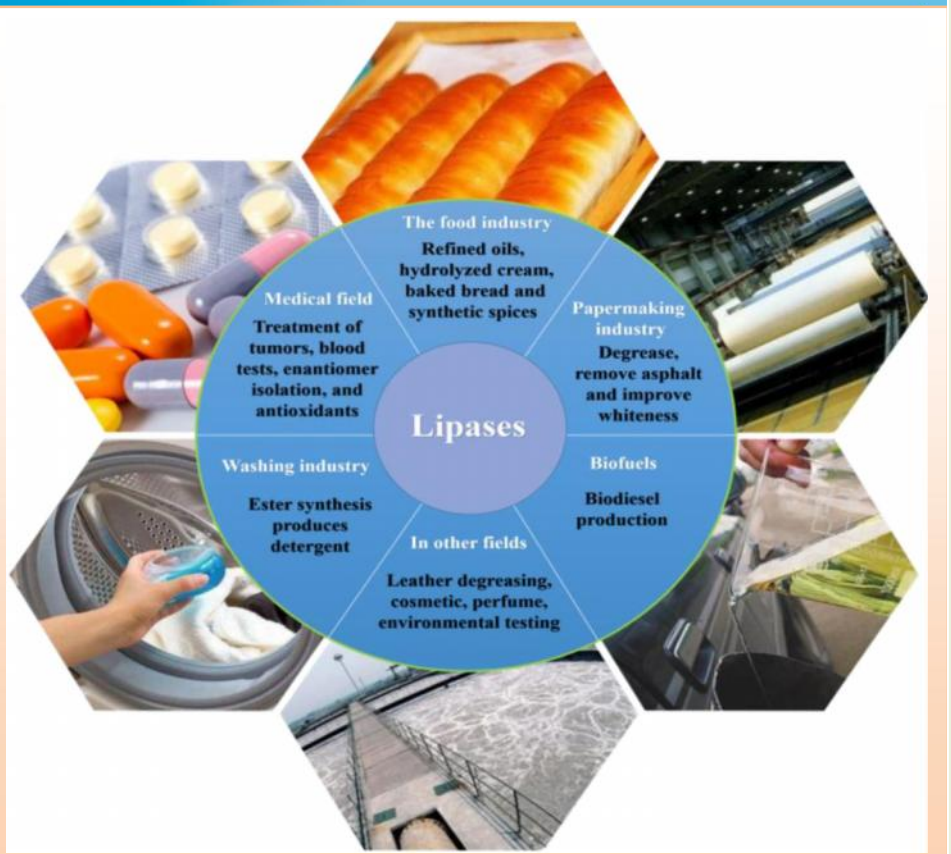

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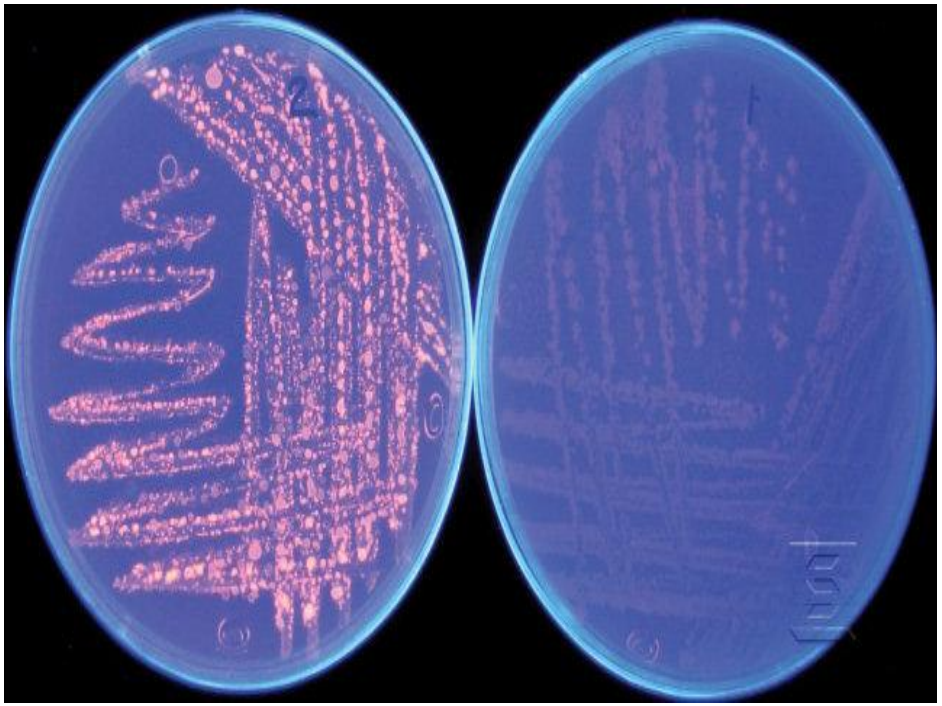
Bacterial lipase from untapped soil of Panikhaiti, Guwahati

First Edition

Dr. Jai Shanker Pillai HP,
Dr. Laishram Shantikumar Singh,
Miss. Risa Debbarma,
Dr. Venkat M Shinde



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E-mail: narennarayanasamy@gmail.com

About the Authors

Dr. Jai ShankerPillai H P.pursued his Doctor of Philosophy in Microbiology from Gulbarga University, Kalaburagi, Karnataka, India. He is currently serving as the Associate Professor in the Department of Microbiology, Faculty of Science Assam downtown University, Guwahati, Assam. He has 15 years of teaching experience at University level, 12 years of research experience and 2 years of Industrial experience. Dr. Pillai is a recognized research supervisor for Microbiology and interdisciplinary doctoral research program, currently guiding 3 research fellow. Dr. Pillai taught the students of Microbiology not only in the universities in India, he also disseminated his knowledge of microbiology to the overseas students in various countries including Thailand, Myanmar, Nepal, Armenia and Barbados. He also ignited the minds of medical students with his excellency in teaching medical microbiology abroad. His research contributions are particularly in the field of Environmental, Medical and Agricultural Microbiology. He has publishedone Indian patent on "Nanofilter System based Biomass conversion and recycling process" and filed one Indian copyright on "A Novel treatment of Cervical Cancer Using *Astralgus* roots, *Rosmarnius officinalis*, *Garcninia nujiangenesis*. He published 7 review articles and 20 research articles in internationally reputed journals, 2 in national journals. He has also published 5 books and one book chapter. Dr. Pillai is successful in organizing the various numbers of National and International webinars, conferences, symposiums, Seminars and workshops in the hot and emerging fields of Microbiology. Considering his contributions in the field of biological sciences, Tamil Nadu Scientific Research Organization (TNSRO) awarded him "HarGobind Khorana Young Scientist Award; 2011-12". Owing to his significant contribution, he was awarded as Distinguish Fellow of Bose Science Society; 2012-13. Dr. Pillai was also honored with Dr. A.P.J AbulKalam Distinguish Fellow award; 2016 -17 at a National conference on Natural Science held at Holy Cross College, Tiruchirappalli, Tamil Nadu, India. Dr. Pillai is an active life member of Association of Microbiologists of India, National Environmental Science Academy, Bose Science Society, Deccan Environmental Research Organization and International Society for Applied Life Sciences. INSO recognized the dedication of Dr. Pillai in the field of Microbiology research and honored him as Best Researcher on 14.05.2022 at Chennai, Tamil Nadu. Dr. Pillai also honoured with DR. Sir C V Raman National Sceince Day Award on 28 February 2023 by the organization VOICE, Tamil Nadu.

Dr. **Laishram Shantikumar Singh** earned his graduate, post degree in Microbiology from Bangalore University, Bengaluru, India and PhD degree in the field of Microbial Biotechnology and Applied Microbiology from Dibrugarh University, India through research work at Council of Scientific & Industrial Research (CSIR) - NEIST, Jorhat, Assam, India. Currently he is serving as Associate Professor and Program head, Department of Microbiology, Assam Down Town University, Guwahati, Assam, India. Prior to his present assignment Dr. Laishram had worked as Scientist, Microbial Biotechnology and Microbial Resources group at Institute of Bioresources and Sustainable Development, Sikkim Centre, Department of Biotechnology (DBT), Govt. of India, Gangtok, Sikkim, India. Dr. Laishram has also worked in renowned corporate R & D sectors of Tata Tea Research Centre in APPL as Assistant Manager R & D and Advisor Microbiologist where he was actively instrumental in setting up of the state-of-the-art Biofertilizer & Biopesticide unit of the Centre at Teok, Jorhat, Assam. After completing his doctoral research, he has worked on post-doctoral research at the University School of Biotechnology at Guru Govind Singh Indraprastha University, Delhi. Dr. Laishram had served as lecturer at NFG College (present Brindavan College), under Bangalore University, where he taught UG and PG courses of Microbiology and Biotechnology. He has also taught Microbiology courses as visiting faculty at The College of Agricultural Engineering and Post Harvest Technology (CAEPHT), CAU, Ranipool, Gangtok, Sikkim, India. His research focuses on Microbial biotechnology, microbial bioprospecting, plant endophyte relation and crosstalk, endophyte ecology, microbial secondary metabolites, bioactive compounds, antimicrobial agents and fight against multidrug pathogens as well as opportunistic pathogens. Other research interests include ethnomedicinal plants and their validation. He is the recipient of several awards and recognitions for his academic and research achievements, including Group Technology Award 2009 given by DRL, Defence Research and Development Organization, Ministry of Defence, Government of India. He has been awarded Junior Research Fellowship and Senior Research Fellowship during the course of his Ph.D. work by DRDO, Govt. of India. He has been the recipient of certificate of appreciation from Asian PGPR society and American Society for Microbiology, Uzbekistan unit. Dr. Laishram has been awarded as the best faculty of the month at Assam Down Town University for endeavor in teaching and research front. He has more than 40 research papers publish in National and International Journal of repute and 4 book chapters. He has presented research articles in many National and International conferences. He has

published more than 20 research data in NBCI Genbank database. So far 24 students have carried out their M.Sc. dissertation project under his guidance. He is a recognized Ph.D. guide and supervising six Ph.D. students at present. Dr. Laishram has worked and participated in many R & D projects funded by government agencies and at present is working on research projects funded by DBT, Government of India. Dr. Laishram is active member of national scientific bodies as well as societies and editorial member in several reputed journals. He is member of several departmental and institutional committees for society and scientific and served as external expert in quite many panels of college and institution. Dr. Laishram has delivered many lectures as invited speakers in conferences and seminars.

