPA levels ranging from 70 to 90 μg per 1 kg of the medium in preserved foods or model liquids **(Talpade *et al.*, 2018).** The breakdown of synthetic polymers, incineration of household waste, and substantial deposition of refuse are among the key activities contributing to the influx of BPA into soil, aquatic, terrestrial, and atmospheric environments; these mechanisms collectively contribute to the proliferation of BPA contamination **(Crain *et al.*, 2007).** Microbes play a crucial role as mediators of soil metabolic potential and serve as reliable indicators of its biological quality; consequently, they have become indispensable in the development of sustainable management practices **(Ba´cmaga *et al.*, 2021).**

2.3.3. Effect of BPA on The Animals:

Owing to their markedly lipophilic characteristics, a significant portion of BPA tends to amass within animals and animal-derived edibles like dairy, eggs, and meat product; this accumulation primarily stems from animals' exposure to BPA contained in their dietary intake; given the pivotal role of feed in animal husbandry, commercial feed products are typically encased to shield them against moisture and external pollutants, thereby facilitating their handling, transportation, and storage; the predominant materials employed in the

fabrication of feed packaging are polyethylene (PE) and polypropylene (PP), prized for their cost-effectiveness and practicality; BPA serve as ubiquitous crosslinking agents in the formulations of these polymer compounds **(Garcia-Moreno *et al.*, 2006).** Indeed, there is a potential risk of BPA migration from packaging materials into animal feeds; for instance, **Xia and Rubino (2015)**, documented the migration of BPA from PE films into simulated foodstuffs, highlighting the potential for BPA transfer from packaging to the contents within.

Exposure to various environmental endocrine-disrupting substances like BPA leads to an increased occurrence of cystic ovaries in bovines, often accompanied by irregular estrus cycles or anestrus; environmental estrogens notably impact the development of ovarian follicles and the onset of follicle growth within the framework of bovine ovarian physiology **(Erler and Novak, 2010).**

The declining fertility observed in dairy cattle in recent years has become a significant concern in the agricultural sector; while numerous factors could potentially contribute to this trend, emerging evidence indicates that the reproductive difficulties experienced by high-yielding dairy cows might be associated with prolonged or acute exposure to EDCs; despite estrogen's crucial role in female reproductive processes, the risk posed by EDCs to bovine fertility has received relatively little attention until now; this is particularly troubling because EDCs have the ability to mimic or disrupt estrogen's natural actions, thus compromising the delicate mechanisms essential for successful reproduction in dairy cattle **(Fortune and Yang, 2011; Sheng *et al.*, 2012).**

These physiological events occur during the early to mid-gestational phases in cattle; the impact of exposure to EDCs during fetal development extends to the regulation of early ovarian follicular development, which could potentially

affect the female reproductive lifespan and contribute to the onset of cystic follicles in vivo, ultimately leading to decreased fertility; the knowledge gained from these findings could provide valuable insights for dairy producers and veterinarians, enabling the development of enhanced herd management and nutritional strategies to minimize inadvertent exposure of cattle to endocrine-disrupting chemicals; this, in turn, could improve overall reproductive performance in dairy herds **(Fortune and Yang, 2011).** Figure (2-4).

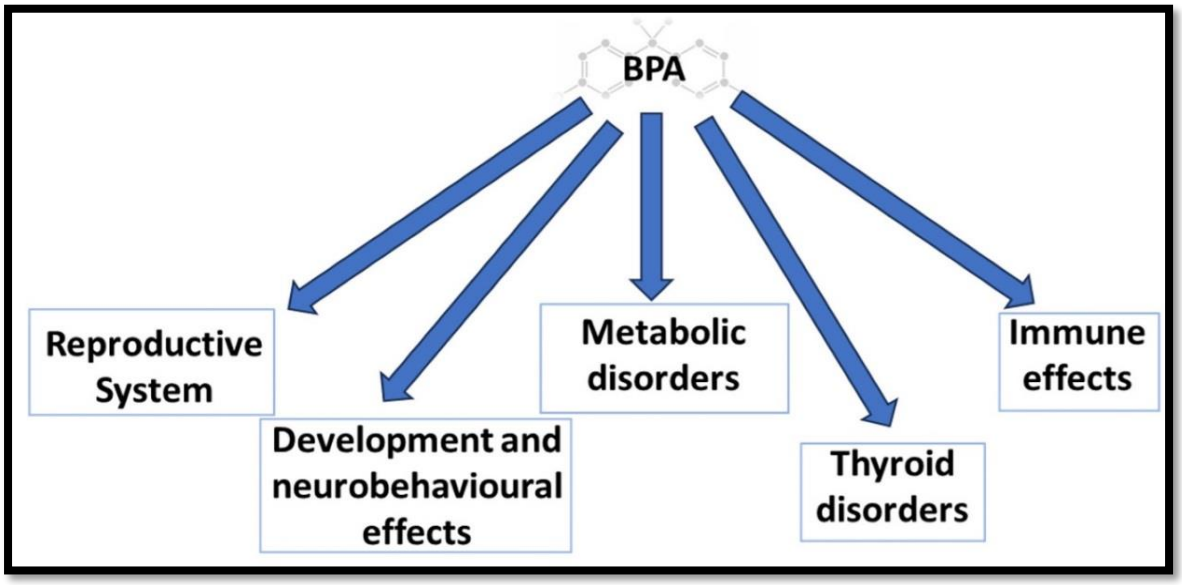


Figure (2-4): The physiological effect of BPA on different criteria. Molina-López *et al.*, (2023).

In the realm of livestock agriculture, it is imperative not to overlook the looming threat posed by animals inadvertently consuming or encountering microplastics; furthermore, there is a legitimate concern regarding the potential infiltration and accumulation of these minuscule particles within animal-derived goods such as meat, milk, and eggs **(Filho *et al.*, 2021).** Bisphenol A (BPA) consistently leaches from plastic equipment into animal feed during repetitive feeding or watering routines; among goats, exposure to minimal BPA levels commonly initiates apoptosis in spermatogenic and Sertoli cells, whereas

heightened concentrations often result in necrosis within these specific cell populations **(Talpade *et al.*, 2018).**

Research conducted through in vitro studies and animal trials indicated that exposure to BPA could impact not only reproductive and cardiovascular systems but also thyroid functions; moreover, there are indications that BPA might be linked to metabolic disorders and oxidative stress, including conditions like diabetes and obesity; furthermore, documented evidence has highlighted various adverse effects of BPA on wildlife, including developmental inhibition, malformations, and disruptions to reproductive systems **(Catenza *et al.*, 2021).**

In experimental studies, it was shown that, exposure to BPA has harmful effects on the reproductive system; this includes negative impacts on female reproduction, as well as neonatal and perinatal stages; exposure to BPA has been observed to cause significant changes in the structure of the reproductive organs, disrupt the menstrual cycle, reduce fertility, and alter hormone levels throughout adulthood **(Molina-López *et al.*, 2023).** Rivers and streams, vital components of aquatic ecosystems, act as reservoirs for a range of pollutants, including BPA; while BPA typically doesnot endure extensively in aerobic environments, reports indicate incomplete breakdown during wastewater treatment, posing risks to aquatic habitats that receive treated effluent; hence, prioritizing strategies that expedite and ensure complete BPA degradation is crucial for wastewater treatment **(Yu *et al.*, 2019).** Recent studies suggest that, BPA found in fish can also originate from the release of these compounds from plastic debris in the ocean **(Sadrabad *et al.*, 2023).** Fish exposed to endocrine-disrupting substances can pass on harmful reproductive effects to their offspring for up to three generations; BPA, in particular, activates estrogen receptors, leading to malformations in reproductive organs, decreased sperm quality, delayed sperm maturation, and changes in gender ratios; male red shiners show color variations after being exposed to water contaminated with BPA **(Flint *et al.*,**

In fish, research has accumulated regarding pathological effects associated with the absorption of nano-plastics across the gastrointestinal wall; particularly, ingested microparticles have been found in different organs like the intestine, liver, and kidneys; within the gut, these plastics are implicated in causing changes such as reduced mucosal secretion, intestinal barrier dysfunction, inflammation, and microbiota dysbiosis; additionally, in the liver, the presence of these particles has been linked to inflammation, resulting in disturbances in the blood lipid profile **(Yong and Valiyaveettil, 2020).** Furthermore, BPA exposure has been associated with reproductive toxicity and mortality in aquatic animals; to mitigate the burden of organic pollutants, various strategies have been implemented, including oxidation, biodegradation, photo-electrocatalytic oxidation, and adsorption **(Zhang *et al.*, 2013; Cheng *et al.*, 2016).**

2.4. Biodegradation of BPA:

Due to its high chemical stability and low water solubility, BPA is challenging to degrade naturally, and traditional technologies often struggle to completely remove it from the environment **(Leech *et al.*, 2009).** In recent years, various physical and chemical methods have been developed, including physical adsorption, membrane filtration, photocatalysis, and advanced oxidation, to address this issue **(Ma *et al.*, 2022).** While physical and chemical methods have demonstrated effectiveness in removing BPA, they often come with a significant financial burden; furthermore, the by-products generated from degradation may be more toxic than BPA itself; in contrast, biological methods offer unique advantages in pollutant removal from different sources as compared to physical and chemical degradation methods **(Bharagava *et al.*, 2021).** Figure (2-5).

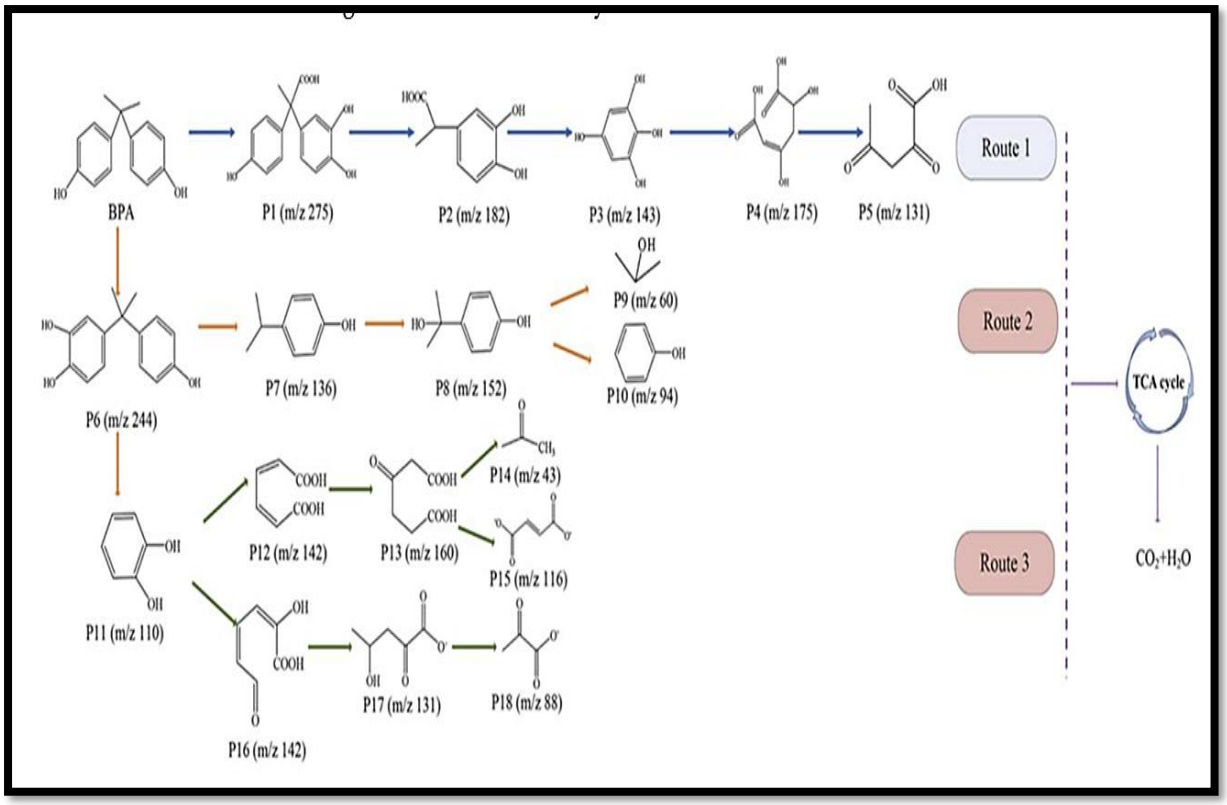


Figure (2-5): The pathways of biodegradation of BPA by isolated strains (Hou and Yang 2022)

2.4.1. Microorganisms Related to Biodegradation:

Bisphenol A (BPA) has been detected in various environments, including soils, landfill leachates and water; the most common methods for removing pollutants from the environment include photodegradation, photo-electrocatalytic oxidation, oxidation, and biodegradation; among these methods, bacterial biodegradation of BPA stands out as the most significant due to its low costs and eco-friendliness compared to other cleanup methods **(Eltoukhy *et al.*, 2020).** The utilization of BPA-degrading microorganisms in bioremediation shows promise for eliminating BPA from contaminated soils and environments **(Kamaraj *et al.*, 2014).** Numerous studies have reported numerous detrimental effects of BPA on both human and animal organs **(Chouhan *et al.*, 2014; Zhou**

***et al.*, 2017).** Interestingly, biodegradation offers an eco-friendly approach with economic efficiency **(Fuentes *et al.*, 2014).**

In contrast to conventional chemical remediation methods, bacterial-mediated biodegradation is cost-effective, safely to the environment, and effective, even at low concentrations **(Jaafar, 2020).** The success of bioremediation largely depends on the ability of selected bacterial strains to thrive in contaminated environments; therefore, the use of bacterial strains obtained from contaminated soils holds promise for addressing the cleanup of harmful and hazardous pollutants **(Saiyood *et al.*, 2010).**

Many organisms, including bacteria, fungi, algae, and plants, demonstrate the ability to degrade BPA in the environment; however, bacterial biodegradation stands out as the most significant focus in biodegradation studies; **(Eltoukhy *et al.*, 2020).** In bacterial bioremediation, the effectiveness of the process relies on bacterial metabolism; bacteria utilize pollutant substances as a source of carbon and a substrate for energy generation; as a result, bacterial biodegradation plays a crucial role in the bioremediation process by facilitating the reduction or removal of environmental concentrations of contaminants **(Noszczyńska and Piotrowska-Seget, 2018).**