Miss Risa Debbarma is graduate in Microbiology and pursuing post graduate in Microbiology from Assam Down Town University, Guwahati, Assam, India. She has completed one-month online course on "DNA DECODED" offered by McMaster University through Coursera and Seven Days Student Development Course on "The Blue Print of Life" organized by Faculty of Science of Assam Downtown University. She is well versed in the field of general and applied microbiology. She has worked on quite many project assignments including "Microbial Spoilage of bread, succession of fungal species in it and their isolation and characterization" under the supervision of Dr. Laishram Shantikumar Singh, Associate Professor, Department of Microbiology, Faculty of Science, Assam Down Town University, Guwahati, Assam, India

Dr.Venkat M Shinde, did his MSc in Botany in the year 2004 from Gulbarga University Kalaburagi, and obtained his PhD in 2009, From Gulbarga University Kalaburagi, presently working as full time guest faculty from 2009 to till date in the Department of Botany Gulbarga University Kalaburagi. He as 14 years of experience in teaching and research. He has significantly contributed in the field of Mycology, Plant pathology and Mycorrhizae and he has published more than 20 research articles in peer reviewed National and International journals. And published 03 three books successfully.Owing to his research contribution in the field of Biological Sciences, he has been awarded as J C Bose best scientist award in 2012-13 by Tamilnadu Scientific Research Organization (TNSRO), based on his significant contribution he was awarded as distinguish fellow of Bose Science Society in the Year 2012-13. He as been recently awarded as International best researcher award for the year 2021-22.

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Chapter – I
Introduction

CHAPTER 1: INTRODUCTION

1.1 Soil

The essential component of the environment and the coordinator of the terrestrial ecosystem is the soil. The physico-chemical and biological characteristics and functions of terrestrial ecosystems are all significantly impacted by soil. In the terrestrial ecosystem, soil is well known for its vital functions viz., providing habitation for variety of organisms, promoting the developments of plant, regulation of hydrologic cycle, recycling of wastes and also serves as a building raw material etc.

Weathering is the process that creates soil. Rocks continually break both physically and chemically as a result of phenomena called weathering. Several factors, such as wind, water, or climate might influence this. The five main elements that affect soil formation are- climate, parent rock, geography, organisms, and time—variate from one soil type to another.

1.2 Soil Profile:

Soil profile is the composition of several layers of soil from the soil's top down to where it meets the rocks below. The texture and color of the soil make it simple to identify different soil profile layers. The soil makes up the topmost layer of the Earth's crust. The most important layer is made up of diverse organic minerals and small pieces of rock. When soil is created, it settles

into several vertical levels. Each layer (strata) is composed differently in terms of colour, texture, and chemical make-up describing the soil profile. The different layers of soil are named as top soil (Horizon A, Sub soil (Horizon B) and Parent rock / Bed rock (Horizon C).

1.1.1 Top Soil (Horizon A)

Top soil is the uppermost layer of the soil, the depth of which is usually in the range between 5-8 cm; in some soil types the depth is between 0.5-1m. The dark brown hue of the top soil generally referred to as humus is due to the partial decomposition of plant and animal remains. Soil humus blended with macroorganisms (Earthworms, Millipedes, and Centipedes etc.) and microorganisms (Bacteria, Actinobacteria and Fungi etc.,) nurture the growth of the plants making the soil rich with its nutrients.

1.2.2 Subsoil (Horizon B)

Horizon B, or subsurface, is the horizon below the topsoil. The topmost layer is thinner and softer than this one. Typically, the topsoil is darker in colour than the subsoil. It contains less humus. Minerals that were transported down from the topsoil are abundant in this layer. It also has metal salts and a significant amount of iron oxide. Additionally, to get the optimum results, farmers combine the topsoil and subsoil when they till their fields in preparation for producing any crop.

1.2.3 Parent rock or Bed rock (Horizon C)

It is an extremely hard layer since it is the parent rock, also known as bedrock, which is made up of rocks and stones. Organic material is absent. It is made up of the consolidated rock or solid material beneath the soil strata. The bedrock layer serves as the base for the soil profile and is often unweathered (Brady 2008) . The area between the parent rock of the earth's surface, the topsoil, and the subsoil is represented by this lowest layer.

1.3 Soil Properties

1.3.1 Texture

The relative abundance of these particles affects the soil's texture. The tiniest sized particles are found in clay. The clay feels smooth because of its extremely small size. Clay particles are smaller than those of silt. Their diameters range from 0.002 to 0.02 mm. Silt doesn't feel smooth, thus. The largest soil particles are sand particles. Their diameter exceeds 0.02 millimeters. The soil's ability to store water is determined by texture. The soil contains some moisture in the form of water. Typically, a thin coating of water surrounds the soil particles. The roots of the plants take it up. The growth of different crops depends on the soil's ability to retain water. Even dry dirt contains some water.

1.3.2 Water absorption

Varying types of soil have varying water-holding capacities. Since soil is porous, it can absorb water. Compared to clay and loamy soil, sandier soil stores less water. Sandier soil doesn't store as much water as clay soil. Clay absorbs more water than sand. Some plants need

more water to develop, and because sand retains less water than clay and loam, less vegetation can be found there.

1.3.3 Color

Different colors are present in soil. They are black, white, and red. The presence of iron oxide is what gives the soil its red hue. Minerals and humus are abundant in the soil's dark tint. Wheat and jowar grow well as a result.

1.3.4 pH

Soil can be neutral, acidic, or alkaline. Some plants, like potatoes and kumara, can thrive in soil that has an acidic pH (below 7). A neutral pH (7.0) is preferred by lettuce and carrots in soil. As minerals are washed away over time, soil becomes more acidic. Aluminum and manganese are typically found in higher concentrations in soils with high acidity, and sodium carbonate is typically found in larger concentrations in soils with high alkalinity.

1.3.5 Percolation Rate

Percolation of water refers to the process through which liquid moves slowly through soil. However, not all types of soils permit water to percolate at the same rate. Clay soil allows for the least amount of water percolation whereas sandy soil allows for the most. Rainwater seeps into the ground and accumulates above the bedrock. The water table refers to this depth of underground water. Since sandy soil is rather loose, the rate of water percolation is higher there than in clay soil, which is quite solid.

1.3.6 Air content

The spaces between the soil granules contain air. The oxygen needed for plant roots and other organisms to breathe is present in this air. Large sand particles are present. Sand particles have a huge gap between them because they cannot fit closely together. Air is present in the substantial void between sand grains. Because of this, sandy soil gives the plant root far more air. However, the close packing of clay particles leaves little room for air. So the plant roots that grow in clayey soil receive substantially less air.

1.3.7 Organic matter

Living plant components, primarily roots, dead organic material, primarily dead plant parts, and soil creatures (microorganisms and soil animals) in various stages of development make up soil organic matter. It significantly affects the soil's chemical, physical, and biological characteristics. Any material created initially by living organisms (plants or animals) and returned to the soil where it undergoes the breakdown process is referred to as soil organic matter. It can contain everything from intact original plant and animal tissues to the significantly decomposed mixture of elements known as humus at any particular moment.

Total soil organic matter content is the soil property most closely associated with soil structure stability. Soil organic matter accumulates over the long term to a steady-state level, which is determined by the amount of biological contributions over time, soil water content and temperature (regulating decomposition) and other factors such as texture. As primary producers in terrestrial ecosystems, plants ultimately can be credited with nearly all of the organic matter added to soil. Direct contributions occur from seasonal shedding of leaves and roots and root exudates as well as the whole plant upon death. The organic compounds added and the microbial

activity and products that result greatly enhance soil structure and improve structural stability. Qualitative differences in soil organic matter and the mechanisms of stabilization involved may account for unexplained differences in aggregate stability associated with plant species. Often much of the short-term increase in organic carbon has been found in the sand-size fraction, which includes fragments of plant tissue (Wilkinson, 2000). According to (Baker et al. 2007), soil organic matter (SOM) content, which is dynamic and responsive to changes in soil management, tillage, and plant production, is the essential building block of environmental quality.

1.4 Types of soil analyzed in this research

1.4.1 Sandy Soil

Sandy soil is chemically inert, clean and coarse. It should be free from any organic or vegetable matter. Usually 3 to 4% clay is permitted. It should contain sharp, angular, coarse and durable grains. It should not contain salts which attract moisture from the atmosphere. It should be well graded i.e., should contain particles of various sizes in suitable proportions. The finest additives for sandy soil are those that improve the soil's capacity to retain water and boost its nutrient levels. It should pass BIS No. 480 mesh sieve and should not pass BIS No. 15 sieve. Sandy soils (containing > 50% sand) are widely distributed worldwide and are characterized by their poor structure, low organic matter, weak hydraulic and nutritional properties, and low crop productivity.(Adrees M et al.,2020)

1.4.2 Silt Soil

Silt is produced when water and ice erode, or wear away, rock. Silt has particles between 0.002 to 0.075 mm in size. Due to its fineness, silt is a material with limited or no

plasticity. Silt soil, not rocky or grainy, is slippery when wet. Rocks like siltstone are created when silt deposits are squeezed and the particles are pushed together. Silts are comparatively resistant to moisture, challenging to compact, and extremely vulnerable to frost heaving. Contrary to clays, which preserve their volume with changes in shape, silt masses experience a change in volume along with a change in shape (a feature known as dilatancy). Silt soil has the characteristics of low natural moisture content and poor viscosity, and the strength and deformation required for foundation engineering can be satisfied by reinforcing and improving the silt. (Lu L et al., 2021).

1.4.3 Clay Soil

The smallest soil particles are clay particles, which are less than 0.002 mm or 2 microns in size. It is made up of minute and sub-microscopic fragments that are the result of the chemical deterioration of rock. Cohesive soil with fine grains is clay. When wet or dry, they readily adhere to one another and take on a sticky or glue-like quality. Clay soils hold a lot of water because of the gaps between clay particles, which compose more than 25% of clay. Clays are weak, highly compressible, and subject to large volumetric changes. It is studied due to its exceptional plasticity, permeability, bearing capacity, and settlement properties.

1.5 Soil Microorganisms

Various microscopic creatures known as soil microorganisms play important roles in soil ecosystems. They comprise bacterial, fungal, archaeal, algal, protozoan, and viral organisms. These microbes are necessary to preserve soil health, nitrogen cycling, organic matter decomposition, and fertility (Nannipieri et al., 2019). It is admirable that matter and

soil microbiology exist. However, it is important to remember that in soil ecosystems, minerals, organic matter, and microbes work together as a single system in ongoing connection and interaction (Huang et al., 2004). By interacting with one another and their surroundings, soil microbes create intricate microbial communities. Factors like soil type, moisture, temperature, pH, the amount of organic matter in the soil, and land management techniques have an impact on these communities (Mendes et al., 2018)

1.5.1. Soil Bacteria

Bacteria carry out crucial tasks in the soil, breaking down organic leftovers from enzymes released into the soil. According to Ingham (2009), the four main functional groupings of soil bacteria are lithotrophs, pathogens, mutualists, and decomposers. Every healthy bacterial community contributes to the recycling of soil nutrients. By using their secretions to bind soil particles together, soil bacteria create micro aggregates in the soil. These micro aggregates function as the foundation for bettering soil structure. Improved soil structure boosts water infiltration and the soil's ability to hold more water (Ingham et al., 2009).

The simple sugars and carbon molecules that are easier to digest are consumed by the decomposers, who also bind soluble nutrients like nitrogen in their cell membranes. In tilled soils, bacteria predominate, but they are only 20–30% effective in recycling carbon (C). The nitrogen (N) concentration of bacteria is larger than that of the majority of microorganisms (10–30% nitrogen, 3–10 C:N ratio) (Islam et al., 2008).