Sphingobacterium sp. and *Pseudomonas aeruginosa* were assessed for their ability to bioremediate plastics within soil environments effectively; *Sphingobacterium sp.* was known to inhabit diverse habitats, including soil, activated sludge, forests, lakes, compost, fecal matter, and various food sources; additionally, these bacterial species have been documented to demonstrate potential in the biodegradation of various pollutants, including plastic mixture wastes especially **(Satti, 2018).** Previous researches had demonstrated that, bacteria such as *Sphingobium spp.*, *Pseudomonas putida*, *Bacillus megaterium*,

Arthrobacter sp., and *Achromobacter xylosoxidans* exhibited the ability to degrade BPA efficiently **(Ren *et al.*, 2016; Eltoukhy *et al.*, 2022).**

Various studies focusing on the biodegradation of BPA in the natural environment, efforts have been made to isolate BPA-degrading microorganisms from various sites, including soils and water bodies receiving industrial waste and landfills; given the significant controversies surrounding the use of BPA, there is a pressing need to bridge the gap between research that identifies the damage caused by BPA and studies aiming to safely remove BPA from the environment using naturally occurring microbes; this integrated approach can provide valuable insights into both the detrimental effects of BPA and effective strategies for its remediation **(Saiyood *et al.*, 2010).**

The study done by who investigated that, the biodegradation ability of various microorganisms from polluted soil has been studied, revealing that an acclimated microcosm was able to completely degrade and transform BPA into several metabolic intermediates; BPA is recognized as one of the endocrine-disrupting chemicals with deleterious estrogenic activity **(Noszczyńska *et al.*, 2021).**

2.4.2. Genes Responsible of Biodegradation of BPA:

Furthermore, an increasing number of bacteria with BPA-degrading abilities have been identified from diverse environmental sources, such as soil, water bodies, sediment, and wastewater treatment facilities; these bacteria include both Gram-negative and Gram-positive strains **(Das *et al.*, 2018).** The scientific approach has proposed several pathways for BPA degradation based on the intermediates detected during the biodegradation process by specific strains of bacteria **(Vijayalakshmi *et al.*, 2018).**

To date, the primary focus of BPA biodegradation research has been on single-strain organisms or consortia of bacteria sourced from sedimentary or aquatic environments, with limited investigation into the biodegradative

capacities of microbial populations inhabiting desert soils and arid locales **(Moussavi and Haddad, 2019).**

The biodegradation of BPA by different microorganisms has been demonstrated as a reliable and cost-effective method for its removal from various environmental settings **(Fu *et al.*, 2019).** Many genes have been studied as potential candidates responsible for BPA biodegradation, with one of the most significant being the Cytochrome P450 (CYP) monooxygenase gene; researchers have discovered the involvement of the *bisdAB* operon, which encodes cytochrome P450 *(bisdAB)* and ferredoxin *(bisdFd)* components of the cytochrome C P450 monooxygenase system, in the breakdown of BPA **(Jia *et al.*, 2020, a).** While significant attention has been devoted to studying the degradation pathways of BPA, the understanding of the metabolic mechanisms involved, including the catalysts and genes implicated, remains limited, as noted by various researchers **(Mtibaà *et al.*, 2018).**

Moreover, research by **Telke *et al.* (2009),** had pinpointed the involvement of an ammonia monooxygenase and an extracellular laccase within *Pseudomonas spp.* in the utilization of BPA; additionally, in *Sphingobium spp*., proteins implicated in protocatechuate transformation are presumably integral to the BPA degradation pathway; furthermore, enzymes such as p-hydroxybenzoate hydroxylase, known for their role in the metabolism and breakdown of xenobiotics, have also been implicated in the degradation of BPA within these bacterial species **(Noszczyńska *et al.*, 2021).**

The analysis of bacterial degradation behavior has predominantly focused on examining degradation effects or pathways; despite extensive studies on BPA degradation pathways, the understanding of the key genes and metabolic mechanisms involved in BPA degradation remains limited; therefore, studying

microbial genomes is a crucial step towards advancing our knowledge, particularly in understanding the defense and degradation mechanisms related to BPA in microorganisms; this will facilitate the development of precise bioremediation technologies for polluted environments **(Li *et al.*, 2023, b).**

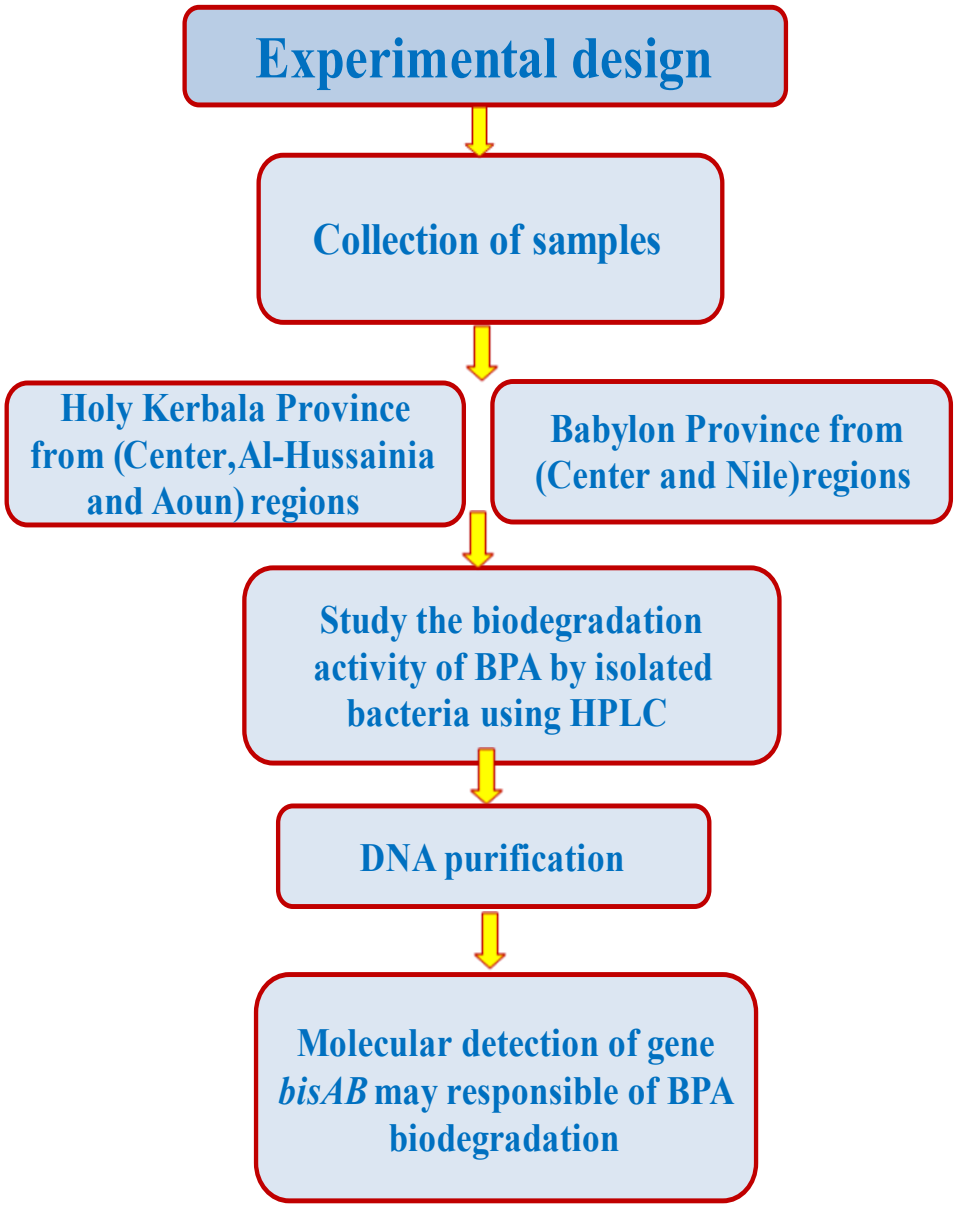
Chapter Three

Methodology

3. Methodology:

3.1. Materials:

3.1.1. Study Design:



3.1.2. Ethical Approval:

The study was done and the samples were collected after getting the agreement of college of Veterinary Medicine by the local committee with the number UOK.VET.HE2023.067.

3.1.3. Laboratory Instruments and Equipment:

The equipment and tools utilized in this study were listed in table (3-1).

Table (3-1): Laboratory Equipment and Instruments

Equipment / Instruments	Company	Country
Incubator	Memmert	Germany
Water bath		
Oven		
Distillator	GFL	
Centrifuge	Hettich	
Micropipettes 5-50 µl ,100-1000 µl, 0.5 – 10 µl + tips	Eppendorf	
DNA extraction tubes 100 µl.		
PCR tubes 50µl.		
Millipore filter (0.45mm)	Satorins membrane Filter Gm, BH, W.	
Glass slides, flasks and Beakers	Hirschman	
Microcentrifuge	Beckman	
Autoclave	Stermite	Japan
HPLC	Shimadzu	
Light microscope	Olympus	
Digital camera	Panasonic	
Sensitive electron balance	A & D	
Vortex	Clever	USA
Gel electrophoresis		

VITEK 2 System	Biomerieux	France
Refrigerator	Concord	Italy
Sequencers equipment	Macrogen	Korea
Micro- centrifuge	Fisons	England
Hood	Labogene	Denmark
Sterile swab	Lab.Service	S.P.A.
Thermocycler	Bioneer	Korea
Eppendrof tubes 1.5ml	BIO BASIC	Canada
Test tubes (10ml).	AFCO	Jordan

3.1.4. Chemicals and Biological Materials:

3.1.4.1 Chemical Materials:

Chemical materials used in this study are listed in table (3-2)

Table (3-2): Chemical Materials

Chemicals	Company/origin
NaCl, $NaHCO_3$ .10 H_2O , K_2HPO_4, KH_2PO_4 , $(NH_4)_2SO_4$, $MgSO_4$ .7H_2O , $CaCl_2$ .2H_2O, $MnSO_4$ .4H_2O , $ZnSO_4$. 7H_2O, (NH4)Mo_7O_2.4H_2O , NaH_2PO_4, H_2O_2, $CuSO_4$ .5H_2O , $CoCl_2$.6 H_2O , $FeSo_4$.7H_2O	BDH/ England
Bisphenol A	High Media Company/ India
McFarland tube standard (0.5)	Mastgroup/England
Oxidase	
99% and 70% alcohol (Ethanol)	Fluka chemika/ Switzerland
Glycerol ($C_3H_8O_3$)	
Ethidium bromide	Promega/ USA
Loading dye (bromophenole blue)	
Tris-EDTA buffer solution(TE)	
Tris-Borate-EDTA (TBE) buffer	
Gram stain set	Crescent/ KSA
Tracking dye	Geneaid /Taiwan

3.1.4.2. Biological Materials:

The biological materials used are listed in table (3-3).

Table (3-3): Biological Materials

Materials	Company/origin
Blood agar base, MacConkey agar, Agar-agar, Nutrient agar, Nutrient broth,	Himedia /India
Agarose	Bio basic/Canada

3.1.5. Molecular and Diagnostic Kits:

The molecular and diagnostic kits utilized in this study were listed in table (3-4) and its appendices, as follows:

Table (3-4): Molecular and diagnostic kits used in the study:

Types of kits	Company/country
Genomic DNA extraction kit from bacteria 1. Lyses cells solutions: - - Nuclei lyses solution - RNase solution 2.Protein precipitation solution 3. DNA precipitation alcohols: - -Isopropanol alcohol - Ethanol alcohol 4.DNA rehydration solution	Gene aid/Korea
Master mix 1. DNA polymerase enzyme (Taq). 2. dNTPs (400µm dATP, 400µm d GTP, 400µm dCTP, 400µm dTTP) 3. $MgCl_2$ (3mM)	Promega/USA

4. reaction buffer (pH 8.3)	
DNA ladder 100bp 1. Ladder consists of 11 double-stranded DNA with size 100-1500bp 2. Loading Dye has a composition (15% Ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50mM EDTA)	Bioneer/Korea
Primers supposed for Bisphenol A-biodegradation	

3.1.6 Primers used in This Study:

The primers used for amplification of a fragment gene were listed in Table(3-5).

Table (3-5): Primers sequences and PCR conditions:

Gene	Primer sequence (5´- 3´)	Size (bp)	PCR conditions	Reference
bisdAB operon	**F**:5'GGAAGCTTGGCCTCCGCACAGC3' **R:**5'AGCTGCAGGCCTACCTCTGACTGC3'	**3171**	**94ºC 5min 1x** **94ºC 1min** **56ºC 1min 30x** **72ºC 2min** **72ºC 10min 1x**	**Badiefar *et al.*, 2015**
16rRNA Universal primer	**F:** 5'AGAGTTTGACCTGGCTCAG3' **R:** 5'ACGGCTACCTTGTTACCGAC T3'	**1114**	**94ºC 1min 1x** **94ºC 1min** **57ºC 1min 30x** **72ºC 1min** **72ºC 7min 1x**	**Vijayalakshmi *et al.*, 2018**

3.2. Methods:

3.2.1. Sampling:

At about five grams of polluted soils from different sites of Kerbala province from (Center, Al-Hussaini and Aoun) regions and Babylon province from (Center and Nile) regions. Those specimens were collected in 6 October 2024and placed in tubes containing 100 ml of normal saline to maintain the specimens moist until taken to laboratory. These samples were inoculated on MacConkey agar, nutrient agar, and blood agar plates and then incubated aerobically and at 37ºC for 24-48hr.

3.2.2. Preparation of Reagents and Solutions:

3.2.2.1. Oxidase Reagent:

It was produced afresh in a dark bottle by dissolving 1gm Tetramethyl Para Phenylene Diamine Dihydrochloride in 100ml distilled water **(Forbes *et al*., 2007).**

3.2.2.2. Catalase Reagent:

Hydrogen peroxide at concentration of (3%) was prepared from stock solution in a dark bottle **(Forbes *et al*., 2007).**

3.2.2.3. Gram Stains Solutions:

The solutions were used according to the requisite microbiological methods, and these solutions included: four solutions crystal violate, iodine, pure alcohol and safranine **(Collee *et al*., 1996).**

3.2.2.4. McFarland Standard Solution:

McFarland standard solution was made by adding 0.05 ml of BaCl2.2H2O (1.175%) to 9.95 ml of H2SO4 (1% concentration). The McFarland standard tube (No. 0.5) was used to compare bacterial cells in suspension, resulting in a cell density of 1.5x108 cells/ml. **(Baron *et.al.*, 1994).**

3.2.2.5. Tris-EDTA Buffer Solution (TE buffer):

The buffer was made by dissolving 0.05 moles Tris-OH and 0.001 moles EDTA in 800ml of distilled water. The pH was adjusted to 8, then completed to one litter, autoclaved at 121^0C for 15 minutes, and stored at 4°C until required **(Sambrook and Rusell, 2001).**

3.2.2.6. Tris Borate-EDTA Buffer Solution (TBE):

The buffer was made by dissolving 0.08 mole Tris-OH, 0.05mole boric acid, and 0.02mole EDTA in 500ml of distilled water; the pH was corrected to 8. Autoclaved at 121^0C for 15 minutes, and kept at 4°C **(Sambrook and Rusell, 2001).**

3.2.2.7. Ethidium Bromide Solution:

It was prepared by dissolving 0.05gram of Ethidium Bromide in 10ml of distilled water and then, stored in dark reagent bottle **(Sambrook and Rusell, 2001).**

3.2.3. Preparation of Culture Media:

A bunch of culture media were prepared according to the company's instructions and serialized by autoclaving at 121°C for 15 minutes.