Four different species of mutualistic bacteria transform atmospheric nitrogen (N2) into nitrogen for plants. Azotobacter, Azospirillum, and Clostridium are three species of

soil bacteria that live freely in the soil and fix nitrogen without the assistance of a plant host.

Clay soil particles (0.2 m) and silt soil particles (2–50 m) are comparable in size to bacteria. In a region known as the rhizosphere, they develop and dwell in thin water films close to roots and around soil particles. Smaller than larger, more complex creatures like fungi, bacteria can proliferate and adapt to changing environmental conditions more quickly. Only nitrogen fixing bacteria can convert atmospheric nitrogen into a form (fixed nitrogen) that plants may utilize. Soil bacteria break down nitrogen in organic substrates. Because these particular bacteria produce the nitrogenase enzyme, nitrogen is fixed. The majority of soil types include a large variety of nitrogen-fixing bacteria, including both free-living soil species and bacteria that depend on plant hosts. Generally speaking, free living species make up a relatively small portion of the entire microbial population and are frequently bacteria strains with weak nitrogen fixation capabilities (Dick et al., 2009).

In soils with a neutral pH and plenty of oxygen, soil microbes thrive. Since soil frequently lacks nitrogen, bacteria feed plants with enormous amounts of nitrogen. In order to make phosphorus more soluble and available to plants, many bacteria produce enzymes into the soil. Since fungi prefer less disturbed, more acidic settings, bacteria typically predominate in tilled or disturbed soils. In flooded areas, bacteria also predominate since most fungi cannot thrive without oxygen. Due to their small size, large population, and capacity to dwell at tiny microsites within the soil where environmental conditions may be favorable, bacteria can survive in both dry and wet environments. When the surrounding environment around these microsites improves, the survivors swiftly grow.

In terms of the individual contributions made by members of those phylogenetic families of bacteria to the overall bacterial communities in soil, these groups are widely dispersed and plentiful. However, due to their apparent inability to grow on or on laboratory substrate, many of these groupings' members were not previously accessible for in-depth study (Buckley et al., 2002).

1.5.2. Enzymes from soil bacteria

It is well recognised that soil bacteria create a large range of enzymes that are essential for the cycling of nutrients, the breakdown of organic matter, and other significant biochemical activities in soil ecosystems. These enzymes are responsible for dissolving complicated organic chemicals into simpler ones that other species can easily use. Soil bacteria produces various types of enzymes such as cellulases, proteases, chitinases, amylases, lipases, phosphatases, laccases etc. (Lynd et al., 2002) .

The pace at which plant leftovers break down and release nutrients for plant uptake is accelerated by soil enzymes. The material that a soil enzyme transforms into is referred to as substrate. For instance, the soil enzyme glucosidase breaks down the ubiquitous plant component glucoside to produce glucose. Enzymes have active sites that bind to the substrate to create a transient complex that is unique to the substrate. A result of the enzymatic process, which could be a nutrient present in the substrate, is produced.

Living and dead bacteria, plant roots and wastes, and soil animals are all sources of soil enzymes. In the soil matrix, stabilised enzymes assemble or take the form of complexes, they are connected with clay, humus-clay compounds, and organic materials (humus), but not with living cells. Since 40–60% of enzyme activity is thought to come from stabilised enzymes, there may not be a strong correlation between enzyme activity and microbial biomass or respiration.

As a result, both long-term microbial activity and activity of the viable population at sampling contribute to enzyme activity. Dehydrogenase, which in theory can only exist in viable cells and not in stabilised soil complexes, is an example of an enzyme that only reflects activity of viable cells (Bandick AK et al., 1999)

1.7 Lipase

Glycerol ester hydrolases known as lipases operate on acylglycerols to release glycerol and fatty acids. Long-chain, water-insoluble triglycerides can be hydrolyzed by lipases into diglycerides, monoglycerides, glycerol, and fatty acids 1,2. Lipases are ubiquitous enzymes that are found in a variety of organisms, including plants, animals, and microorganisms (Veerapagu et al.; 2013). A useful technique for tracking the biodegradation of petroleum hydrocarbons, including diesel oil, in recently polluted soil is soil lipase activity (Margesin et al. 1999, 2002). In both unfertilized and fertilised soil, the residual hydrocarbon concentration correlates adversely with soil lipase activity.

Soil that has been fertilised (Margesin and Schinner 2001). Generally speaking, there is a link between soil lipase activity and other biological variables. The stimulation of this enzyme activity by the pollution is demonstrated by the fact that soil lipase activity rises with increasing initial oil loading rates. The advent of products from hydrocarbon biodegradation, the substrate for hydrolases such esterases and lipases, is credited with this induction. The ability of microorganisms to break down diesel oil and their lipolytic activity have been shown to be strongly correlated (Mills et al. (1978) and Kato et al. (2001). The biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil was not accompanied by an increase in lipase activity (Margesin et al. 2000a). It is also helpful to measure lipase activity to keep track of how carboxyl esters, such lipids and polyesters that degrade in soil (Sakai et al. 2002). The outlined technique can also be used to assess lipase activity in uncontaminated soil and may be helpful for screening soil bacteria that produce lipase.

Chapter – II

Review of Literature

CHAPTER 2: REVIEW OF LITERATURE

2.1 Soil Bacteria

For many years, soil microorganisms have been utilized in crop production. The primary roles of these bacteria (Davison 1988) are to provide nutrients to crops, stimulate plant growth, for example, by producing plant hormones, control or inhibit the activity of plant pathogens, improve soil structure, and bioaccumulate or leach inorganics from the environment (Brierley 1985; Ehrlich 1990). As part of the bioremediation of disturbed soils, microorganisms have also lately been utilised in soil (Middledrop et al. 1990; Burd et al. 2000; Zhuang et al. 2007; Zaidi et al. 2008). In the era of sustainable crop production, the interactions between plants and microbes in the rhizosphere are crucial for transforming, mobilising, solubilizing, etc. nutrients from a finite nutrient pool and, ultimately, allowing plants to absorb vital nutrients to reach their full genetic potential. In an integrated plant nutrition management system, the use of biological techniques as a supplement to chemical fertilizers is growing in popularity right now.

2.1.1 Lipase

To define what lipase exactly is two standards were applied to determine if an enzyme was a "true" lipase (EC 3.1.1.3): (A) It should be interface-activated, meaning that it should become significantly more active as soon as the triglyceride substrate emulsifies. Interfacial activation is the term used to describe this (Sarda and Desnuelle, 1958). (B) It should have a "lid" that is a hydrophobic oligo-peptide surface loop that covers the enzyme's active site and

detaches upon contact with the interface, allowing the substrate to enter the binding pocket right away (Van Tilbeurgh et al., 1993).

2.1.2 Lipase characteristics

The lipases that organisms create can be used for a variety of tasks and come in extracellular, intracellular, immobilized, and regiospecific forms. Extracellular lipase is an enzyme that has been purified using various methods after being isolated from the generating organism. The utilization of the enzyme while it is still present in the generating organism is referred to as intracellular lipase, on the other hand (Robles-Medina et al., 2009). Both extracellular and intracellular lipase could be immobilized using a solid support (Jegannathan et al., 2008). They can also be regiospecific by nature which means they only act on specific bonds of the triglyceride molecule (Robles-Medina et al., 2009)

Extracellular microbial lipases can be made through submerged fermentation or solid state fermentation, and they are primarily produced by the latter. In order to raise the degree of purity and hence improve the biocatalyst activity of the enzyme, the fermentation procedure is typically followed by a purification step (Balaji and Ebenezer, 2008; Barberis et al., 2008). The intricate process of creating extracellular lipase's crucial purification stage is dependent on the lipase's origin and structural makeup (Saxena et al., 2003). Extracellular lipases should be produced at scale in a way that is affordable, quick, simple, and effective.

Extracellular lipases are expensive to purify, so using entire cells as biocatalysts is an alternative strategy to address this issue. An appealing method for mass producing biodiesel and polyesters without the use of enzyme lipase is the use of compact cells for intracellular production of lipases or fungal cells immobilized within porous biomass support particles as a

whole biocatalyst (Iftikhar et al., 2008). Intracellular lipase is the term used to describe how the cells use their own lipase (Robles-Medina et al., 2009). On specific supports, several microorganisms employed as sources of lipase can spontaneously immobilize.

2.2 Enzymes

2.2.1 Microbial enzymes

Enzymes are major components of biological soil processes, such as the degradation of organic compounds, their mineralization and the liberation or recycling of nutrients including nitrogen, phosphorus, sulphur and other essential metals. The activity of hydrolytic enzymes and ligninolytic oxidases and peroxidases directly affects the rates of transformation of soil biopolymers into compounds that are accessible for microorganisms and plants. The study of enzymatic activities in environmental samples (soil, litter, lignocellulose or other matrices) is a useful tool for assessing the functional diversity of soil microbial communities or soil organic mass turnover (Kandeler et al. 1999).

2.2.2 Bacterial enzymes

There are several bacterial enzymes in soil. They are

2.2.2.1 Protease

Proteases are enzymes that disassemble proteins into smaller peptides or amino acids. They are often referred to as proteinases or peptidases. They are created by soil bacteria and are essential for the breakdown of organic matter, such as dead plant and animal matter.

2.2.2.2 Amylase

Amylases are enzymes that break down complex carbohydrates like starch into simpler sugars like glucose. Amylases are created by soil bacteria to use starch as a source of carbon and energy.

2.2.2.3 Lipase

Lipases hydrolyze fats and oils into fatty acids and glycerol. The lipids in organic matter are broken down by bacterial lipases in the soil. They are also capable of reversing the reaction in non aqueous media and they are abundantly present in nature (Singh et al., 2017) . The 3-D structure of lipases from different microbial sources are not exactly alike, they exhibit high sequence diversity.

2.2.2.4 Cellulase

The most prevalent biomass on Earth is cellulose (P. Tomme et al., 1995). The most plentiful renewable bioresource produced in the biosphere, it is the main byproduct of photosynthesis in terrestrial ecosystems (M. Jarvis et al., 2003). Cellulases are enzymes that degrade cellulose into glucose and other simple sugars, as was previously noted earlier. Cellulases are made by bacteria in the soil to break down plant matter and release nutrients.

2.2.2.5 Chitinase

Chitinases are enzymes that break down chitin, a complex polymer present in the exoskeletons of insects and crustaceans as well as the cell walls of fungus. Organic material containing chitin is recycled by bacterial chitinases in soil.

These are only a handful of the bacterial enzymes that can be discovered in soil. In order to carry out multiple biochemical reactions needed in nutrient cycling, organic matter breakdown, and ecosystem functioning; bacteria develop a large number of additional enzymes in the abundant microbial habitat that is soil.

2.2.3 Bacterial Lipase

Bacterial lipases are enzymes that bacteria manufacture that are essential for the metabolism and breakdown of lipids. They are used in a variety of businesses, including those that produce food, detergent, drugs, and biodiesel. Here are a few instances of bacterial lipases and some references for more research:

A thermostable lipase made by Pseudomonas aeruginosa, is one of its products. It is used in the manufacturing of biosurfactants, detergents, and flavoring chemicals (Verma et al., 2015).