3.2.3.1. MacConkey Agar (PH:7.3):

MacConkey agar medium was made using the technique specified by the manufacturer; it was utilized for the primary isolation of most Gram-negative bacteria and to distinguish lactose fermenters from non-lactose fermenters **(Winn *et al*., 2006).**

3.2.3.2 Blood Agar (pH:7.1):

Blood agar media was created by dissolving 40 grams of blood agar base in 1000 ml of distilled water; this media was autoclaved at 121^0C for 15 minutes and then cooled to 50^0C. Then, 5% of fresh blood was added; this medium was used for cultivating bacterial isolates **(Forbes *et al*., 2007).**

3.2.3.3 Nutrient Agar :

Nutrient agar medium was prepared according to the manufacturing company (28 gm/1L). It was used for general experiments, cultivation and activation of bacterial isolates when it is necessary **(McFaddin, 2000).**

3.2.3.4. Minimal Salt Medium (MSM):

3.5 gm of K_2HPO_4, 1.5 gm of KH_2PO_4, 0.5 gm of NaCl, 0.5 gm of $(NH_4)_2SO_4$ and 0.15 gram of $MgSO.7H_2O$ and trace element were added and they contained, 2 gm of $NaHCO_3.10\ H_2O$, 0.3 gm $MnSO_4.\ 4H_2O$, 0.2 gm $ZnSO_4.7H_2O$, 0.02 gm $(NH_4)\ Mo_7O_2.4H_2O$, 0.1 gm CuSO4.5H2O, 0.5 gm $CoCl_2.6H_2O$, 0.05 $CaCl_2.2H_2O$ and 0.5 gm $FeSO_4.7H_2O$ dissolved in 1000 ml of distilled water, and then sterilized in an autoclave at 121^oC for 15 minutes. After cooling the mixture to 50^oC, Bisphenol A was added to the mixture as the only carbon source in the media in concentration 200 mg/L. This media used to detection of Bisphenol A-degrading activity **(Louati *et al*., 2019).**

3.2.4. Laboratory Diagnosis of Bacteria:

3.2.4.1. Microscopic Examination and Colonial Morphology:

According to the diagnostic procedures recommended by**(McFadden, 2000),** a single colony was taken from each primary positive culture and its identification depended on the morphological properties (Colony size, shape, color, nature of pigments, translucency, edge, elevation and texture). Bacterial smear stained with Gram stain was used to check the morphological properties of bacterial cells.

3.2.4.2 Staining:

Gram stain was used to differentiate Gram negative from Gram positive bacteria and to identify their shape and arrangement in steps declared by **(Winn *et al*., 2006).**

3.2.5. Biochemical and Physiological Tests of Bacteria:

3.2.5.1 Oxidase Test:

This test was done by rubbing the tested bacterial colony on the filter paper moistened by several drops of oxidase reagent; when the colony changed into violet color the result was recorded as positive **(Baron *et.al.,* 1994).**

3.2.5.2 Catalase Test:

Nutrient agar medium was streaked with the selected bacterial colonies and incubated at 37ºC for 24 hrs., then the growth was transferred by a wooden stick and put on the surface of a clean slide, then a drop of (3% H_2O_2) was added. Formation of gas bubbles indicates a positive result **(Forbes *et al*., 2007).**

3.2.6. Identification of Bacterial Isolates by VITEK 2 System:

The VITEK 2 System was used to confirm the result of the manual biochemical test, in recent times this system used to identify microorganisms **(Winn *et al,*. 2006).** It was supplied with the required identification data base for all routine identification tests that provide an improved efficiency in microbial diagnosis which reduce the time and the need to do any additional tests, that will be safe for the user of system. This system was performed according to the manufacturer's instructions (Biomerieux-France). This system consists of:

1. A personal computer.

2. Reader/incubator that consisting of multiple internal components including: card cassette, card filler mechanism, cassette loading processing mechanism, card sealer, bar code reader, cassette carousel and incubator.

3. The system also contains: transmittance optics, waste processing, instruments control electronics and firm ware.

This system was performed according to the manufacturer's instructions:

1- Three ml of normal saline were placed in plane test tube and inoculated with a loop full of single colony of overnight culture.

2- The test tube was inserted into dens check machine for standardization of colony to McFarland's standard solution (1.5×10^8 cell/ml).

3- The standardized inoculums were placed into the cassette.

4- Then a sample identification number was entered into the computer software via barcode. Thus, the VITEK 2 card was connected to the sample ID number.

The cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader/incubator module.

3.2.7. Estimation of Bisphenol A -3 by Using HPLC:

Bisphenol A was added to the medium as the only carbon source in the media in concentrations 200 mg/L. Each sample was inoculated directly into medium. The samples were incubated aerobically at 37°C for 4 days. And the concentration of Bisphenol A was measured in the containing media using HPLC on a C18 column with gradient elution with water or acetonitrile at elution rate 1 ml per minute at absorption 220 nm. with a total running time of 25 min and a flow rate of 1 mL/min, at elution rate 1 ml per minute at absorption. And the retention time was 3.9-6 minute with wavelength 220nm. **(Louati *et al.,* 2019).**

Each culture was centrifuged for 10 min at 12,000 rpm. The resulting supernatant was filtered through a 0.22 µm filter to remove potential insoluble component. The filtrate was used for the quantification of the residual BPA concentration **(Vijayalakshmi *et al.,* 2018).**

This analytical technique grounded on the separation of molecules due to the differences in their composition and/or structure; these involves moving of the sample through the system over the stationary phase, the molecules in the sample

will have dissimilar interactions and affinities with the stationary phase, that leading to separation of molecules (Kupiec, 2004). The sample components that exhibit stronger interactions with the stationary phase will move more slowly through the column, than components with weaker interactions; so different compounds can be separated from each other when they move through the column **(Gerber *et al.*, 2004).**

The schematic of an HPLC instrument typically involves a sampler, pumps and a detector; the sampler transports the sample mixture into the mobile phase stream which conveys it into the column; the pumps supply the desired flow and composition of the mobile phase through the column; while the detector creates a signal proportional to the amount of sample component emerging from the column, therefore allowing for quantitative analysis of the sample components **(Xiang *et al*., 2004).**

3.2.8. Genotyping Assay:

3.2.8.1. Genomic DNA Extraction from Bacterial cells:

The procedure is achieved according to the method recommended by the manufacturing company (Favorgen) with some modification (by special communication):

1. An overnight bacterial culture was harvested and added to 200 µl of phosphate buffer saline (FBS) and added to a microcentrifuge tube (not provided).

2. If RNA-free genomic DNA is required, 4 µl of 100 mg/ml RNase A is added to the sample and incubated for 2 min at room temperature (Optional).

3. 20 µl Proteinase K and 200 µl FABG Buffer are added to the sample. And mixed thoroughly by pulse-vortex. Proteinase-K did not add directly to FABG Buffer.

4. Incubated at 60 ºC for 15 minutes to lyse the sample. During incubation, the sample is mixed every 3-5 minutes.

5. The tube is briefly rotated to remove drops from the inside of the lid.

6. 200 µl ethanol (96- 100 %) is added to the sample. Mixed thoroughly by pulse-vortexing for 10 sec.

7. The tube is briefly rotated to remove drops from the inside of the lid.

8. FABG Mini Column was placed a to a Collection Tube. The mixture is transferred (including any precipitate) carefully to the FABG Mini Column. Centrifuge at 6,000 x g for 1 min then FABG Mini Column was placed to a new Collection Tube.

9. 400 µl W1 Buffer is added to the FABG Mini Column and centrifuged at full speed (18,000 x g) for 30 sec then discarded the flow-through.

10. 750 µl Wash Buffer is add to the FABG Mini Column and centrifuged at full speed for 30 sec then discarded the flow-through. Make sure that ethanol has been added into Wash Buffer when first open.

11. Centrifuged at full speed for an additional 3 minutes to dry the column.

Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

12. The FABG Mini Column is placed to a Elution Tube.

13. 50 ~ 200 µl of Elution Buffer or ddH2O (pH 7.5- 9.0) is added to the membrane center of FABG Mini Column. Standed FABG Mini Column for 3 minutes.

- Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

14. Centrifuged at full speed for 1 minutes to elute total DNA.

15. Total DNA is stored at 4 °C or -20 °C.

Note: all centrifugation process is done at 14000 rpm.

3.2.8.2. Thermal Cycling Conditions:

A Polymerase Chain Reaction (PCR) is performed in order to make a large number of copies of a gene. Otherwise, the quantity of DNA is insufficient and cannot be used for other methods of testing. A PCR is performed on an automated

cycler, which heats and cools the tubes with the reaction mixture in a very short time. PCR is performed for 30-40 cycles, in three major steps: **1)** denaturation **2)** annealing and **3)** extension 4)final extraction

3.2.8.3. Preparation of Primers:

The lyophilized oligonucleotide upstream and downstream primers are prepared according to the manufacturing company (Bioneer, USA) and kept in -20ºC.

3.2.8.4. The Reaction Mixture:

Amplification of DNA is carried out in a final volume of 20 µl containing the following as mentioned in Table (3-6)**.**

Table (3-6): Contents of the reaction mixture:

No.	Contents of reaction mixture	Volume
1	Master mix	12.5 µl
2	Upstream primer	2.5 µl
3	Downstream primer	2.5 µl
4	DNA template	5 µl
5	Nuclease free water	2.5 µl
Total volume		25 µl

3.2.8.5. Detection of Amplified Products by Agarose Gel Electrophoresis:

Successful PCR amplification is confirmed by agarose gel electrophoresis (Lodish *et al.*, 2004). Agarose gel is prepared by dissolving 2gm of agarose powder in 100ml of TBE buffer (pH:8) in boiling water bath, allowed to cool to 50ºC and ethidium bromide at the concentration of 5µ/ml was added.

The comb is fixed at one end of the tray for making wells used for loading DNA sample. The agarose is powered gently into the tray, and allowed to solidify at room temperature for 30 min. The comb is then removed gently from the tray.

The tray is fixed in an electrophoresis chamber which was filled with TBE buffer covering the surface of the gel, 5µl of DNA sample is transferred into the

signed wells in agarose gel, and in one well we put the 5µl DNA ladder mixed with 1µl of loading buffer.

The electric current is allowed at 70 volts for 60 min. UV transilluminator is used for the observation of DNA bands, and gel is photographed using a digital camera. The amplified products are determined by comparison with a commercial 100- 1500 bp ladder (Bioneer).

3.2.8.6. Sequence Analysis of Pure Colonies (General Protocol):

The PCR products were purified by using a clean kit **(Favorv PREPTM PCR Clean–Up Mini (FAVORGEN Biotechnology CORP., KOREA)** according to the manufacturer's instructions.

1- 10-100 µl of PCR product was transferred to micro-centrifuge tube, and 5 volumes of FAPC buffer were added and mixed well by vortex.

2- A FAPC column was inserted into a collection tube and paced.

3-Fill the FAPC column halfway with the sample mixture, centrifuge for 30 seconds at 11,000 rpm, and discard the flow–through.

4- 600 µl of wash buffer (ethanol added) were added to the FAPC column. Then, centrifuge at 11,000 rpm for 30 sec, and then discard the flow through. Then Centrifuge again at full speed (18,000 x g) for an additional 3 minutes to dry the column matrix.

5-An elution tube was connected to the FAPC column (provided).

6-40 µl of elusion buffer was added or doubled distilled water (ddH2O) to the membrane center of the FAPC column. Stand the FAPC column left for 1min. **7-** At full speed (18,000 xg) centrifuged or 1min to eluted DNA. After that, the purified produ

cts and primers 27F and 1492R with concentration of 10 pmol were sent for sequencing at Macrogen laboratories (Macrogen, Inc., South Korea). Sequence results were then submitted to a BLAST search in GenBank **(http://**

blast.ncbi.nlm.nih.gov/Blast.cgi) to compare with the closest known alignment identities for the partial 16S rRNA sequences.

3.2.8.7. Statistical Analysis:

The statistical program Graph Pad Prism 8.0 the t-test was used, P≤0.05 was chosen as the standard of significance. The data points were shown as mean± SD **(Motulsky *et al*., 2003).**

Chapter Four

Results and Discussion

4. Results and Discussion:

4.1. Isolation of Bacterial Strains:

Bisphenol A , an organic chemical substance, included with industrial significance, which serving as a crucial raw material in the synthesis of various products; the extensive utilization of these chemical compounds results in substantial quantities of BPA being released directly into terrestrial and aquatic environments, posing a severe toxicity threat to numerous types of organisms (Inoue *et al.,* 2008).

fifteen samples were taken in October 2023 from polluted soil with plastic wastes of different sites in Karbala province (Center, Al-Hussainia and Aoun) regions and Babylon province (Center and Nile) regions, 10 samples were taken from each regiones, **Appendix (I).** These samples were prepared and cultured on different media such as (MacConkey agar, blood agar, nutrient agar) for traditional isolation and identification. And these isolates were subjected to examine their ability to degraded BPA, by using MSM with BPA as sole carbon source for bacterial growth. Table (4-1). **Appendix (II).**

Table (4-1): Positive bacterial growth grown in Bisphenol-a containing media

No. of isolates	Positive bacterial growth	Negative bacterial growth	*P value*
50	31 (62%)	19 (38%)	< 0.002

P value significant < 0.05

Due to the low costs and less disruption for the contaminated soils, when compared to other cleanup methods; various studies demonstrated several BPA-degrading bacteria and their BPA-degrading performance **(Suyamud *et al.*, 2018; Suyamud *et al.*, 2020).**

The results of distribution of bacterial isolates, percentage in different studical regions table (4-2).

Table (4-2): The distribution of positive bacterial growth according to the regions

No.	Regiones	Positive bacterial growth	*P* value
1	Karbala (Center)	8 (26%)	≥ 0.082
2	Karbala (Al-Hussainia)	5 (16%)	
3	Karbala (Aoun)	4 (13%)	
4	Babylon (Center)	9 (29%)	
5	Babylon (Nile)	5 (16%)	
Total: 31 (100%)			

***P* value significant < 0.05**

The results showed that, there were no significant differences among the number of bacteria with BPA-biodegradability isolated from different regiones. In spite of that, the urban regiones (the center of Kerbala and Babylon) had a greater number of isolates with BPA-degradation compared with rural regiones (Al-Hussainia, Aoun, and Nile). The findings of other research conducted that; the urban areas had higher plastic waste compared to rural areas; in spite of there were higher plastic waste care behavior in urban as comparing to that in rural areas **(Partono *et al.,* 2020).** Plastic pollution generated by large cities affects nearby rural communities through the air–water–soil nexus **(Mihai *et al.,* 2022).** Increased urban population growth is proportional to the use of plastic as a medium used in everyday life, and disposable plastic material is an important part of most people; this is because it is easy to carry, portable, strong, abundant in size and shape, inexpensive, and easy to obtain; therefore, the use of plastic as a drink bottle is a simple example that is widely used, in urban or rural areas (**Khoironi *et al,* 2019).**

After inoculation the bacterial isolates to the MSM with bisphenol A. Each isolate that could grow in the Bisphenol A-containing media subjected to identify by VITEK 2 techinque, as shown in the figure (4-1) and (4-2). As well as in **appendix (III and IV).** The distribution of these bacteria according to the regiones under study shown in table (4-3).

bioMérieux Customer: Microbiology Chart Report Printed December 6, 2023 10:27:41 PM GMT-06:00

Patient Name: 1 Patient ID: 612232
Location: Physician:
Lab ID: 612232 Isolate Number: 1

Organism Quantity:
Selected Organism : Serratia plymuthica
Source: swab **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.85 hours	**Status:**	Final
Selected Organism	92% Probability	**Serratia plymuthica**		
	Bionumber:	4601734470240200		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	(+)	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure (4-1): The results of VITEK2 system to investigated *Serratia plymuthica* bacteria

bioMérieux Customer: Microbiology Chart Report Printed December 6, 2023 10:21:35 PM GMT-06:00

Patient Name: 13 Patient ID: 6122310
Location: Physician:
Lab ID: 6122310 Isolate Number: 1

Organism Quantity:
Selected Organism : Pantoea spp
Source: swab **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.78 hours	**Status:**	Final
Selected Organism	94% Probability	**Pantoea spp**		
	Bionumber:	4601710570040200		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure (4-2): The results of VITEK2 system to investigated *Pantoea spp* bacteria

Table (4-3): The bacterial isolates that grow in Bisphenol A-containing media according to identification by VITEK 2. And their distribution according to the regiones.

No.	Bacterial isolates	Number of isolates	Distribution	Number according to Region
1	*Serratia plymuthica*	**5**	(Kerbala center) (Aoun) (Babylon center)	**2** **1** **2**
2	*Pantoea spp*	**3**	(Al-Hussainia) (Nile)	**2** **1**
3	*Shingomonas paucimobilis*	**5**	**(** Babylon center) (Kerbala center)	**3** **2**
4	*Acinetobacter haemolyticus*	**2**	(Aoun) (Nile)	**1** **1**
5	*Acinetobacter lwoffii*	**2**	(Al-Hussainia) (Babylon center)	**1** **1**
6	*Pseudomonas aeruginosa*	**4**	(Babylon center) (Kerbala center)	**2** **2**
7	*Escherichia coli*	**4**	(Al-Hussainia) (Nile) (Aoun)	**2** **1** **1**
8	*Bacillus spp.*	**3**	(Al-Hussainia) (Nile) (Aoun)	**1** **1** **1**
9	*Proteus spp.*	**3**	(Babylon center) (Nile)	**2** **1**
Total number: 31				

Despite advancements in various remediation techniques, it was noteworthy that biodegradation by the bacteria remained the predominant method for addressing BPA contamination; this underscores the significance of harnessing the metabolic capabilities of bacteria in effectively breaking down and mitigating the environmental impact of BPA **(Usman *et al.*, 2019).**

4.2. Biodegradation Ability of Bacterial Isolates:

After 3-4 days of incubation of bacterial isolates in MSM with BPA. The broth centrifugated to filtrate it from bacterial growth. As well as, Millipore filter used to ultra-filtration. And the residues of BPA in these solutions calculated by HPLC, by using specific column, retention time and mobile phase. The results of figure (4-3), showed the control tube (that was replicated three time for precise results). The tube without bacterial inoculation and determined as a control with area under curve 100% and height 100%; all other tubes that inoculated with bacterial isolates compared to this tube to detect the residues of BPA.

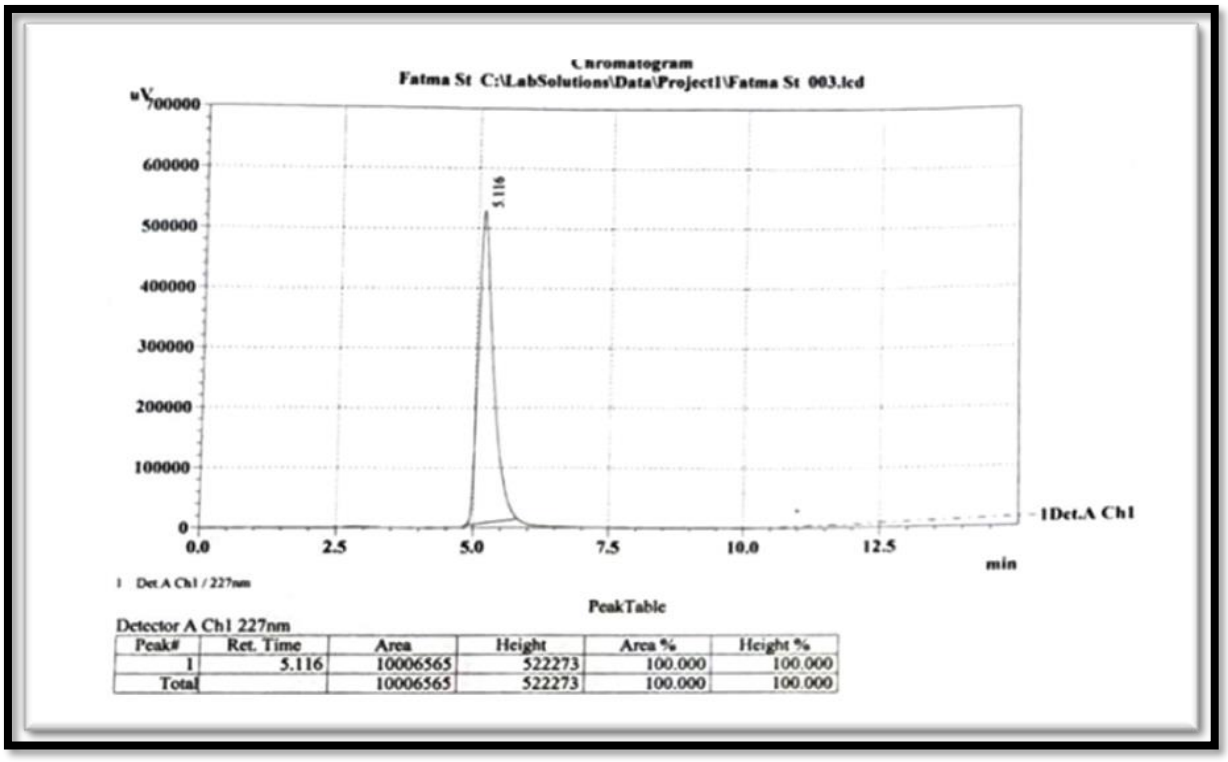
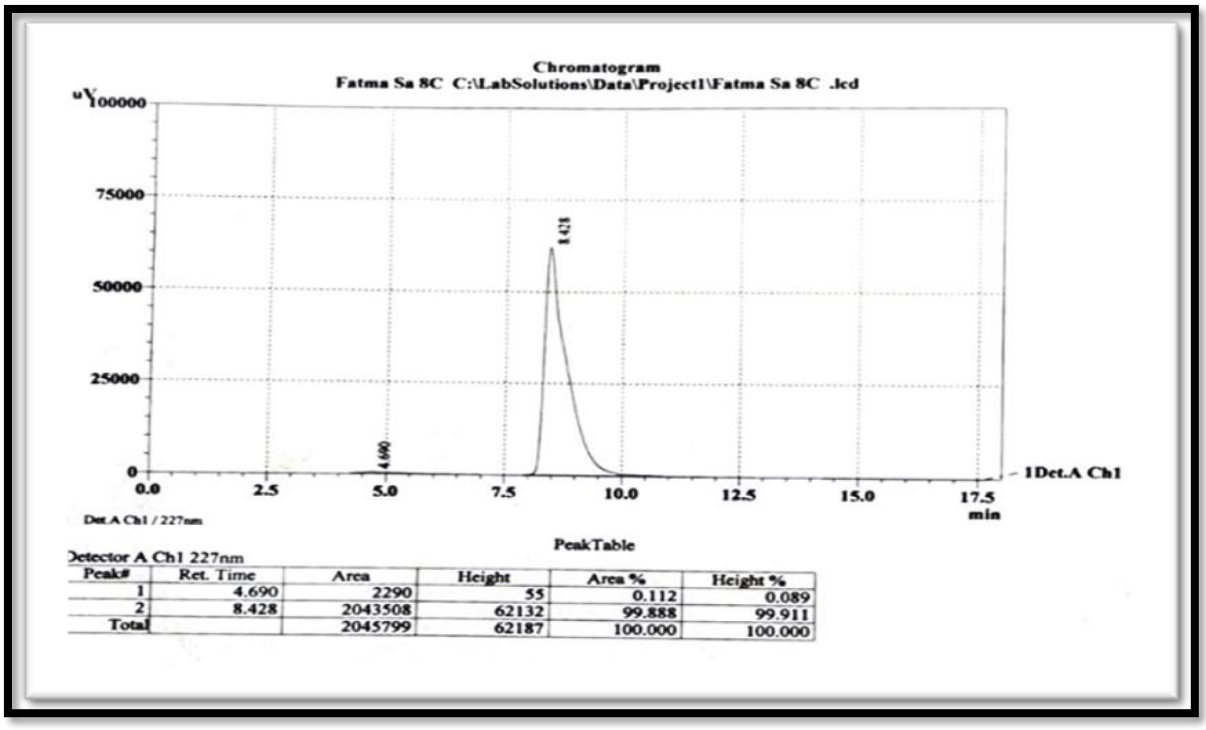


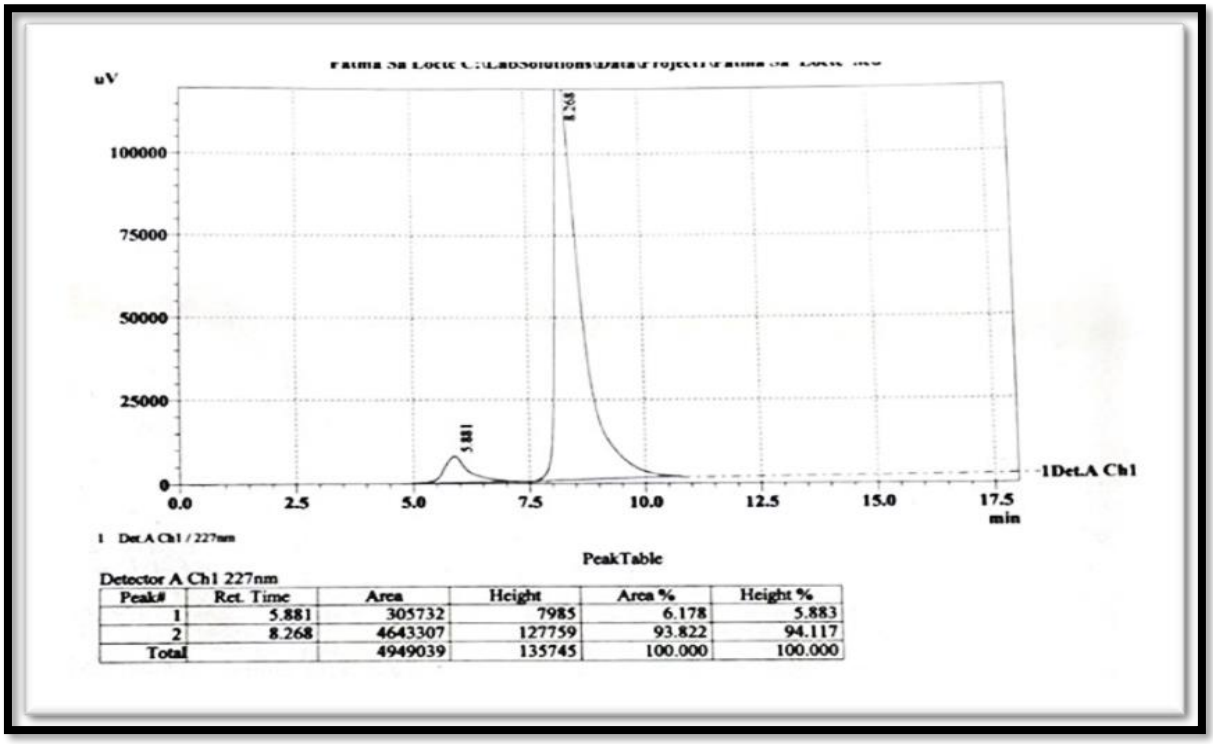
Figure (4-3): Curve of HPLC for Bisphenol A control tube. The peak appeared at 5.11 min with 100% area and height under curve

The results of BPA biodegradation ability of bacterial strains, showed in Figure (4-4) and (4-5); and **appendix (V, VI and VII).**



Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.690	2290	55	0.112	0.089
2	8.428	2043508	62132	99.888	99.911
Total		2045799	62187	100.000	100.000

Figure (4-4): Curve of HPLC for the ability of *Shingomonas paucimobilis* to biodegrade BPA.



Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.881	305732	7985	6.178	5.883
2	8.268	4643307	127759	93.822	94.117
Total		4949039	135745	100.000	100.000

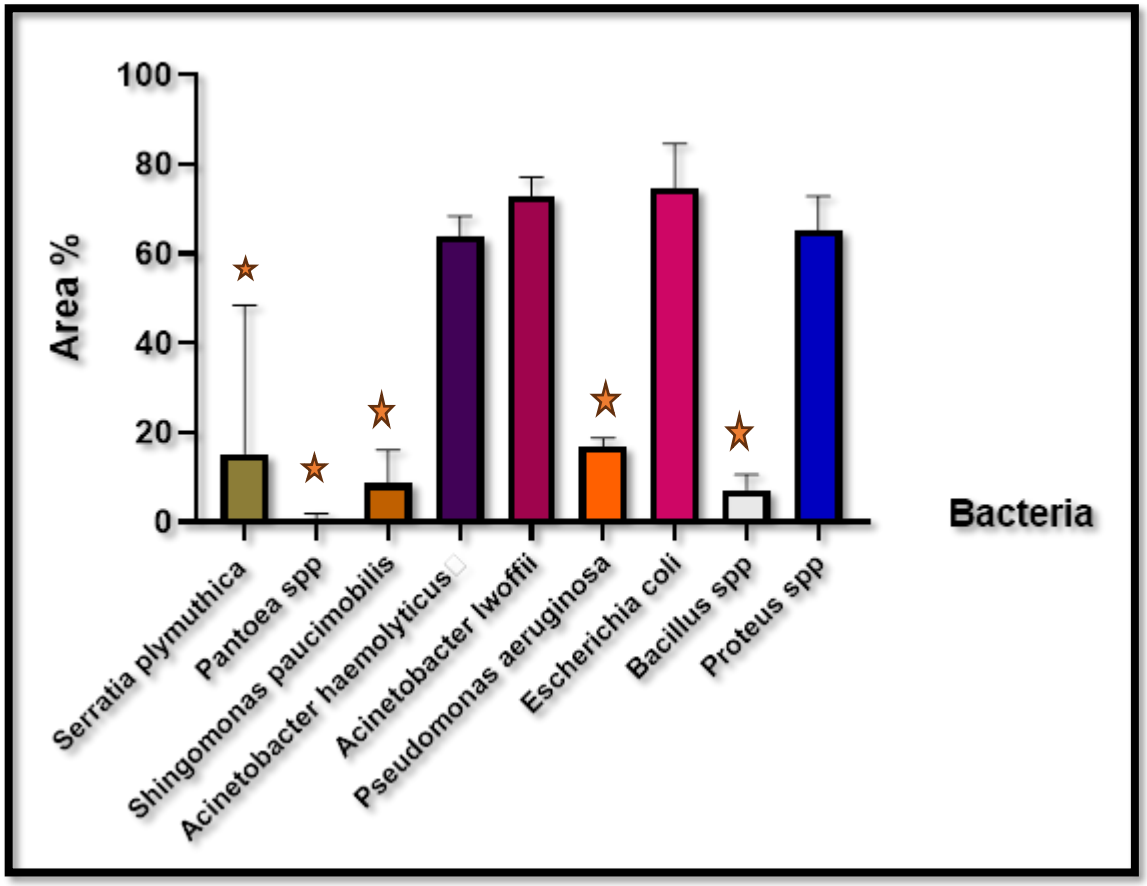
Figure (4-5): Curve of HPLC for the ability of *Bacillus spp.* to biodegrade BPA.

The results of capacity of biodegradation of BPA of all isolates illustrated in the table (4-4), figure (4-6) and **appendix (VIII)**.

Table (4-4): The Percentage of area, height and biodegradation capacity of bacterial isolates according to HPLC results.

No.	Bacteria	Area %	Height %	Biodegradation capacity
1	*Serratia plymuthica*	0.046	0.486	99.9 %
		0.128	0.565	99.8%
		0.680	1.396	99.3%
		0.821	1.757	99.1%
		74.718	85.274	25.2%
2	*Pantoea spp*	0.410	1.246	99.5%
		2.068	1.457	97.9%
		0.051	0.343	99.5%
3	*Shingomonas paucimobilis*	6.131	5.237	93.8%
		20.026	24.741	79.9%
		0.112	0.089	99.8%
		11.197	9.564	88.8%
		6.131	5.237	92.3%
4	*Acinetobacter haemolyticus*	**67.868**	91.164	32.1%
		65.243	90.341	34.7%
5	*Acinetobacter lwoffii*	**74.160**	93.420	25.8%
		72.166	93.001	27.8%
6	*Pseudomonas aeruginosa*	19.459	28.122	80.5%
		15.182	19.967	84.8%
		17.124	20.235	82.8%
		14.451	19.11	85.5%
7	*Escherichia coli*	**81.262**	89.833	18.7%
		75.458	77.999	24.5%
		80.821	88.122	19.1%
		57.564	85.999	42.4%

8	*Bacillus spp.*	6.178	5.883	93.8%
		5.111	4.044	94.8%
		13.212	15.761	86.7%
9	*Proteus spp.*	**75.904**	91.071	24.0%
		56.21	88.232	43.7%
		66.573	90.122	33.4%
Total Bacterial isolates: 31				



***P* value significant < 0.05**

Figure (4-6): The efficiency of the bacterial isolates to degraded BPA

The results found that, the bacterial isolates could degrade the BPA in different ranges started from 18.7% to 99.9%. And there were significant differences of the

ability of bacteria to remediate BPA such as *Serratia plymuthica*, *Pantoea spp*, *Shingomonas paucimobilis*, *Pseudomonas aeruginosa* and *Bacillus spp*., comparing with other isolates of the recent study such as, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Escherichia coli* and *Proteus spp.,* according to the BPA biodegradation capacity.

Pantoea bacteria encompass stains that have the ability to degrade BPA and reports for the first time that, these can use BPA as the sole carbon source **(Louati *et al.,* 2019).** Biotransformation by *Pantoea* could generate beneficial compounds, whereas biodegradation eliminates hazardous chemicals such as BPA (**Yang *et al.,* 2023).**

Serratia spp. capable of growing on BPA as a sole source of carbon and energy; and could completely degrade 115 mg/l BPA in the media containing it **(Zhang *et al.,* 2013).** Furthermore, *Serratia spp, Pseudomonas aeruginosa* and *Escherichia coli* had been studied for their ability of BPA removal from the environment, and biofilm formation on activated granule carbon (**Mita, *et al.,* 2015)**.

In the pursuit of degradation of BPA, beyond chemical and physical treatment methods, microbial degradation had emerged as a focal point of scientific attention; in recent, microbial treatment had gained prominence as an environmentally friendly alternative, offering sustainable approaches to the degradation of plastics, including BPA; this shift towards microbial degradation underscores the potential of harnessing microorganisms to address pollution of plastics and highlights the importance of eco-friendly solutions in strategies of environmental remediation **(Yu *et al.,* 2019).**

The BPA-degrading bacterium *Shingomonas paucimobilis* had demonstrated the capability to mineralize about 60% of the total carbon content of BPA into carbon dioxide (CO2), additionally, it could assimilate 20% of BPA

into bacterial cells, while the remaining 20% is transformed into soluble organic compounds **(Badiefar *et al.*, 2015).**

The analytical study conducted by (**Yue *et al.*, 2021),** revealed that, while *Sphingomonas spp*. exhibited the capability to fully degrade BPA, the process was deemed inefficient as intermediates tended to accumulate; in contrast, *Pseudomonas sp*. demonstrated more rapid utilization of these intermediates, thereby facilitating the overall mineralization of BPA within the microbial community; this observation cleared the intricate dynamics of microbial interactions and the significance of specific bacterial strains in optimizing the efficiency of BPA degradation pathways.

At least there were four potential pathways for BPA degradation had been identified; the cytochrome P450 monooxygenase system had a notable enzyme complex playing a pivotal role in this process; this system comprises cytochrome P450, ferredoxin and also, ferredoxin reductase; notably, the functions of cytochrome P450 and ferredoxin in this system exhibited similarities to those found in *Sphingomonas* bacteria, as reported by **Das *et al.* (2018).** According to bacterial biodegradation, the efficacy of the process hinges on bacterial metabolism, wherein bacteria utilized pollutant molecules as solely carbon source and substrate for the energy generation by the bacteria; consequently, bacterial biodegradation emerges as a valuable tool in the bioremediation processes, contributing to the removal or reduction of environmental concentrations of contaminants **(Farias and Krepsky, 2022).**

The pivotal role of *Pseudomonas aeruginosa* in the biodegradation of various polymers, encompassing degradation of the xenobiotic compounds; moreover, its involvement in the breakdown of plastics, dyes, and oils had been extensively documented; similarly, *Sphingobacterium* had been reported to exhibit notable biodegradation of diverse pollutants potentially; including the

ability to contribute to the degradation of mixed plastic wastes; this emphasized the versatility of these bacterial strains in environmental remediation efforts targeting a range of pollutants and substances of polymer-based **(Satti *et al.*, 2019).**

The ability of biodegradation demonstrated by (**Vijayalakshmi *et al.*, 2018)** observed that, the bacterium *Pseudomonas aeruginosa* demonstrated the ability to thrive in a nutrient broth medium containing varying concentrations of BPA, spanning from 5 mM reaching to 35 mM. The highest BPA elimination rates, amounting of 100% and 96%, were noted under conditions characterized by an initial bacterial amount of 15 ml and a salinity level of 2 percent for concentrations of BPA at 10 and 20 mg/L respectively; furthermore, the degradability of the effluent reached 69%, accompanied by a concurrent reduction in toxicity to 7% at all **(Neisi *et al.*, 2020).** That was agreed with the results obtained in this study.

Furthermore, *Bacillus spp*. had the capability to utilizing BPA as the only carbon source; isolated from the creek sediment located within recycling electronic-waste sites, these bacteria demonstrated remarkable efficiency by completely removing 100 percent of 5 mg/L BPA under an optimal aerobic condition; this underscores the efficiency of *Bacillus spp*. in serving as effective agents for BPA biodegradation, especially in environments associated with electronic-waste contamination **(Zhang *et al.*, 2013).** On the other hand, Park and Chin reported that, *Bacillus sp.* abled to grow in a BPA-enriched MSM; remarkably, Bacillus sp. demonstrated the ability to degrade 84.68% of BPA within a 72-hour period, employing an initial BPA concentration at about 25 ppb; this finding highlights the proficiency of these bacteria in efficiently degrading BPA under controlled environmental conditions **(Park and Chin, 2023).** *Enterobacter,* when cultivated in MSM with a singular carbon source of 200 mg/L BPA, exhibited a significant capability for BPA degradation; subsequent to 8, 24, and finally 48 hours of cultivation, the residual quantity of BPA within the cultures

was assessed through analysis using HPLC; so, remarkably, these bacteria demonstrated the capacity to biodegrade 53.50±0.153 mg/L of BPA within the 48-hour period, surpassing the degradation observed in the control medium **(Zhang *et al.*, 2007).**

The toxicity bioassay indicated that, the degradation of BPA by *Acinetobacter spp.* not only led to elimination of this component, but also correlated with a reduction in its harmful effects; this suggests that, the tested strains of *Acinetobacter spp*. had served as effective tools for BPA removal in the context of wastewater treatment, emphasizing their significance in mitigating the environmental impact of this pollutant **(Noszczyn´ska *et al.*, 2021).** *Acinetobacter sp*. demonstrated the ability to eliminate 20±3% of BPA at an initial level about 100 mg/L; considering the profound influence of pH values on the activity of the numerous enzymes; their impact on the capacity of BPA degradation by the bacteria were systematically evaluated; it was noted that pH variations might impede the remediation process by blocking the most active sites of the degradation enzymes potentially, thereby inhibiting the efficiency of the BPA degradation processes **(Go´rny *et al.*, 2019).**

As a result, a strain exhibiting significant BPA breakdown capability was isolated and identified as *Proteus mirabilis* throughout this study. Subsequently, it was observed that, these bacteria were able to decomposing 1 mg/L and 20 mg/L of BPA by 98.22% and 66.77%, respectively, within a 72-hour period; this highlighted the efficacy of *Proteus mirabilis* in BPA degradation and expressed its potential applicability in environmental remediation processes **(Hou and Yang, 2022).**

The interactions between BPA and microorganisms throughout the biodegradation process are indeed intricate; Microorganisms exhibited a range of metabolic control mechanisms aimed to countering and neutralizing BPA-induced

stress; these mechanisms were orchestrated by the microbial community to adapted to and efficiently degraded BPA, thereby mitigating its adverse effects on the environment totally **(McDonough *et al.,* 2021; Tian *et al.,* 2022).** The examination of bacterial degradation behaviour had predominantly focused on the impact of metabolites such as enzymes and polysaccharides; hence, there are inherent limitations in elucidating the breakdown mechanism of BPA solely by using these methods **(Han *et al.,* 2023; Li *et al.,* 2023 a).** The identification of several species of *Bacillus,* and *E. coli* among other species of microorganisms related to plastic degradation in the soil samples led to speculate that they could colonize the polymers and contribute to their fragmentation and degradation (**Raposo *et al.,* 2022).**

Indeed, molecular analysis held the promise of providing a more profound and comprehensive understanding of cognition; this approach allowed for detailed exploration of various aspects, as identifying the metabolites involved in bacterial degradation functions, elucidating the pathways employed by bacteria for degradation of pollutants and uncovering the modes of bacterial transport to pollutants; by delving into these molecular intricacies, researchers can gain deeper insights into the mechanisms and processes underlying bacterial degradation of pollutants, contributing to more nuanced comprehensions of cognitive functions in this context **(Xiao *et al.,* 2020; Zhu *et al.,* 2023).** Hence, there remained a notable gap in research concerning the evaluation of biodegradation mechanisms from a sufficiently comprehensive perspective **(Koh and Khor, 2022).**

4.3. Detection of *bisdAB* operon in BPA-degrading Bacteria:

After DNA purification of isolated bacteria that shown BPA biodegradability and using specific primer for this gene. The results obtained revealed in the figure (4-7).

Serratia plymuthica line 1-3 had positive results, *Pantoea spp* line 4-5 had positive results, *Shingomonas paucimobilis* line 6-8 had positive results, *Acinetobacter haemolyticus* line 9 had positive results, *Pseudomonas aeruginosa* line 10 had negative results while line 11-12 had positive results, *Acinetobacter lwoffii* line 13 had negative results, *Proteus spp.* line 14-15 had positive results, *Escherichia coli* line 16 had positive results while line 17 had negative results, and finally *Bacillus spp.* line 18 had positive results , and line 19-20 had negative results.