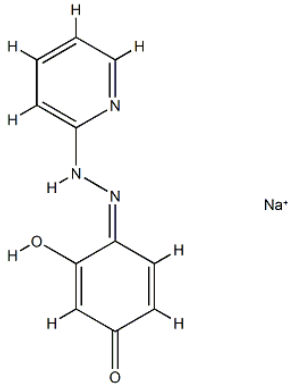
Bacillus subtilis is a strong lipase with a wide range of substrate specificity. It is used in a variety of biotechnological processes, such as the synthesis of pharmaceuticals, the generation of biodiesel, and food processing (Gupta et al., 2004).

Staphylococcus aureus lipase is the bacterium produces an enzyme called lipase that increases its pathogenicity. It contributes to the hydrolysis of host lipids, which helps bacteria survive and infect their host (Sebum J et al., 2019)

Lipase from Streptomyces rimosus is frequently employed in a variety of industrial applications because of its stability and capacity to function under a variety of circumstances. It has uses in the production of medicinal intermediates, food processing, and detergent compositions. (Hasan et al., 2006)

Bacterial lipases are enzymes that help break down triglycerides into glycerol and fatty acids. They are generated by bacteria. They are crucial to many industrial processes, including the manufacture of medicines, detergents, biodiesel, and food.

2.3 Structure of Lipase



(Courtesy:

Lipases are essential for the oxidation and metabolism of lipids (fats). Triglycerides are hydrolyzed into fatty acids and glycerol by means of their catalysis. Numerous creatures, including animals, plants, and microbes, include lipases, which are highly relevant to the domains of biochemistry, biotechnology, and industrial processes.Depending on the parent organism and the particular lipase isoform, the structure of lipases can change. However, the /hydrolase fold, a typical structural fold found in lipases, is shared by all of them. A central -sheet is at the centre of this fold, which is bordered by helices. The enzyme's active site, where the catalytic activity occurs, is typically found in a large hydrophobic pocket. Pancreatic lipase is one well-studied example of a lipase that is essential for the digestion of lipids in the small intestine of vertebrates. X-ray crystallography and other methods have been used to thoroughly characterise the structure of pancreatic lipase. A single polypeptide chain with a molecular

weight of around 50–55 kDa makes up the protein.Pancreatic lipase exhibits a characteristic / hydrolase fold in its crystal structure. It is made up of a core eight-stranded beta sheet encircled by beta helices. The active site is situated at the intersection of the cap domain, which protects it and controls access to it, and the / domain. Serine, aspartate, and histidine, which make up the catalytic triad found in the active site, are essential for lipase's enzymatic activity.

2.4 Sources of lipase

Table 1: List of lipase producing bacteria

Microorganisms	Sources	References
Acinetobacter radioresistens	Bacterial	Li et al.
Pseudomonas sp.	Bacterial	Kiran et al.
Pseudomonas aeruginosa	Bacterial	Ruchi et al. Mahanta et al.
Staphylococcus caseolyticus	Bacterial	Volpato et al.
‘Biopetro-4’	Bacterial	Carvalho et al.
Bacillus stearothermophilus	Bacterial	Abada
Burkholderia cepacia	Bacterial	Fernandes et al.
Burkholderia multivorans	Bacterial	Gupta et al.
Serratia rubidaea	Bacterial	Immanuel et al.
Bacillus sp.	Bacterial	Ertugrul et al., Shariff et al., Nawani and Kaur
Bacillus coagulans	Bacterial	Alkan et al.
Bacillus subtilis	Bacterial	Takaç and Marul

2.5 Industrial applications of Lipase

Microbial lipases are a significant class of biotechnologically useful enzymes, primarily because of the flexibility of their applications and the simplicity of their mass manufacturing. Microbial lipases are especially appealing for industrial applications because of their wide range of enzymatic characteristics and substrate specificity. Based on total sales volume, lipases are regarded as the third largest group after proteases and carbohydrases. According to Jaeger et al. (1999), the commercial use of lipases is a multibillion dollar industry with numerous distinct uses.

According to Cheetham (1995), traditional aerobic submerged fermentations, which offer more control over the growth conditions than solid-state fermentations, are employed to manufacture the majority of the enzymes utilised in industrial applications. The present literature is becoming more and more populated with information regarding lipases, a sign that lipases are currently receiving more attention. Because they function under benign circumstances, are very stable in organic solvents, have a wide range of substrate specificities, and frequently exhibit excellent regio- and/or stereoselectivity in catalysis, lipases are prized biocatalysts (Snellman et al., 2002).

Bacterial lipase's physiological and physical characteristics are what make it useful in science and business. Purified lipase might become widely accessible, making it simple to produce in huge quantities. Animal or plant lipases are typically less stable than bacterial lipases. Because lipases are active at room temperature, the energy required to carry out reactions at high pressures and temperatures is greatly decreased, which also greatly reduces the risk of labile reactants and products being destroyed.

2.5.1 In food industry

In the recent past, lipases have been employed frequently in the manufacture of a wide range of goods, from the fermentation of vegetables to the creation of fruit juices (Pandey et al., 1999). According to Sharma et al. (2001), lipases make it easier to remove fat from meat and fish products. Despite the very low quantities of the substrate acylglycerols present in the formulations, adding lipase to noodles causes them to have noticeably softer textural properties (Undurraga et al., 2001). In the development of a fat product in the confectionary industry that contained a high concentration of 1,3-disteraroyl-2-monoloein, 1,3-regioselectivity of lipases was utilised (Macrae 2000). When creating cocoa butter equivalents, this fat could be utilised in place of sheastearine. These kinds of enzyme esterification reactions have also led to the production of fats intended to prevent bloom formation in chocolate products (Macrae 2000).The production of ice cream, single-cell proteins, life-saving pharmaceuticals, carbohydrate esters, and amino acid derivatives not possible through conventional chemical synthesis are just a few of the many uses for C. rugosalipases in the food and flavour industry (Benjamin and Pandey 1998). In the dairy business, lipases are widely employed to hydrolyze milk fat. To improve the flavours of different cheeses, the dairy industry uses lipases to change the fatty acid chain lengths. The acceleration of cheese ripening and the lipolysis of butter, fat, and cream are further current applications (Sharma et al., 2001; Ghosh et al., 1996).

Enzyme utilisation in the oils and fats sector is new, offering a number of remedies to the sector's issues as well as the key to creating unique oils and fats. With their great selectivity and ability to catalyse reactions under mild conditions (such as the industrial hydrolysis of fats and

oils or the formation of fatty acid amides), lipases can be employed to produce high-value compounds for both food and industry at reasonable costs **(**Nakajima *et al*., 2000) .

2.5.2 In detergent industry

The addition of hydrolytic lipases to detergents, which are mostly utilised in residential and commercial laundry as well as in household dishwashers, is the most commercially significant application for these enzymes. Since all detergents use the same detergency mechanisms and share a lot of the same chemicals, their cleaning effectiveness appears to have peaked. Modern heavy duty powder detergents and automated dishwasher detergents frequently include one or more enzymes such lipase, amylase, cellulase, and protease to increase detergency (Ito et al., 1998). Detergent lipases are specifically chosen to satisfy the following criteria: In order to hydrolyze fats of different compositions, an enzyme must have the following characteristics: (1) a low substrate specificity; (2) the ability to withstand relatively harsh washing conditions (pH 10–11; 30–60°C); and (3) the ability to withstand damaging surfactants and enzymes (such as linear alkyl benzene sulfonates and proteases), which are key components of many detergent formulations. Furthermore, the removal of grease stains has become more difficult as washing temperatures have been lowered, especially for cotton and polyester clothing. According to Jaeger and Reetz (1998), some specialised lipases can remove greasy stains like lipstick, frying fats, butter, sauces, etc.

Enzymes can also lessen the impact of detergent on the environment because they biodegrade quickly and without leaving any hazardous residues, don't interfere with sewage treatment procedures, or endanger aquatic life.

2.5.3 In Pharmaceutical industry

A number of medications are made using microbial lipases, which are utilised to enhance PUFAs from animal and plant lipids (Dong et al., 1999). Because of their advantages for metabolism, PUFAs are increasingly exploited as food additives, medicines, and nutraceuticals. For the correct synthesis of prostaglandins and lipid membranes, several PUFAs are necessary. PUFAs are extracted from lipids derived from plants and animals, such as menhaden oil, tuna oil, and borage oil, using microbial lipases. Subsequently, a variety of medicines are made using free PUFAs and their mono- and diacylglycerides (Sharma et al., 2001). By delivering drugs to specific locations and avoiding anatomical obstacles and drug waste inactivation, liposomes are employed in the medical field to maximise the impact of medications (Linko and Wu 1996).

2.5.4 In paper and pulp industry

Triglycerides and waxes, which are hydrophobic parts of wood, are collectively referred to as "pitch." Paper mills frequently contain pitch and other compounds that are problematic (Jaeger and Reetz, 1998). These issues manifest as gummy buildup in the paper machines and might result in holes and blemishes in the finished paper. But when pulp is created during the paper-making process, pitch is removed using lipases (Jaeger and Reetz, 1998). According to Jaeger and Reetz (1998), lipases hydrolyze up to 90% of the triglycerides in pitch into glycerol, monoglycerides, and fatty acids, which are much less sticky and more hydrophilic (easier to wash). The Candida rugosa fungal lipase is used in a pitch control technique developed by Nippon Paper Industries in Japan to hydrolyze up to 90% of the triglycerides in wood (Jaeger and Reetz, 1998).

2.5.5 In cosmetic industry

Isopropyl myristate, isopropyl palmitate, and 2-ethylhexyl palmitate are currently produced by several cosmetic businesses for use as emollients in personal care products including skin and sun-tan creams, bath oils, etc. Immobilised Rhizomucor miehei lipase was extensively exploited as a biocatalyst in this instance. In contrast to the frequently employed acid catalyst, using enzymes results in substantially higher-quality products with a minimum amount of downstream refining. Wax esters (esters of fatty acids and fatty alcohols), which are likewise created by the action of lipase derived from C. cylindracea employing batch bioreactor, have comparable uses in personal care products. This technique of production has a little greater total cost than the widely used conventional method, but the expense is offset by the higher quality of the finished good. Immobilised lipase was used in a catalytic reaction to create water-soluble retinol compounds (Maugard et al., 2002). In the preparation for hair waving, lipases have been utilised (Saphir, 1967). Additionally, lipases have been administered orally (Smythe, 1951) and as a component of topical anti-obesity lotions (August, 1972).

2.5.6 In energy production

Long-chain fatty acid and short-chain alcohol esters are what make up the liquid biofuel known as biodiesel. According to Vicente et al. (2004), the direct transesterification of vegetable

oils and lipids with short chain alcohols (such methanol and ethanol) produces biodiesel molecules . In a procedure analogous to hydrolysis, transesterification is the displacement of one alcohol from an ester by another alcohol using an alcohol as the solvent (Srivastava et al., 2000). Methanol and ethanol are the most often utilised short chain alcohols, with methanol being used more frequently due to its low cost and physicochemical advantages (Clark et al., 1984).