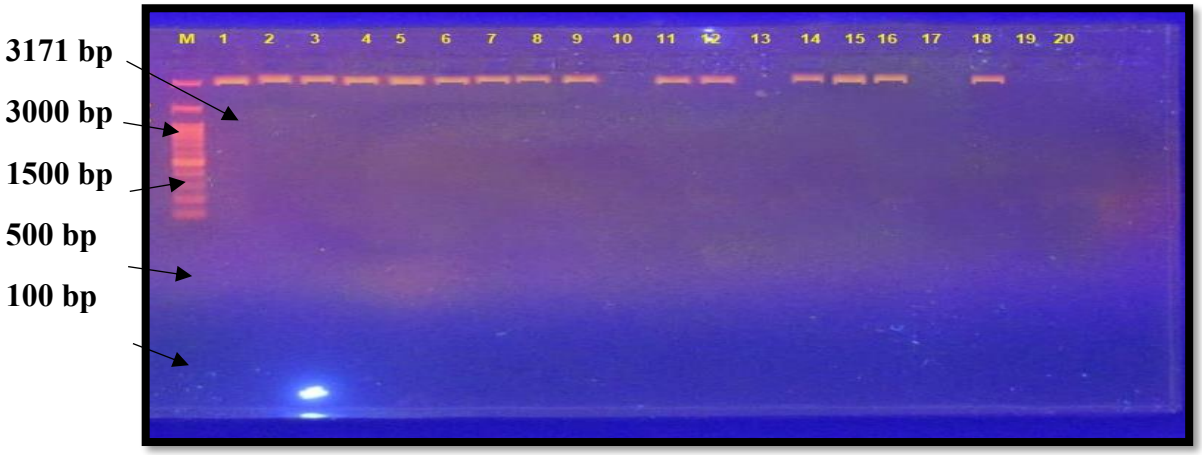


Figure (4-7): Gel electrophoresis of *bisd AB* operon, the isolates no. (1-9, 11-12, 14-16, and 18) had positive results, and isolates (10, 13, 19 and 20) had negative results.

The *bisdAB* operon from *Sphingomonas spp*. was cloned into the bacteria *Enterobacter gergoviae*,**(Badiefar *etal.*,2015)** and the impact of its expression and degradation activity of BPA had been investigated; so, strains carrying the *bisdAB* operon were successfully isolated on media with 25 µg/mL BPA as the only carbon source, confirming the presence of the *bisdAB* operon and its role in BPA degradation activity **(Eltoukhy *et al.*, 2022).**

The expressing the *bisdAB* genes in various strains of *E. coli* grown on BPA-containing medium increased the degradation activity of BPA, from 10 mg/L to 30-90 mg/L within 18 hours; these engineered strain of *E. coli* exclusively converted BPA into 1,2-bis,4-hydroxyphenyl-2-propanol (byproduct1), a cytochrome P450 monooxygenase activity product; additionally, another product of BPA degradation by these modified cells was detected as byproduct II, which was not previously observed in degradation pathway of BPA in these strains **(Sasaki *et al.,* 2005).** The proposed degradation pathway of BPA outlined specific enzymes as well as, encoded genes; further verification solidified the cytochrome C P450 (CYP450) role, in degradation of BPA; a notable reduction in BPA degradation was observed in the presence of a CYP450 inhibitor; subsequently, a CYP450 *bisdAB*-deficient strain exhibited a loss in its ability to transform BPA compared to the wild type strain; moreover, introducing *bisdAB* into *E. coli* enabled the degradation of 66 mg/L of BPA within 24 hours; these findings underscore the importance of CYP450 in the BPA biodegradation **(Eltoukhy *et al.,* 2022).**

Bacteria carrying the *bisdAB* gene had been reported to acquire the capability to degrade BPA; conversely, the *bisdAB* gene knockout strain had no ability to remediate BPA, highlighting the crucial role of P450bisdB for BPA metabolism as an essential initiator in bacterial strains; therefore, for remediation BPA-polluted soil, strains containing this operon significantly enhanced the biodegradation of BPA in conjunction with the soil microbial community; these findings suggested that, such strains hold promise as effective microbes for BPA removal, showcasing significant application potential **(Jia *et al.,* 2020 a).**

Some bacterial isolates yielded negative results for the *bisdAB* gene, indicating a potential absence of this specific metabolic pathway for BPA degradation in those bacteria; this observation suggested the possibility of an alternative BPA metabolic pathway existing in these bacterial strains.

Amino-benzotriazole (ABT), a commonly used as an inhibitor of CYP, was employed to diminish the activity of CYP enzymes; its application is often employed to assess whether a reaction is mediated by CYP enzymes **(Hayashi *et al.,* 2007; Mtibaa *et al.,* 2018).** Following 9 hours of incubation, varying concentrations of ABT (0.1, 0.5, 1, and 2 mmol/L) resulted in the disappearance of 46.8%, 12%, 6.5%, and 3.4% of BPA, respectively, compared to 85.5% in cultures lacking ABT, the presence of ABT significantly hindered BPA degradation, with degradation efficiency declining as ABT concentration increased, indicating the involvement of CYP in BPA degradation; these observations support previous research indicating the role of CYP in the transformation of xenobiotics **(Wei *et al.,* 2018).** Though, the inclusion of ABT resulted in a minor reduction in the rate of BPA removal and that indicating, the significant involvement of CYP in the conversion process **(Jia *et al.,* 2020 b).**

The results indicate that specific strain of bacteria exhibited robust soil remediation capabilities; during the initial phase of BPA degradation; this strain, equipped with CYP enzymes, takes on a predominant role; Meanwhile, other microbes lacking genes responsible for the initial breakdown of BPA but possessing pathways for its further degradation contribute to the transformation process; this synergistic substrate exchange between BPA-degrading and non-degrading microbes enhances pollutant removal in the environment; given its promising potential strain, emerges as a viable candidate for environmentally-friendly bioremediation applications **(Eltoukhy *et al.,* 2020).**

In soil, radio-labelled BPA displayed rapid dissipation (< 3 days) according to **(Fent *et al.*,2003).** The primary dissipation pathway involved the creation of non-extractable residues, accounting for 76.0 - 81.6% of residues after 120 days across four tested soils; Only a minor fraction, less than 20% of the applied radioactivity, was recoverable; subsequent research by (**Ying and Kookana, 2005)** affirmed the

swift dissipation (at 7 days) of BPA in soil under aerobic conditions; however, no degradation was observed under anaerobic conditions.

The study conducted by **(Eltoukhy *et al.*, 2022),** presented evidence to indicate the involvement of *CYPbisd* in BPA degradation; however, this evidence alone cannot definitively conclude that no other genes can perform the same function; therefore, it became necessary to investigate whether the deletion of *bisdB* from the bacteria studied, would impact its ability to metabolize BPA; consequently, a *bisdB* knockout strain was created, and assessed for BPA degradation; intriguingly, the bacteria lacking *bisdB* completely lost its capacity to degrade BPA and was unable to thrive when BPA was the sole source of carbon and energy; this suggested that, the *bisdB* gene is essential for initiating the catabolism of BPA.

Most research on bacterial degradation has centered on examining its effects and pathways; while the breakdown of BPA has received considerable attention, our comprehension of the pivotal genes and metabolic processes involved remains restricted; thus, delving into microbial genomes emerges as a crucial stride toward enhancing our understanding, particularly in terms of how microorganisms defend against and degrade BPA; such investigations will facilitate the development of targeted bioremediation strategies for contaminated ecosystems **(Li *et al.*, 2023 a).** The effectiveness of PBA degradation heightened with greater concentrations of microorganisms. The highest level of removal occurred when the BPA concentration was 10 mg/L and the seed size was 15 mL **(Neisi *et al.*, 2020).** BPA-degrading bacteria have been isolated from various environmental substrates, showing a range of capabilities; the efficacy of BPA degradation by these bacteria varies among different strains; and the involvement of the cytochrome P450 system enables these bacteria to thrive in basal salt mineral medium using BPA as their sole carbon source **(Matsumura *et al.*, 2009).**

4.4. Identification of Bacterial Isolates by Sequencing *16rRNA* Gene:

Only 31 bacterial isolates detected to had BPA-degrading bacteria. Identified biochemically and morphologically. These isolates were identified further by using Vitek2 system as shown earlier. After that for more precise identification, (10 isolates) were selected to identified using sequencing for 16rRNA gene by using universal primer for this gene, to confirm their identification. The PCR products shown in figure (4-7).

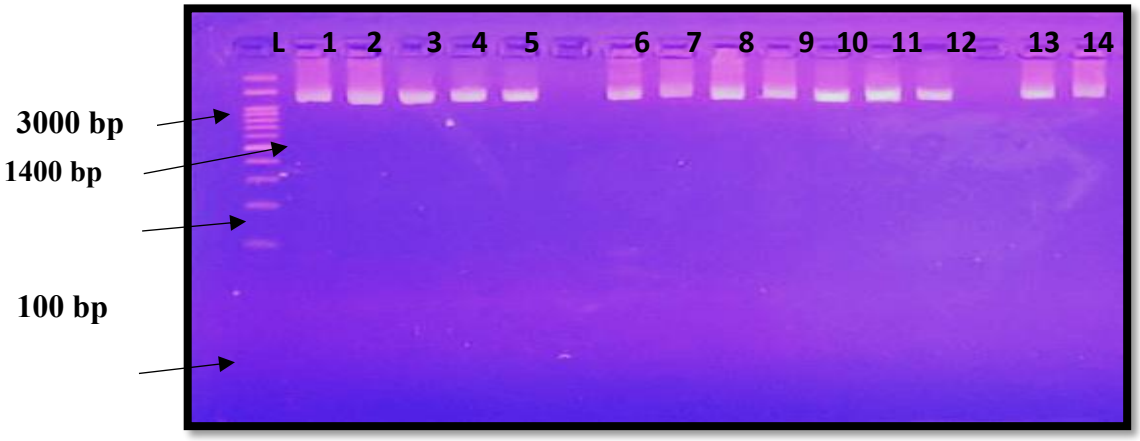


Figure (4-8): Amplefication product of *16rRNA* gene. Product size= 1400 bp and, L=ladder

Then the PCR products sent for sequencing and the results of sequences were illustrated in Table (4-5) and **Appendix (IX, X, XI and XII).**

Table (4-5): The sequencing analysis results of bacterial isolates

16 rRNA identified bacteria	VITEK2 Analysis	Percentage of bacterial isolate
Bacillus licheniformis	*Shingomonas paucimobilis*	99%

Brevibacterium borstelensis	*Serratia plymuthica*	97%
Brevibacterium borstelensis	*Acinetobacter haemolyticus*	98%
Bacillus aerius	*Bacillus spp.*	90%
Bacillus sp. (firmicutes)	*Proteus spp.*	48%
Sphingomonas Bisphenolicum	*Pseudomonas aeruginosa*	92%
Bacillus licheniformis	*Bacillus spp.*	97%
Enterobacter gergoviae	*E. coli*	77%
Achromobacter xylosoxidans	*Serratia plymuthica*	93%
Bacillus licheniformis	*Pantoea spp*	95%

The study that conducted by **(Park and Chin, 2023),** unveiled that 12 *Bacillus* species (including *B. aerius*, *B. licheniformis*, and *B. paralicheniformis*) exhibited the capability to degrade BPA; moreover, the findings demonstrated significant variability in BPA removal capacity among strains even within the same species; so, among the isolates, *licheniformis* were the most dominant, and their maximum BPA degradations were 6.2–54.5%. Biodegradation emerges as the most promising avenue for addressing plastic pollution, as microorganisms efficiently utilize low-density polyethylene as their sole carbon source. This signifies a groundbreaking approach to tackling the issue of plastic waste. Bacillus licheniformis, identified as the isolate in this context, holds potential for implementing this approach **(Rani *et al.*, 2022).**

The bacterium *B. borstelensis* strain, isolated from soil, demonstrated the capability to utilize branched low-density polyethylene as its exclusive carbon source, effectively degrading it; and incubation of polyethylene with *B.*

borstelensis for 30 days at 5degrees resulted in a reduction of its gravimetric and molecular weights by 11% and 30%, respectively **(Hadad *et al.*, 2005).**

It has been reported that supplementation with *Sphingomonas bisphenolicum* was able to significantly improve the BPA decomposition activity of the microbial community in soil **(Matsumura *et al.*, 2015).** Also, *Pseudomonas spp*. could accelerate the degradation of BPA with *Sphingomonas spp* **(Jia *et al.*, 2020 b).** The metabolic pathways of *S. bisphenolicum* were also examined, revealing its capacity to enhance the efficiency of BPA degradation **(Matsumura *et al*., 2015).** Further investigation into Sphingomonas bisphenolicum revealed the involvement of the cytochrome P450 monooxygenase system in BPA biodegradation **(Sasaki *et al.*, 2008).**

Enterobacter gergoviae, isolated from petrochemical wastewater outlets, exhibited tolerance to up to 2000 mg of bisphenol A/L and degraded approximately 23 mg of BPA/L within 8 hours in a basal medium; various metabolic pathways of BPA biodegradation led to the production of different metabolites **(Cydzik-Kwiatkowska *et al.*, 2020).** *Achromobacter xylosoxidans* exhibited maximum BPA degradation at pH 7. Furthermore, increasing the size of the inoculum resulted in enhanced degradation of BPA by these strain isolated from compost leachate of municipal solid waste **(Zhang *et al.*, 2007).**

Chapter Five

Conclusions and Recommendations

5. Conclusions and Recommendations:

5.1. Conclusions:

1- Many types of bacterial that isolated from polluted soils with plastics could convert the toxic organic compound Bisphenol A to other metabolite in the media-containing it as only carbon source.

2- There was no significant difference for BPA degradation ability, among the bacteria isolated from urban and rural regiones.

3- The study concluded that, there were significant differences of the ability of bacteria to remediate BPA such as *Serratia plymuthica*, *Pantoea spp*, *Shingomonas paucimobilis*, *Pseudomonas aeruginosa* and *Bacillus spp*., comparing with other isolates of the recent study such as, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Escherichia coli* and *Proteus spp.,* according to the BPA biodegradation capacity.

4- According to current study we could employee this Bactreial activity to eliminate these materials safely from the polluted soils and water efficiently than using chemicals that might be toxic to environment, human being and animals.

5- The operon of *bisAB* gene detected in most isolates and this gene suggested to involved in BPA degradation.

5.2. Recommendations:

1- Examine the biodegradation activity of the bacteria at different concentrations of BPA and different concentration of biomass to detect the most appropriate one.

2- Identify other genes may include in BPA-degradation activity

3- Manipulate Cytochrome C gene (bisAB) in bacteria lacking it, and detect its ability to remediate BPA.