2.5.7 In leather industry

Proteins and lipids are found in the collagen fibres of hides and skins. These chemicals need to be eliminated in part or whole before the hides and skins are tanned. Soaking is the initial treatment. By doing so, the common salt is eliminated and the hide is made clean and free of dirt and blood. Protease activity must also be used to remove non-fibril proteins that hold the fibres together. By hydrolyzing the fat cell wall, proteolytic enzymes aid in the emulsification of natural fat as well as the soaking procedure. Lipases exclusively break down fat; they do not harm the leather. In the degreasing process, lipases stand for the fat removal technique with the least negative environmental effects. Lipases enable tensile to be entirely replaced in bovine skins. Solvents are frequently used on sheepskins, however lipases and surfactants can also be used in their place.

2.5.8 In textile industry

The removal of size lubricants using lipases results in a fabric with increased absorbency for enhanced levelness in dyeing, which is a common practise in the textile industry. In the

denim abrasion systems, it is also utilised to lessen the incidence of streaks and cracks. At a commercial scale, lipases and alpha amylase are utilised to shrink denim and other cotton materials (Rowe et al., 2001). Polyester has a number of important benefits for the textile industry, including increased strength, a soft hand, stretch resistance, stain resistance, machine washability, wrinkle resistance, and abrasion resistance. Enzymes have been used to process and modify synthetic fibres so they can be used to make yarns, fabrics, textiles, rugs, and other consumer goods. It has to do with altering a polyester fiber's physical properties such that these polyesters are more susceptible to post-modification treatments.

2.6 Bacterial screening for lipase production

Numerous bacteria and higher eukaryotes manufacture lipases. The majority of usable lipases for commerce come from microorganisms. Lipase-producing microbes have been discovered in a variety of settings, including compost piles, coal ash heaps, hot springs, vegetable oil manufacturing plants, dairies, soil contaminated with oil, oilseeds, and decaying food (Sztajer et al., 1988).

2.6.1 Qualitative screening

Based on the zone of hydrolysis created, a microbe's ability to breakdown lipids is utilized as a criterion to determine whether or not to produce lipase. Results that are positive indicate that the isolate has demonstrated a zone of hydrolysis around their colony, while results that are negative indicate that there is no discernible zone surrounding the microbial colonies. Production of intracellular enzymes may be the cause of the lack of a clean zone. By measuring the activity of enzymes quantitatively, it was further supported (Verma et al., 2014)

2.6.2 Quantitative screening

The isolates were evaluated based on a quantitative assessment of enzyme activity. The colony having greater zone of hydrolysis shows that extracellular enzyme activity (Verma et al., 2014)

Chapter – III

Experimental Methods

CHAPTERE 3: EXPERIMENTAL METHODS

3.1 Selection of sites and Collection of soil sample

In selection of sites, the sites were selected from the campus of Assam Down Town University, from the excavated soil and the soil is undisturbed. The sites that is selected is purely new and beneath the soil. The sites were selected from different parts of Assam Down Town University Campus. Most of the sites selected from the under construction is purely new.

The soil samples were collected from the selected sites of the Assam Down Town University campus. The upper layer of the soil is first extracted 2cm in depth before the collection of soil sample, as per the standard procedure (Skinner, 1951) with the help of the spatula and collected in a clean zip bag and brought in laboratory.

3.2 Processing of collected soil sample

The soil sample goes through the process of removal of unwanted particles. It is achieved through the process of sieving where the fine particles are separated from the larger particles. After sieving, the soil is left with fine particles and ready for further process.

3.3 Analysis of soil sample

The soils samples that are collected from the Assam Down Town University campus, were subjected for soil analysis (Margesin and Schinner, 2005) . The soil analysis includes pH, Carbon, Nitrogen, Phosphorous and Nitrogen.

3.3.1 Determination of pH of soil

The reagents used in determining pH are Standard buffer (4.0) and standard buffer (9.2) . The apparatus used in determining the pH are electronic pH meter with glass and electrode. In a beaker, 20gm of air dried soil was taken and 50ml of distilled water was added and stirred at regular intervals. The pH meter was then switched on for 15-20minutes to warm up. The temperature of the pH was adjusted as well as the temperature of the machine at 4.0 and 9.2. After that pH of sample was measured in suspension to pH meter.

3.3.2 Determination of organic content, organic matter and nitrogen content of soil

The reagent used in the determination of organic content, organic matter and nitrogen content of soil are 1N Potassium Dichromate Solution, 0.5 N Ferrous Ammonium Sulphate (Mohr's salt), concentrated sulphuric acid, orthophosphoric acid(85%) and diphenylamine indicator. The apparatus involved in this test are conical flask, pipette, burette and analytical balance.

0.5 gm of soil was taken in a conical flask and 5ml of 1N $K_2Cr_2O_7$ and 10ml of con.H_2SO_4 was added and mixed together. It is then kept for 30minutes for the reaction to occur

and cool. 100ml distilled water was poured into the mixture and 5ml of orthophosphoric acid and 5ml of sodium fluoride was added and mixed. 2-3 drops of diphenyl amine indicator was added. 0.5 N $Fe(NH_4)_2(SO_4)_2.6H_2O$ was titrated till the color changes from blue to bright green. The reading is then noted.

3.3.3 Determination of available phosphorous in soil (Bray's method)

The reagents used in the determination of available phosphorous in soil are 1N of Ammonium Flouride (NH_4F), 0.5N HCl, extracting solution (NH_4F working solution), dickman and bray's reagent, stannous chloride(stock solution) and stannous chloride working solution. The apparatus used in this are photoelectric, colorimeter, pipette, volumetric flask(25ml), funnel and whatman no 42 or 44 filter paper.

5ml of soil was taken in 100ml of conical flask and 50ml of NH_4F working solution and 1 st. spoon of ash was added. The content is then shaked for exactly 5minutes and filtered through Whatman 42. 5ml of eliquot was taken into 25ml volumetric flask and 5ml of NH4F working solution + 5ml of dickman reagent + 1ml of stannous chloride solution was added. Distilled water was added and the volume was made upto 25ml. A blank was prepared with water and the extract to colorimeter was feeded at 60mm after 5minutes. The reading was noted and plotted in standard curve.

3.3.4 Determination of K_2O in soil

The reagents used in determination of K_2O in soil are neutral 1N NH_4OAc solution and potassium chloride. The apparatus used in this test are conical flask, flame photometer, tube,

whatman 42 filter paper and funnel. 5gm of soil was taken in a conical flask and 25ml of NH_4OAc was added. The contents is then shaken for 5min and filtered. The filtrate is then feeded into the atomizer of flame photometer after standardizing 0-100 with distilled water and 40ppm KCl. The reading is then noted and concentration on standard curve of K was located.

3.4 Isolation of bacteria by serial dilution plate culture technique

The standard serial dilution technique (El – Nakeeb and Lechevalier, 1963) was employed to isolate the microorganisms from the soil under the construction site of Assam Down Town University campus. Adequate serial dilutions ranging from 10^{-1} to 10^{-6} were prepared from the sample. Then 0.5ml of the samples taken from the respective dilution was plated on nutrient agar medium. The plate is then streaked inside a sterile laminar air flow. After streaking the plates were incubated at 37 degree Celsius for 24hours.

3.5 Colony and microscopic features of the bacterial isolates

For the characterization of the colonies, the colonies were grown on starch agar medium on were identified based on the size, shape, odor, texture, and color (Venkataramana kandi 2015). The microscopic features of the microorganism were identified with all the staining method including gram staining were observed under the microscope as per the methods describe in the Bergeys Manual of Determinative Bacteriology (Goodfellow, 1989).

3.6 Qualitative Screening of bacterial isolates for the production of Lipase

With 10 g/L peptone, 20 g/L agar, 5 g/L NaCl, and 0.1 g/L CaCl22H2O in distilled water, Tween80 agar medium pH7.0 was created. The medium was then autoclaved for 15 minutes at

121 °C under 15 lb of pressure before being chilled to 45 °C. Tween 80 was added to the chilled media at a rate of 10 ml/L. Transferring aliquots to petri dishes, they were given time to harden. Each pure culture was streaked separately onto a Tween80 agar plate, and the infected plates were then incubated at 37 °C for 48 hours. A white precipitate around the colony after incubation shows lipase activity.

3.7 Biochemical Characterization of the promising isolates

The isolates were further proceeded for biochemical characterization such as urease test, catalase test, TSI test, IMViC test, gelatin test, starch hydrolysis, sugar fermentation, oxidase test and casein test as per the standard protocol prescribed in Bergeysmanual of systemic bacteriology (2012); Gottlieb (1960).

3.8 Quantitative screening of isolates for the production of isolates

The ability to produce lipase was tested in all of the chosen bacterial cultures that produce the enzyme. The 5 g/L peptone and 5 g/L beef extract in distilled water used to create the production media (pH 7.0) were autoclaved for 15 minutes at 15 lb pressure (121 °C), cooled to about 60 °C, and then 10 ml/L olive oil was added. Selected lipase-producing bacterial cultures that had been cultivated overnight were injected separately in 100 ml of production media in 250 ml Erlenmeyer flasks and allowed to grow for 24 hours at 35 °C. After incubation, the culture was centrifuged at 10,000 rpm for 10 min. The supernatant was then utilized to test for lipase (Ilesanmi et al., 2020) .

3.9 Lipase Activity Assay

Utilizing p-nitrophenyl laureate (p-NPL) as a substrate, lipase activity was assessed. 2 L of 0.03 g/L p-NPL, 6 L of 0.05 M phosphate buffer, and 2 L of enzyme extract (lipase) were included in the reaction mixture, which was incubated at 30 ℃ for 10 min. 30 L of pure ethanol were added to stop the reaction. For the lipase reaction, absorbance was measured using a Nanodrop spectrophotometer at a wavelength of 380 nm. The definition of a unit of lipase activity is an enzyme that releases 1 mol of free p-nitrophenol every minute. (Ilesanmi et al., 2020) .

3.10 Optimization of process condition for the optimized production of lipase

Variable enzyme optimisation factors were seen, including the ideal incubation period, carbon supply, ideal pH, ideal inoculum amount, optimal nitrogen source, and molecular characterization.

3.10.1 Influence of still and shaking conditions on the production of lipase by JRD11

By incubating the enzyme production medium with the inoculation culture in an orbital shaking incubator at 36°C with variable agitation speed from 120 rpm to 200 rpm for 24 hours, the effect of agitation on lipase production was examined. After incubation, the enzyme was measured using a colorimetric technique (Veerapagu et al., 2013).

3.10.2 Influence of incubation period on the production of lipase by JRD11

At 36°C and 150 rpm, an inorbital shaker was used to cultivate the isolates in Tribuytrin broth with yeast extract, NaCl, peptone, and 1% (w/v) olive oil. By centrifuging the culture broth at 10,000 g for 30 min. at 4°C, the culture broth was harvested every 8 hours. The recovered supernatant was utilized as a crude enzyme solution, and the activity of the enzymes was measured (Sirisha et al., 2010)

3.10.3 Influence of different temperature conditions on the production of lipase by JRD11

With the exception of the incubation period, which was standardized above, the incubation temperatures ranging from 22 °C to 42 °C were chosen to determine the optimal temperature for isolates to produce lipase (Golani et al., 2016)

3.10.4 Influence of different pH conditions on the production of lipase by JRD11

By replacing the buffer in the reaction mixture with several buffers for various pH levels, the effect of pH on lipase action was examined. By adding Na2CO3, the pH of the acetate buffer, phosphate buffer, and pH-neutral buffer was changed. Thus, a pH range of 4.0 to 9 was scanned, with the exception of the incubation period and temperature, which were tuned, to find the pH at which the isolate would produce the most lipase (Golani et al., 2016)

3.10.5 Influence of different inoculums size on the production of lipase by JRD11

Optimum inoculum concentration for lipase enzyme production was studied by preparing the inoculum as described and varied inoculums concentration (1% to 10 %) were added to the enzyme production medium in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 36°C for 24 hrs and the enzyme was assayed (Veerapagu et al., 2013)

3.10.6 Influence of different carbon source on the production of lipase by JRD11

Tween20, Tween80, olive oil, glucose, and fructose were some of the carbon sources chosen since they might stimulate the formation of lipase. Each type of carbon source was present in the nutrient broth where the bacterial culture was injected. Cultures were cultured at 30 °C for 12 hours, with samples taken every 3 hours for a lipase assay (Ilesanmi et al., 2020) .

3.10.7 Influence of suitable nitrogen source on the production of lipase by JRD11

In order to avoid interfering with the creation of lipase, various nitrogen sources (including yeast extract, peptone, casein, ammonium nitrate, and potassium nitrate) were utilised in the production media. Each type of nitrogen source was added to nutritional broth before being infected with bacterial culture. Cultures were cultured at 30 °C for 12 hours, with samples taken every 3 hours for a lipase assay (Ilesanmi et al., 2020) .

3.11 Molecular characterization

From the culture, DNA was isolated. On a 1.0% Agarose Gel used to test the DNA's quality, a single band of high-molecular weight DNA could be seen. A 16s rRNA gene fragment was amplified by PCR. When the PCR amplicon was resolved on agarose, just one distinct band was seen. To get rid of impurities, column purification was used to clean the PCR amplicon. Using the BDT v3.1 Cycle Sequencing Kit and an ABI 3500xl Genetic Analyzer, a DNA sequencing reaction of a PCR amplicon was performed. A BLAST search was done using the 16s rRNA sequence against the NCBI GenBank database. The first ten sequences were chosen and aligned using various sequence alignment software tools based on greatest identity score (GeneXplore).

Chapter – IV

Results and Discussion

CHAPTER 4: EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Selection of sites and Collection of soil sample

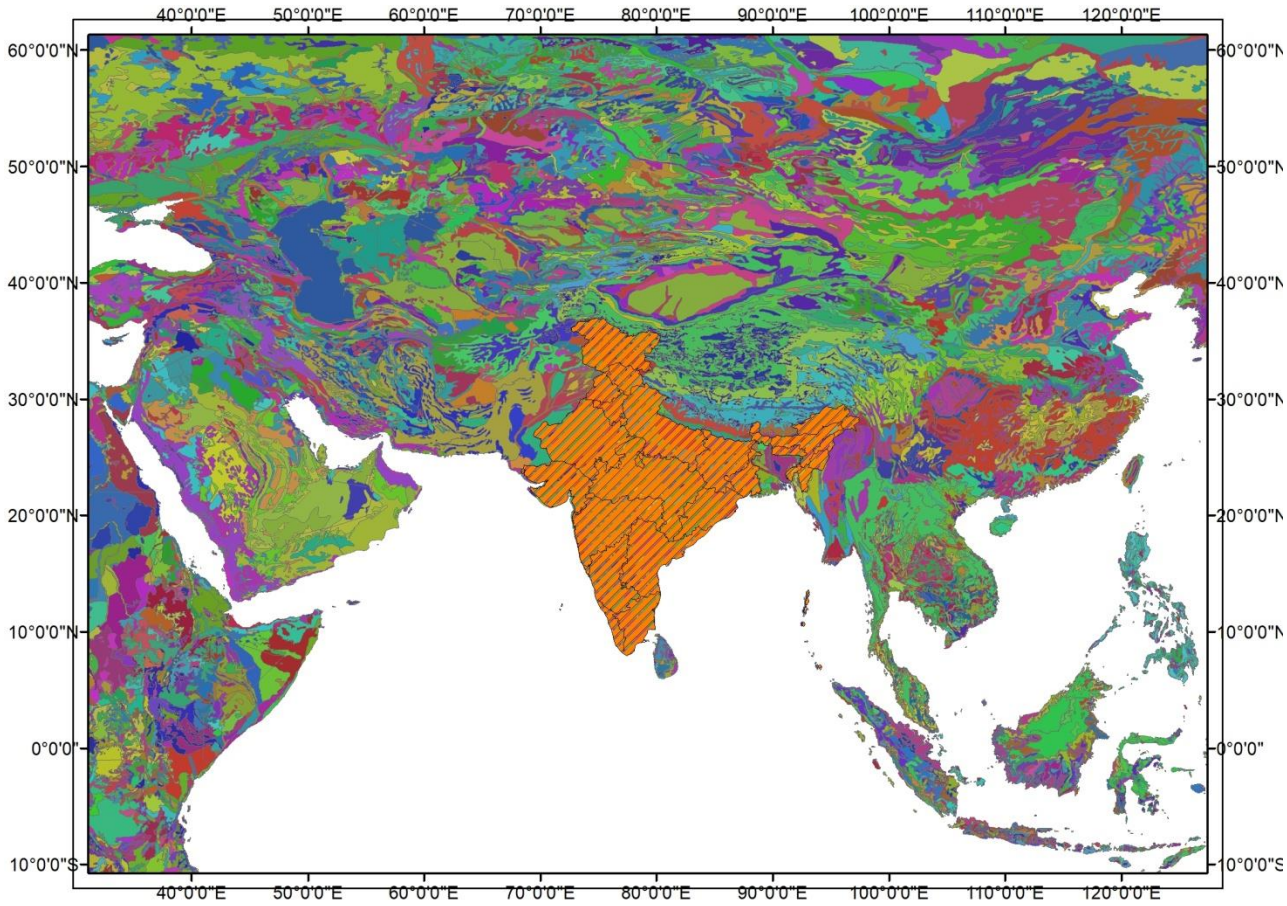


Fig 1: World Map

Here, the map depicts world map which highlights India map

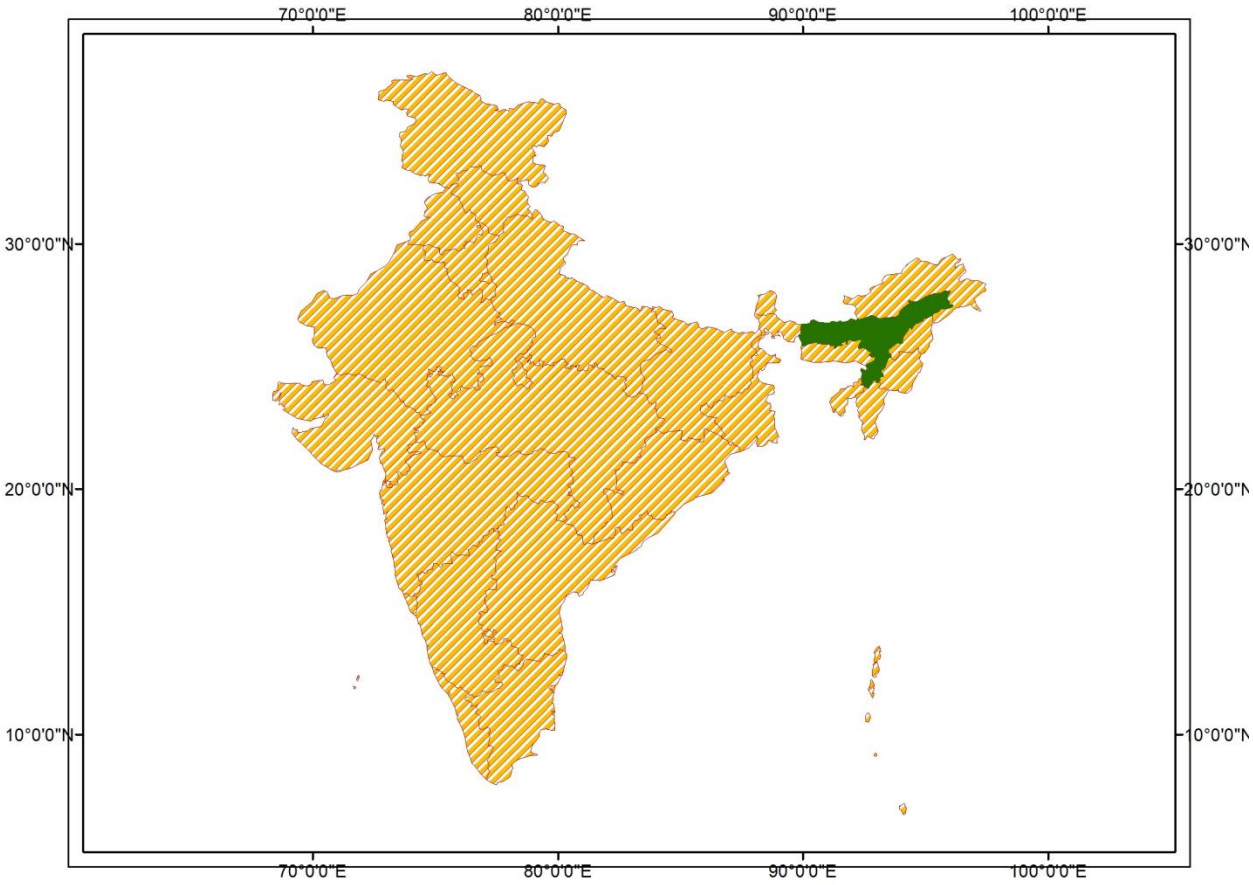


Fig 2: India Map

Here the map depicts India map which highlights Assam map

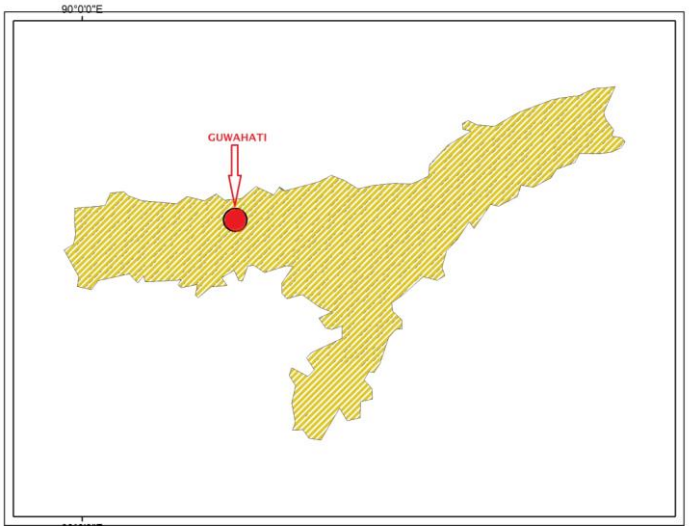


Fig 3: Assam Map

Here, the map depicts Assam map which points Guwahati

Fig 4: Site from where the soil sample was collected

The above figure shows the site from where the soil sample collected from Assam Down Town University Campus, Panikhaiti