4- Extract the enzyme cytochrome C dehydrogenase and applied it in BPA degradation.

5- Using of Glasses instead of Plastic.

Chapter Six

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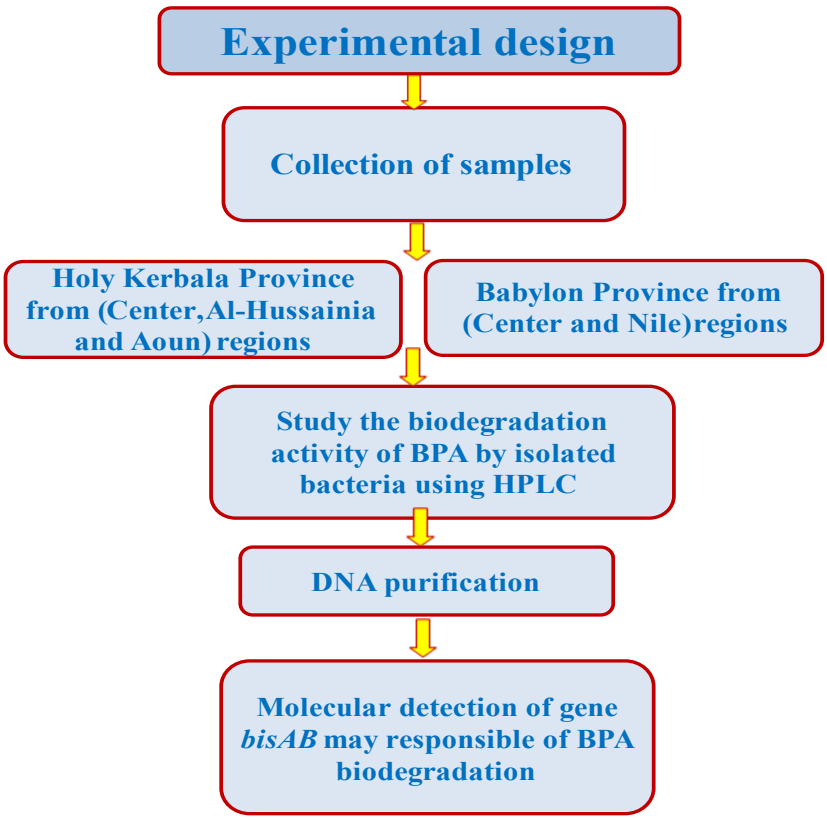
Appendices

Appendix I:



Region of Samples Collection

Appendix II:

Bisphenol A-containing Media with Positive and Negative Growth of Bacteria

Appendix III:

Patient Name: 8 ب
Location:
Lab ID: 612234

Patient ID: 612234
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Sphingomonas paucimobilis
Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.57 hours	**Status:**	Final
Selected Organism	96% Probability	**Sphingomonas paucimobilis**		
	Bionumber:	4601200000040000		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	+	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

The results of VITEK2 system to investigated *Sphingomonas paucimobilis*

Appendix IV:

Patient Name: 6 ج
Location:
Lab ID: 612239

Patient ID: 612239
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Acinetobacter haemolyticus
Source: swab

Collected:

Comments:	

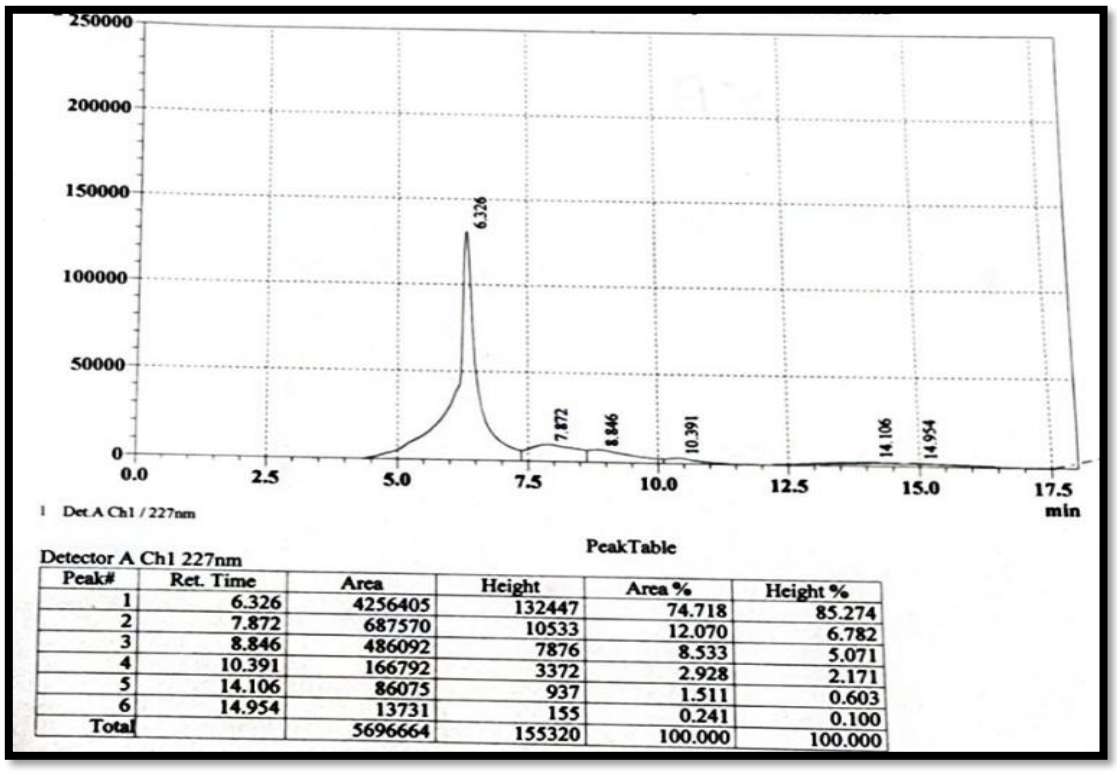
Identification Information	**Analysis Time:**	9.93 hours	**Status:**	Final
Selected Organism		**Acinetobacter haemolyticus**		
	Bionumber:	0201000100000200		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	(+)	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	(-)	62	ELLM	-	64	lLATa	-			

The results of VITEK2 system to investigated *Acinetobacter haemolyticus*

Appendix V:



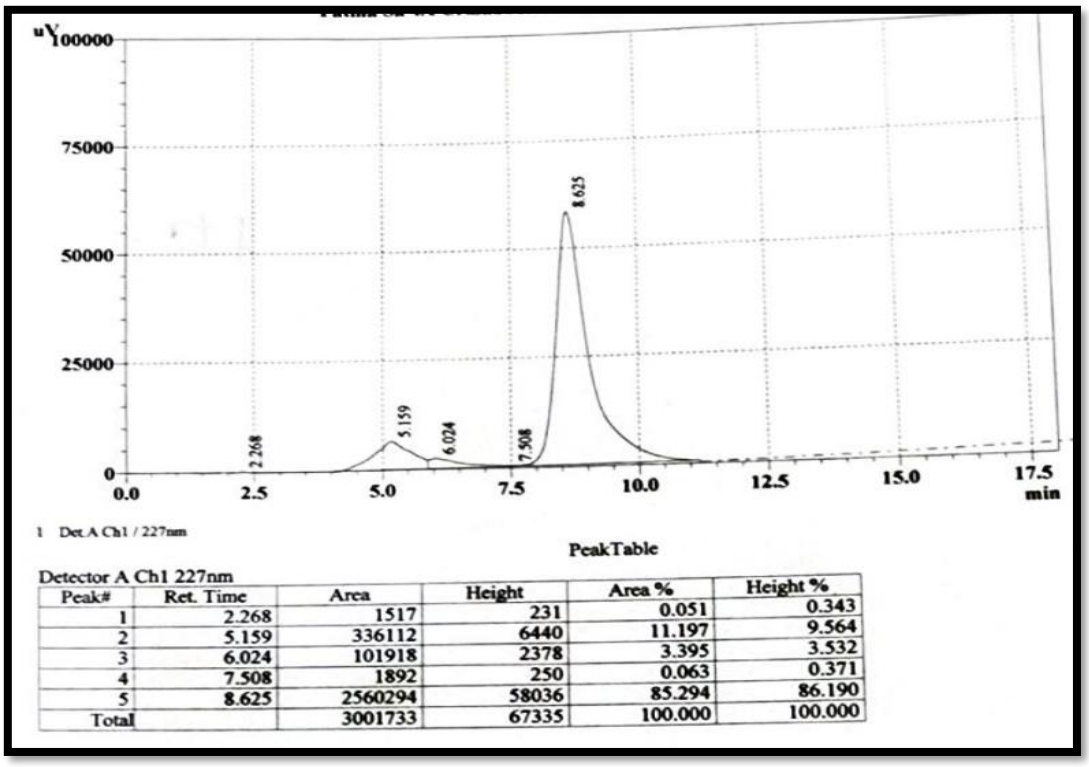
PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.326	4256405	132447	74.718	85.274
2	7.872	687570	10533	12.070	6.782
3	8.846	486092	7876	8.533	5.071
4	10.391	166792	3372	2.928	2.171
5	14.106	86075	937	1.511	0.603
6	14.954	13731	155	0.241	0.100
Total		5696664	155320	100.000	100.000

Curve of HPLC for the ability of *Serratia plymuthica.* to biodegrade BPA.

Appendix VI:



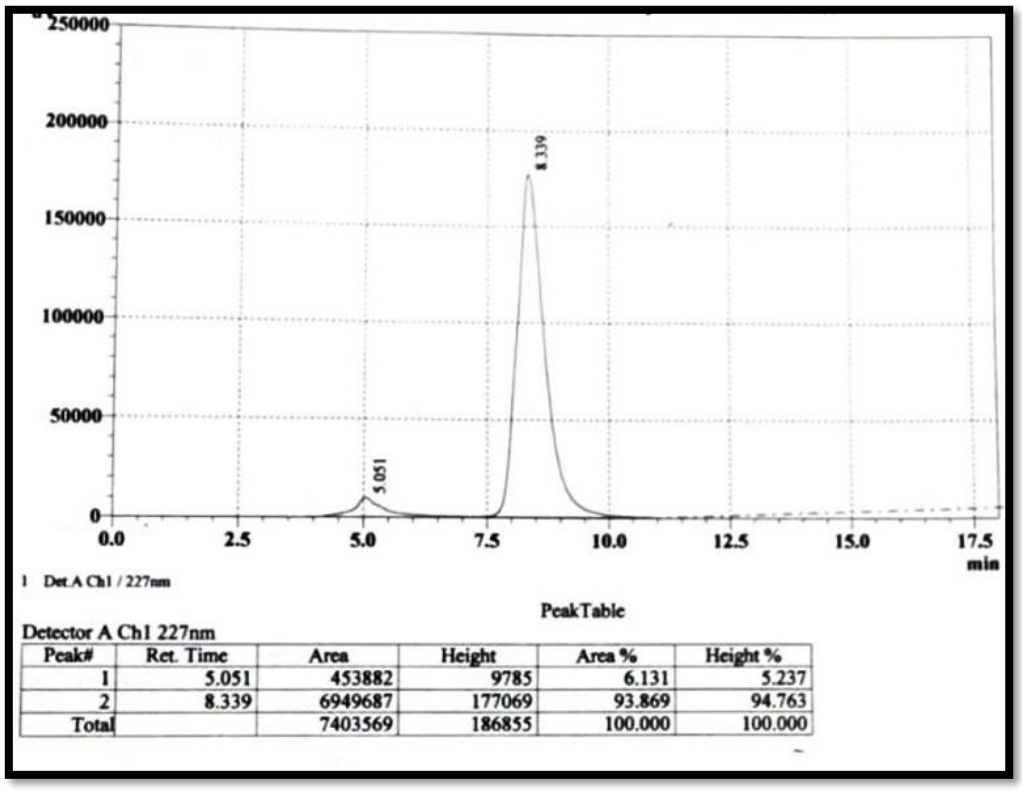
PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.268	1517	231	0.051	0.343
2	5.159	336112	6440	11.197	9.564
3	6.024	101918	2378	3.395	3.532
4	7.508	1892	250	0.063	0.371
5	8.625	2560294	58036	85.294	86.190
Total		3001733	67335	100.000	100.000

Curve of HPLC for the ability of *Pantoea spp* to biodegrade BPA

Appendix VII:



Curve of HPLC for the ability of *Shingomonas paucimobilis* to biodegrade BPA

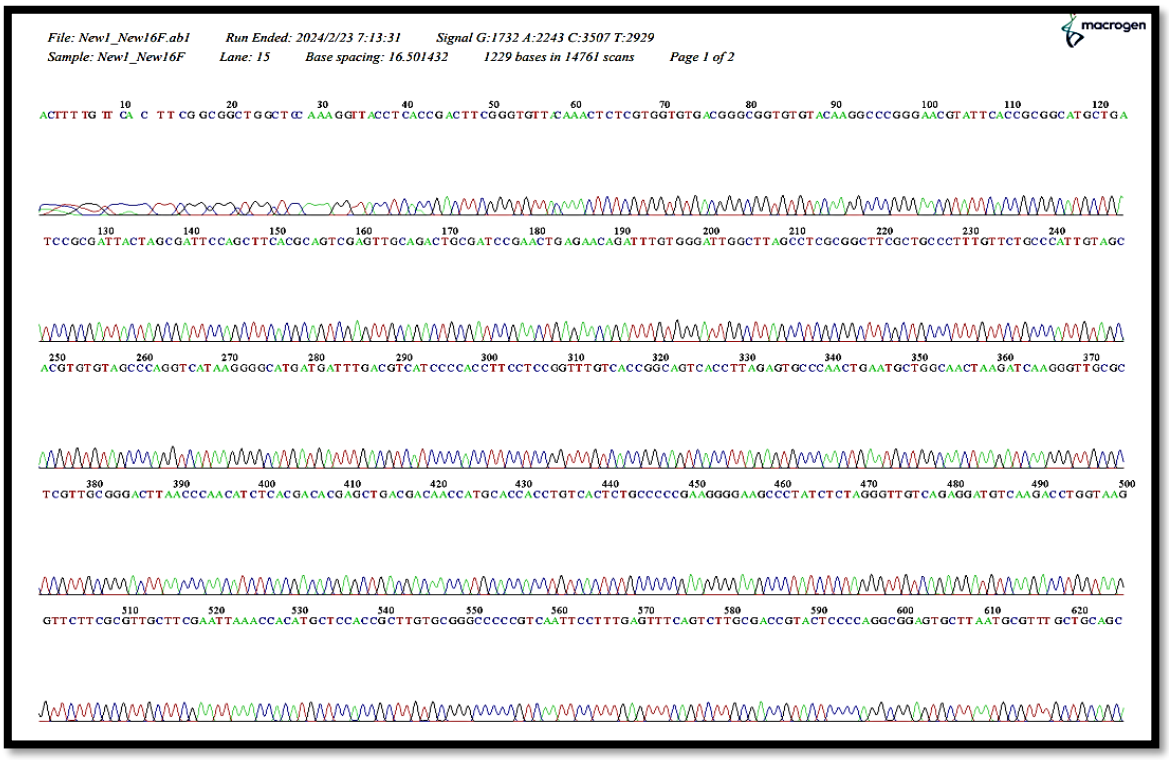
Appendix VIII:

Table: The comparison among the ability of bacterial strains to degraded BPA

Test details	Mean 1	Mean 2	Significant?	Summary	*P* Value
Serratia plymuthica vs. Pantoea spp	15.28	0.8970	No	ns	0.6546
Serratia plymuthica vs. Shingomonas paucimobilis	15.28	8.719	No	ns	0.9946
Serratia plymuthica vs. Column D	15.28	63.99	Yes	****	<0.0001
Serratia plymuthica vs. Acinetobacter lwoffii	15.28	72.81	Yes	****	<0.0001
Serratia plymuthica vs. Pseudomonas aeruginosa	15.28	16.85	No	ns	>0.9999
Serratia plymuthica vs. Escherichia coli	15.28	74.75	Yes	****	<0.0001
Serratia plymuthica vs. Bacillus spp	15.28	7.243	No	ns	0.9802
Serratia plymuthica vs. Proteus spp	15.28	65.29	Yes	****	<0.0001
Pantoea spp vs. Shingomonas paucimobilis	0.8970	8.719	No	ns	0.9832
Pantoea spp vs. Column D	0.8970	63.99	Yes	****	<0.0001

Pantoea spp vs. Acinetobacter lwoffii	0.8970	72.81	Yes	****	<0.0001
Pantoea spp vs. Pseudomonas aeruginosa	0.8970	16.85	No	ns	0.5242
Pantoea spp vs. Escherichia coli	0.8970	74.75	Yes	****	<0.0001
Pantoea spp vs. Bacillus spp	0.8970	7.243	No	ns	0.9957
Pantoea spp vs. Proteus spp	0.8970	65.29	Yes	****	<0.0001
Shingomonas paucimobilis vs. Column D	8.719	63.99	Yes	****	<0.0001
Shingomonas paucimobilis vs. Acinetobacter lwoffii	8.719	72.81	Yes	****	<0.0001
Shingomonas paucimobilis vs. Pseudomonas aeruginosa	8.719	16.85	No	ns	0.9786
Shingomonas paucimobilis vs. Escherichia coli	8.719	74.75	Yes	****	<0.0001
Shingomonas paucimobilis vs. Bacillus spp	8.719	7.243	No	ns	>0.9999
Shingomonas paucimobilis vs. Proteus spp	8.719	65.29	Yes	****	<0.0001
Column D vs. Acinetobacter lwoffii	63.99	72.81	No	ns	0.9653
Column D vs. Pseudomonas aeruginosa	63.99	16.85	Yes	****	<0.0001
Column D vs. Escherichia coli	63.99	74.75	No	ns	0.8986
Column D vs. Bacillus spp	63.99	7.243	Yes	****	<0.0001
Column D vs. Proteus spp	63.99	65.29	No	ns	>0.9999
Acinetobacter lwoffii vs. Pseudomonas aeruginosa	72.81	16.85	Yes	****	<0.0001
Acinetobacter lwoffii vs. Escherichia coli	72.81	74.75	No	ns	>0.9999
Acinetobacter lwoffii vs. Bacillus spp	72.81	7.243	Yes	****	<0.0001
Acinetobacter lwoffii vs. Proteus spp	72.81	65.29	No	ns	0.9868
Pseudomonas aeruginosa vs. Escherichia coli	16.85	74.75	Yes	****	<0.0001
Pseudomonas aeruginosa vs. Bacillus spp	16.85	7.243	No	ns	0.9438
Pseudomonas aeruginosa vs. Proteus spp	16.85	65.29	Yes	****	<0.0001
Escherichia coli vs. Bacillus spp	74.75	7.243	Yes	****	<0.0001
Escherichia coli vs. Proteus spp	74.75	65.29	No	ns	0.9484
Bacillus spp vs. Proteus spp	7.243	65.29	Yes	****	<0.0001

Appendix IX:



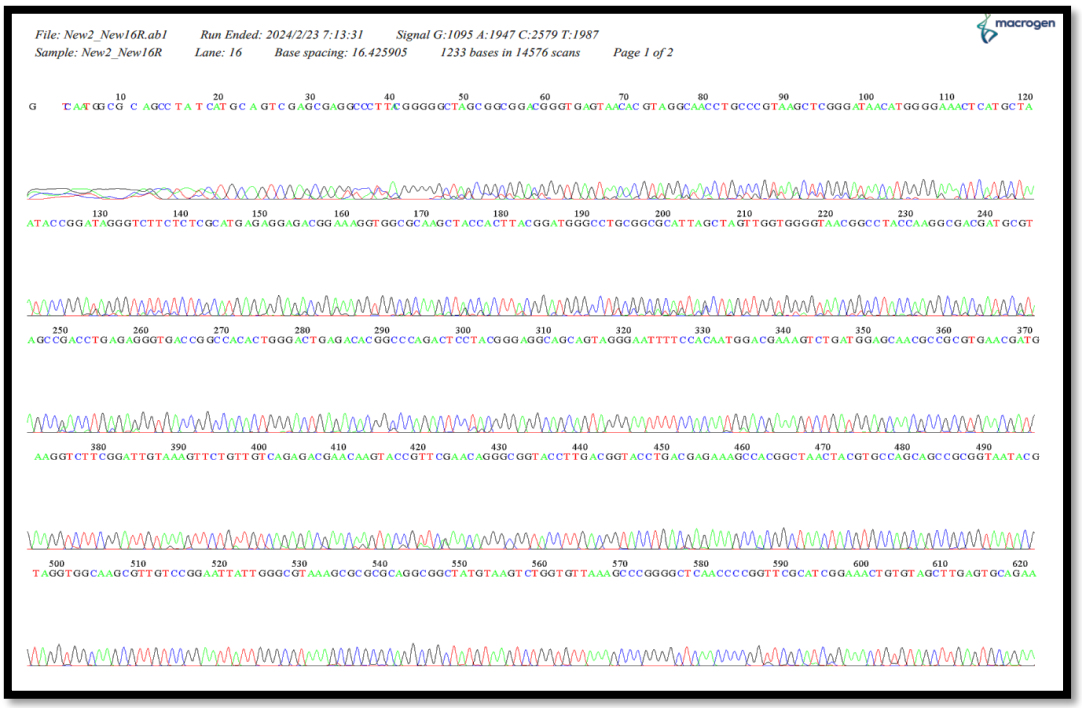
Sequence analysis of *Bacillus licheniformis*

Appendix X:

ACTTTTGTTCACTTCGGCGGCTGGCTCCAAAGGTTACCTCACCGACTTCG
GGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGA
ACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTC
ACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA
TTGGCTTAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGCAC
GTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACC
TTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCT
GGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCT
CACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGA
AGGGGAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAG
GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCG
GAGTGCTTAATGCGTTTGCTGCAGCACTAAAGGGCGGAAACCCTCTAACA
CTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTT
CGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGC
CTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT
GGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACC
CTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGC
GCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTA
CGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCA
AGGTGCCGCCCTATTCGAACGGGACTTGTTCTTTCCTAACAAAAAATTTT
ACGATTCGAAAAAACTTCTTTACTCACGCGGTCGGTGGCTCCCTCCCGAA
CTTTCTTCCCCTTGGCGGGAAAAAATCCCACCTGTTGGCTCCCCCCGGAA
GATGGGGGGCGGGGGGGCGTCCCCAGGGGGGGGGGGAAAACCCCCTCTTC
GGCGGGGGAAGACAAACACCCCCCTTGGG

Sequence analysis of *Bacillus licheniformis*

Appendix XI:



Sequence analysis of *Brevibacterium borstelensis*

Appendix XII:

GGGCCGTTAAGGGCAAGCATAGCGTGTGAGTCCAGCGGATTACTTAACAG
ACTTCGCGGTGTTACAAACTCCCGTGGTGTGACGGGCGGTGTGTACAAGG
GCCGGGAACTGGGGGCCGCGTGATGCTGATCCACGATTACTAGCGATTCC
GACTTCATGCCGGCGAGTTGCAGCCTGCAATCCGAACTGAAACTGGTTTT
AAGAGATTGGCTTACTCTCGCGAGCTAGCTTCCCGTTGTACCAGCCATTG
TAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATC
CCCGCCTTCCTCCGTCTTGTCGACGGCAGTCTCTCTAGAGTGCCCAACTG
GATGCTGGCAACTAAAGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCA
ACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACCGCTG
CCCCGAAGGGAAGCCCTATCTCTAGGACGGTCAGCGGGATGTCAAGACCT
GGTAAGGGTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTG
TGCGGGCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGAGCGTACTCCC
CAGGCGGAGTGCTTATTGCGTTAGCTGCGGCACTGAGGGTATTGAAACCC
CCAACACCTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAAT
CCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAA
AGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCT
GCACGTGGAATACCGCTTTCCTCTTCTGCACTCAAGCTACACAGTTTCGA
TGCGAACCGGGGTTGAAGCCCGGGCTTTAACACCAGACTACATAGCCGCC
TGCGCGCGCTTTACGCCCAATAATCGGACAACGCTTGCCACCTACGTATT
ACCGCGGCTGCTGGCACGTAGTTAGCGTGCTTTCTCGTCAGGTACCGTCA
AGGTACCGCCCTGTTCGAACGTACTTGTTCGTCTCTGACAACAGAACTTA
CATCGAAGACTCATCGTCACGCTGCGTGCTCATCAGACTTCGTCATTGAG

Sequence analysis of *Brevibacterium borstelensis*

الخلاصة

البسفينول أ(BPA) ، المعروف أيضًا باسم 2،2 (هيدروكسي فينيل) بروبان ، هو مادة صناعية تستخدم على نطاق واسع في صناعة البلاستيك، وخاصة البولي كربونات وراتنجات الإيبوكسي على مدى سنوات تتواجد هذه المادة على نطاق واسع في التربة ,الرواسب وكذلك المياه الجوفية والسطحية. تهدف هذه الدراسة إلى عزل انواع مختلفة من البكتريا من الترب الملوثة بالبلاستك وبيان قدرة هذه البكتيريا على التحليل الحيوي تجاه البسفينول أ.

في هذه الدراسة تم أخذ خمسين عينة من الترب الملوثة بالنفايات البلاستيكية من مواقع مختلفة في محافظة كربلاء المقدسة من مناطق (المركز والحسينية وعون) ومحافظة بابل من مناطق (المركز والنيل). بواقع عشر عينات من كل منطقة.

تم استخدام الاوساط الملحية الدنيا (MSM) للتعرف على قدرة هذه العزلات على تحلل مادة البسفينول أ باستخدام تقنية HPLC. حيث تم زراعة البكتريا في هذه الاوساط الحاوية على البسفينول أ كمصدر وحيد للكربون. وأظهرت النتائج التي تم الحصول عليها أن 31 عزلة كانت لها القدرة على النمو في هذا الوسط من أصل 50 عينة مأخوذة من التربة الملوثة. توصلت النتائج إلى عدم وجود فروق ذات دلالة معنوية بين عدد البكتيريا ذات القابلية للتحلل الحيوي BPA المعزولة من مراكز المدن والمناطق الريفية. على الرغم من ان مناطق (مركز كربلاء وبابل) كانت فيها عدد أكبر من العزلات المحللة للبسفينول أ مقارنة بالمناطق الريفية (الحسينية، و عون، والنيل).

كما وأظهرت النتائج أن العزلات البكتيرية قادرة على تحليل مادة البسفينول أ بنسب مختلفة بدأت من 18.7% إلى 99.9%. وكانت هناك اختلافات معنوية في قدرة البكتيريا على معالجة مادة BPA مثل *Serratia plymuthica* و *Pantoea spp* و *Shingomonas paucimobilis* و *Pseudomonas aeruginosa* و *Bacillus spp.* مقارنة مع العزلات الأخرى للدراسة الحاليه مثل *Acinetobacter haemolyticus* و *Acinetobacter lwoffii* و *Escherichia coli.* و *Proteus spp.* ، وفقًا لقدرتها على التحلل الحيوي لهذه المادة.

بعد تنقية الحمض النووي، تم التعرف على أوبرون *bisdAB* في البكتيريا المحللة لـلبسفينول أ، وأظهرت النتائج أن هذا الجين موجود في أكثر عزلات الدراسة. وكما أعطت بعض العزلات البكتيرية نتائج سلبية لجين *bisdAB*، مما يشير إلى احتمال اختلاف المسار الأيضي المحدد لتحلل البسفيول أ في تلك البكتيريا؛ وتشير هذه الملاحظة إلى إمكانية وجود مسار استقلابي اخرللتحلل موجود في هذه السلالات البكتيرية.

تم التعرف على عشر عزلات بكتيرية باستخدام تسلسل الجين rRNA16، وقد أعطت النتائج وصفا أكثر دقة لهذه العزلات البكتيرية .حيث تم الكشف عن عمل الجين *bisAB* في معظم العزلات، ويقترح أن يكون هذا الجين له الدور الكبير في تحلل هذه المادة.

نستنتج من نتائج الدراسة أن العديد من العزلات البكتيرية يمكنها تحويل المركب العضوي السام البسفينول أ إلى مستقلب آخر في الوسط الذي يحتوي عليه كمصدر للكربون فقط. ووفقاً لدراسة حديثة يمكن توظيف هذا النشاط لإزالة هذه المواد بشكل آمن من التربة والمياه الملوثة بكفاءة أكبر من استخدام المواد الكيميائية التي قد تكون سامة للبيئة والإنسان والحيوان .

وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الصحة العامة

المعالجه الحيويه

المعالجه الحيويه للتربه الملوثه بالبسفينول أ باستخدام انواع مختلفه من البكتريا

رسالة مقدمة إلى

مجلس كلية الطب البيطري/ جامعة كربلاء كجزء من متطلبات درجة ماجستير في الطب البيطري / الصحة العامة البيطريه

اعداد

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