4.2 Analysis of Soil samples

Table 2: Percent composition of components of collected soil sample

Sl. No.	Soil Sample Code	Sand %	Silt %	Clay %	Texture
1	SDB	28.40	20.28	51.32	Clay
2	RDL	12.60	7.17	80.32	Clay
3	SDR	61.12	6.26	32.62	Sandy
4	VCG	20.30	21.88	57.82	Clay
5	SKY	76.74	2.84	20.42	Sandy
6	SMB	87.30	2.38	10.32	Loamy Sand

The percent composition of different components viz., Sand, Silt and Clay of six soil samples collected was shown in the Table 1. The samples were designated as SDB, RDL, SDR, VCG, SKY and SMB respectively. The abundance and dominance of certain species are influenced by the texture of soil (Mason et al., 2017). Soil texture could also play an essential role in the abundance and dominance of certain species. Each of the six different soil samples was known to contain varied percentage of Sand, Slit and Clay. The Sample SDB had 28.40 %, 20.28 % and 51.32 % of Sand, Silt and Clay respectively and showed the clay texture. Likewise RDL and VCG also showed highest clay percentage of 80.32 and 57.82 respectively. The sand and the silt percentage of VCG were observed to be greater (20.30 & 21.88) than RDL (12.60 & 7.17). The other two soil samples designated SDR and SKY appeared sandy in texture. The SKY sample showed high sand percentage of 76.74 whereas SDR showed only 61.12 percentage of sand. The Clay and Silt percentage was higher in SDR (32.62% & 6.26 %) than in SKY (20.42% &2.84). The soil sample designated as SMB was confirmed to be in the loamy sand

texture due to its higher percentage of sand i.e. 87.30%. The clay and silt percentage of SMB was found to be 10.32 % and 2.38 % respectively.

The sandy soils namely SMB, SKY and SDR found to have higher than 50% sand i.e. 87.30, 76.74 and 61.12 respectively. These sandy soils are reported to have low crop productivity (Adrees M et al., 2020) The low soil fertility and low crop yield of Clay particularly Quaternery Red Clay soil was reported (Su JQ, et al., 2014) .Soil texture could also play an essential role in the abundance and dominance of certain species.

Table 3: Nutrient properties of the collected soil samples

Sl. No.	**Particulars**	**pH**	**O.C. (%)**	**Av. N_2 (Kg/ha)**	**Av. P_2O_5 (Kg/ha)**	**Av. K_2O (Kg/ha)**
1	SDB	5.6	0.52**(M)**	173.02**(L)**	17.95**(L)**	218.40**(M)**
2	RDL	10.1	0.32**(L)**	106.20**(L)**	5.12**(L)**	282.40**(M)**
3	SDR	6.4	0.27**(L)**	92.93**(L)**	11.54**(L)**	97.44**(L)**
4	VCG	5.8	0.83**(M)**	278.79**(M)**	11.54**(L)**	920.64**(H)**
5	SKY	5.6	0.11**(L)**	39.82**(L)**	17.95**(L)**	40.32**(L)**
6	SMB	5.9	0.015**(L)**	53.10**(L)**	17.95**(L)**	67.20**(L)**

Note: H = High, M = Medium & L= Low
O.C. = Organic Content, Av. N_2 = Available Nitrogen, Av. P_2O_5 = Available Phosphorous Pentaoxide, Av. K_2O = Available Di Potassium Oxide
Kg/ha = Kilogram Hectare

The nutrient properties of different types of collected soil samples are shown in the Table 2. The soil samples namely SDB, RDL, SDR, VCG, SKY and SMB were known to contain varied percentage of pH, organic content, available nitrogen, phosphorous and potassium. The only soil sample RDL was alkaline in nature with a pH value of 10.1, whereas the SDR sample was with a near netural pH of 6 .4. All the remaining four soil samples were found to be acidic

in nature. Out of the four soil samples, two samples namely SMB & VCG were found to have a pH of 5.9 and 5.8 respectively. Both the soil samples i.e. SDB and SKY showed the same acidic pH value of 5.6 .

It was reported that the soil nutrients are significantly affected by the spatial structure and thickness of the soil (Kang et al. 2021). It was known that pH of the soil acts as a prime driving factor in structuring the microbial communities in the soil (Yashiro et al. 2016; Wang et al. 2018). The organic content of VCG was found to be the highest (0.83%) in comparison to all other samples tested. The observed organic content of the samples viz., SDB, RDL, SDR, SKY and SMB were in the order of 0.52%, 0.32 %, 0.27 %, 0. 11 and 0.015 respectively.

The highest available nitrogen observed was 278.79 Kg/h with respect to the sample VCG whereas for SKY being the least of 39.82 value. The available nitrogen content for SDB and RDL is 173.02 Kg/ha and 106.20 Kg/ha respectively. The sample SDR and SMB has available nitrogen of value 92.93 Kg/ha and 53.10 Kg/ha respectively. It is observed that among six of the collected soil samples five of the soil samples show less value of available nitrogen except VCG. The amount of available nitrogen is more than the rest of the samples.

The available phosphorous has the same value for the three samples out of the six. The samples are SDB, SKY and SMB of the value 17.95. The sample SDR and VCG also shows the same value of 11.54 respectively whereas, RDL showing the least out of all the six samples of value 5.12.

Low phosphorus fertilizer input has been demonstrated to hinder plant growth and reduce agricultural production in those areas. P deficiency caused a significant decrease in net photosynthesis rate and efficiency of the PSII reaction centre in many crops, including rice,

sunflower, maize, sugar beet, tobacco, oat, sheepgrass, Tibetan wild barley, and tea (Yoneyama K et al., 1993; Rao IM et al., 1995; Fredeen AL et al., 1990; Cordell D et al., 2009; Li L et al 2019). It is observed that in all the six soil sample the available phosphorous show low value.

The available potassium is highest in VCG of the value 920.64 than SKY of the value 40.32 being the least. The sample SDR, RDL and SMB shows 282.40, 218.40 & 67.20 value.

4.3 Isolation of bacteria

Table 3 represents colony characterization of the 45 isolates obtained from the six different soil samples. A total of 45 bacterial isolates were obtained by serial dilution at the factor of 10^{-1} to 10^{-6} by pooling all the six soil samples. The isolated were coded as JRD and numbered from 1 to 45 to differentiate individual unique bacterium based on their phenotype. Thecolony characteristics such as size, shape, color, elevation, margin and texture of all the isolated showed great variations. The size of the isolates was ranged from pin point, small, medium and large. Out of 45 obtained isolated, 3 were pin point sized, 18 were small sized, 21 were medium sized and 3 were large sized.

Fig 5: Pure culture of JRD11

Table 4: Obtained Bacterial Isolates and their colony characteristics

Sl. No.	Isolates	Size	Shape	Color	Elevation	Margin	Texture
1	JRD1	Medium	Round	White	Flat	Entire	Butyrous
2	JRD2	Medium	Round	White	Flat	Undulate	Butyrous
3	JRD3	Medium	Round	White	Raised	Entire	Mucoid
4	**JRD4**	**Small**	**Rod**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
5	JRD5	Medium	Round	White	Flat	Undulate	Butyrous
6	**JRD6**	**Small**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
7	JRD7	Small	Round	Greenish	Raised	Undulate	Mucoid
8	JRD8	Large	Filamentous	Greenish	Flat	Undulate	Butyrous
9	**JRD9**	**Medium**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
10	JRD10	Small	Round	Greenish	Raised	Entire	Mucoid
11	**JRD11**	**Medium**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
12	JRD12	Pinpoint	Round	White	Raised	Entire	Butyrous
13	JRD13	Small	Round	White	Flat	Undulate	Butyrous
14	JRD14	Medium	Filamentous	White	Flat	Entire	Mucoid
15	JRD15	Small	Filamentous	Greenish	Raised	Entire	Mucoid
16	JRD16	Medium	Round	Greenish	Raised	Entire	Mucoid
17	JRD17	Medium	Round	White	Raised	Undulate	Butyrous
18	JRD18	Medium	Filamentous	White	Flat	Undulate	Butyrous
19	JRD19	Small	Round	White	Raised	Entire	Butyrous
20	JRD20	Small	Round	White	Raised	Entire	Mucoid
21	JRD21	Large	Round	White	Raised	Undulate	Butyrous
22	JRD22	Small	Round	White	Flat	Undulate	Butyrous
23	JRD23	Medium	Round	White	Flat	Entire	Butyrous

24	**JRD24**	**Medium**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
25	JRD25	Medium	Filamentous	White	Raised	Entire	Butyrous
26	JRD26	Small	Round	White	Raised	Undulate	Butyrous
27	JRD27	Small	Round	White	Flat	Entire	Mucoid
28	JRD28	Small	Round	Greenish	Flat	Entire	Mucoid
29	JRD29	Pin point	Filamentous	Greenish	Raised	Undulate	Mucoid
30	JRD30	Small	Filamentous	Greenish	Raised	Undulate	Mucoid
31	JRD31	Medium	Filamentous	White	Raised	Entire	Butyrous
32	JRD32	Medium	Round	White	Flat	Undulate	Butyrous
33	JRD33	Medium	Round	White	Flat	Entire	Butyrous
34	JRD34	Small	Round	Greenish	Raised	Entire	Mucoid
35	JRD35	Small	Round	Greenish	Raised	Entire	Mucoid
36	JRD36	Medium	Filamentous	Greenish	Raised	Entire	Mucoid
37	JRD37	Medium	Filamentous	White	Flat	Entire	Mucoid
38	JRD38	Small	Filamentous	White	Raised	Undulate	Butyrous
39	**JRD39**	**Large**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
40	JRD40	Pin point	Round	White	Raised	Entire	Mucoid
41	JRD41	Small	Round	White	Flat	Undulate	Mucoid
42	JRD42	Small	Round	White	Flat	Undulate	Butyrous
43	JRD43	Medium	Filamentous	White	Flat	Entire	Butyrous
44	**JRD44**	**Medium**	**Rod shaped**	**Blue-green**	**Raised**	**Undulate**	**Butyrous**
45	JRD45	Medium	Round	White	Raised	Entire	Butyrous

The shape of the isolates ranges from round, irregular to filamentous. There are 12 irregular shaped isolates, 26 round shaped isolates and 7 rod shaped isolates were observed. The colors of the isolates observed are white, buff and red. There are 27 white colored isolates, 7

blue-green colored isolates, 11 greenish colored isolates are observed. The elevations of the isolates observed are flat and raised. There are 28 raised and 17 flat isolates. The margins of the isolates observed are entire and undulate. There are 22 entire margin and 23 undulate margin isolates were observed. The textures of the isolates observed are butyrous and viscid. There are 28 butyrous textured 17 mucoid textured isolates were observed.

4.4 Microscopic features of the bacterial isolates

Table 5: Microscopic features of bacterial Isolates

Sl. No.	Isolates	Cell Shape	Gram Staining	Capsule Staining	Flagella Staining
1	JRD1	Cocci	Positive	Negative	Motile
2	JRD2	Bacilli	Negative	Positive	Motile
3	JRD3	Pleiomorphic	Negative	Negative	Non-motile
4	**JRD4**	**Bacilli**	**Negative**	**Positive**	**Motile**
5	JRD5	Cocci	Positive	Positive	Non-motile
6	**JRD6**	**Bacilli**	**Negative**	**Positive**	**Motile**
7	JRD7	Bacilli	Positive	Positive	Non-motile
8	JRD8	Cocci	Negative	Positive	Motile
9	**JRD9**	**Bacilli**	**Negative**	**Positive**	**Motile**
10	JRD10	Cocci	Positive	Negative	Non-motile
11	**JRD11**	**Bacilli**	**Negative**	**Positive**	**Motile**
12	JRD12	Cocci	Positive	Positive	Motile
13	JRD13	Cocci	Positive	Positive	Motile
14	JRD14	Cocci	Positive	Positive	Motile
15	JRD15	Bacilli	Negative	Negative	Non-motile
16	JRD16	Bacilli	Positive	Negative	Non-motile
17	JRD17	Bacilli	Negative	Positive	Non-motile

18	JRD18	Pleiomorphic	Negative	Positive	Motile
19	JRD19	Pleiomorphic	Positive	Positive	Motile
20	JRD20	Cocci	Positive	Positive	Motile
21	JRD21	Cocci	Positive	Negative	Motile
22	JRD22	Cocci	Negative	Negative	Motile
23	JRD23	Bacilli	Negative	Negative	Non-motile
24	**JRD24**	**Bacilli**	**Negative**	**Negative**	**Motile**
25	JRD25	Bacilli	Positive	Positive	Motile
26	JRD26	Bacilli	Positive	Negative	Non-motile
27	JRD27	Cocci	Negative	Negative	Non-motile
28	JRD28	Cocci	Positive	Negative	Non-motile
29	JRD29	Cocci	Positive	Negative	Motile
30	JRD30	Pleiomorphic	Negative	Positive	Motile
31	JRD31	Pleiomorphic	Negative	Positive	Non-motile
32	JRD32	Pleiomorphic	Positive	Positive	Non-motile
33	JRD33	Cocci	Positive	Positive	Motile
34	JRD34	Cocci	Positive	Negative	Motile
35	JRD35	Bacilli	Positive	Negative	Non-motile
36	JRD36	Bacilli	Positive	Negative	Motile
37	JRD37	Bacilli	Positive	Positive	Motile
38	JRD38	Pleiomorphic	Negative	Positive	Non-motile
39	**JRD39**	**Bacilli**	**Negative**	**Positive**	**Motile**
40	JRD40	Cocci	Negative	Negative	Motile
41	JRD41	Cocci	Negative	Negative	Motile
42	JRD42	Cocci	Positive	Negative	Non-motile
43	JRD43	Pleiomorphic	Positive	Negative	Non-motile
44	**JRD44**	**Bacilli**	**Negative**	**Positive**	**Motile**
45	JRD45	Bacilli	Positive	Positive	Non-motile

Table 4 determines the microscopic characterization of obtained isolates. The number of isolates are 45 from JRD1 to JRD45 which features the cell shape and three types of staining (gram staining, capsule staining, flagella staining). There are three types of cell shape observed i.e. Bacilli, Cocci, Pleiomorphic. There are 18 cocci shaped bacteria, 19 bacilli shaped bacteria, 8 pleiomorphic shaped bacteria were observed.

Different types of staining was performed that are gram staining, capsule staining, flagella staining. Among 45 isolates in gram staining, there are 24 positive staining and 21 negative staining. In capsule staining, out of the 45 isolates 20 isolates are capsule positive staining and 25 isolates are capsule negative. In flagella staining among 45 isolates, there are 27 motile flagella and 18 non-motile flagella staining are observed.

4.5 Screening of isolates for the production of Lipase

Table 6: Qualitative screening of isolates for the production of lipase

Sl. No.	Isolates	Zone of Hydrolysis
01	JRD4	+ +
02	JRD6	+
03	JRD9	+ + +
04	**JRD11**	**+ + + +**
05	JRD24	+ +
06	JRD39	+
07	JRD44	+ + +

Fig. 6 pure culture

Table 7 indicates the Qualitative screening of the isolates, among the 45 isolates 7 isolates were selected for qualitative screening test. From the 7 isolates, JRD 11 showed excellence in the zone of clearance or hydrolysis , and all other 6 isolates show poor to very good growth.

Notes: poor(+), good(++), very good (+++), and excellence(++++).

4.6 Biochemical Characterization

Table 7: Biochemical characterization of the obtained isolates

Sl. No.	Samples	Urease Test	Catalase Test	TSI Test	Indole Test	MR-VP Test	Citrate Test	Gelatin Test	Starch Hydrolysis	Sugar fermentation			Oxidase Test	Caesin Test
										G	L	S		
1	JRD4	-	+	+	+	+ +	-	-	-	A	A	+	-	-
2	JRD6	+	+	+	-	- -	+	+	+	AG	A	-	+	+
3	JRD9	-	+	+	+	+ +	-	-	+	AG	A	+	-	-
4	JRD11	+	+	+	-	- -	+	+	+	AG	-	-	+	+
5	JRD24	+	+	+	-	- -	+	-	+	A	-	+	+	+
6	JRD39	-	-	+	+	-+	-	+	+	AG	-	+	+	+
7	JRD44	-	+	-	+	-+	-	+	+	A	-	+	-	+

Note: Positive = (+), Negative = (-)

A= Acid, AG= Acid Gas formation, Negative= (-)

Table 5 determines the biochemical characterization of lipase producing bacterial isolates. A total of 9 biochemical tests namely test for Urease, Catalse, TSI, IMViC, Gelatin, Starch Hydrolysis, Sugar fermentation (Glucose, Lactose, Sucrose), Oxidase, Casein were performed. Among the 7 isolates tested 3 were positive (isolate name) for urease, 6 were positive for catalase, isolates and 4 urease negative isolates respectively. In Catalase Test, among the 7 isolates there are 6 catalase positive isolates and 1 i.e. JRD39 catalse negative isolates are observed.

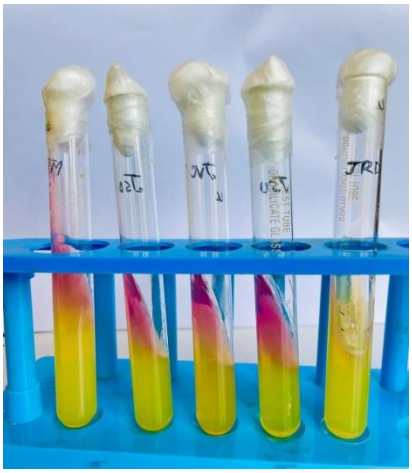


Fig 7: Urease test

In TSI Test, among the 7 isolates 6 TSI positive isolates and 1 TSI negative isolate is observed i.e. JRD44.

Fig: 8. TSI Test

The IMViC Test stands for Indole, Methyl Red, Voges Proskauer and Citrate Test. It is divided into three parts:- Indole, Methyl Red- Voges Proskauer and Citrate Test. In indole test among the 7 isolates, JRD4, JRD9, JRD39 and JRD44 are positive isolates and 3 are indole negative i.e. JRD6, JRD11, JRD24. The MR-VP test, JRD6, JRD11 and JRD24 show negative MR-VP test. In Citrate test among the 7 isolates, it is observed that JRD6, JRD11 and JRD24 show positive citrate test and the other 4 isolates show citrate negative isolates.

In Gelatin Test among the 7 isolates there are 4 gelatin positive and 3 gelatin negative isolates are observed. In starch hydrolysis test it is observed that among the 7 isolates, 6 isolates obtain starch hydrolysis and the other one isolates does not obtain starch hydrolysis.

The sugar fermentation test includes three sugars, those are glucose, lactose and sucrose. In glucose test among the 7 isolates it is observed that 3 isolates show acid formation and 4 isolates show acid gas formation. In lactose test, it shows that among the 7 isolates, 3 isolates show acid formation and 4 isolates show negative lactose formation. In sucrose test it

shows that among the 7 isolates, 5 isolates show positive in sucrose and 2 isolates show negative in sucrose.

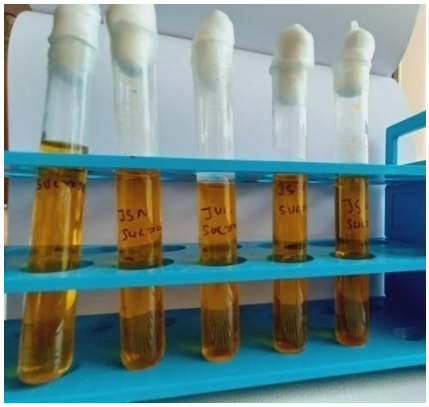


Fig: 9. Sucrose Test

In oxidase test among the 7 isolates, 4 isolates show positive in oxidase test and 3 isolates show negative in oxidase test. In casein hydrolysis test among the 7 isolates, 5 isolates show casein positive and 2 isolates show casein negative.

4.7 Physiological Characterization

Table 8: Effect of sodium chloride (NaCl) on the growth of lipase producing isolates

Sl. No.	Samples	Concentration Of NaCl								
		(0)	(0.5)	(1)	(1.5)	(2)	(2.5)	(3)	(3.5)	(4)
1	JRD4	-	+ + + +	+ + +	+ +	+	-	-	-	-
2	JRD6	-	+ + + +	+ + +	+ +	-	-	-	-	-
3	JRD9	-	+ + + +	+ + + +	+	+	-	-	-	-
4	**JRD11**	**-**	**+ + + +**	**+ + + +**	**+ +**	**+**	**+**	**-**	**-**	**-**
5	JRD24	-	+ + + +	+ + +	+ +	+	-	-	-	-
6	JRD39	-	+ + + +	+ + +	+ +	+	-	-	-	-
7	JRD44	-	+ + + +	+ + +	+ +	+	-	-	-	-

Note: Excellence = + + + + ; Very Good = + + + ; Good = + + ; Poor = +

Table 6 represents the physiological characterization of the isolates obtained from the different soil sample. The physiological characterization includes effect of NaCl and effect of pH. In effect of NaCl, the physiological characterization of effect of NaCl is determined by the concentration of NaCl used for the growth of culture in the petri dish. In a petri dish different concentration of NaCl is taken along with the media required for the growth of culture. The concentration of NaCl are :- 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 . All the 7 isolates were grown in different NaCl concentration and observed. In 0.5% and 1% concentration of NaCl all the 7 isolates show excellent growth (++++). The isolates are JRD4, JRD6, JRD9, JRD11, JRD24, JRD39, JRD44. Some of the isolates show very good to good growth. In 1.5% NaCl concentration, among the 7 isolates, the isolate JRD9 show poor growth. In 2% NaCl concentration among the 7 isolates JRD6 shows no growth while in 2.5% NaCl concentration JRD11 shows poor growth while all the other 6 isolates show no growth. the rest of the. In 3%, 3.5% and 4% NaCl concentration none of the 7 isolates have shown growth. It is seen that as the concentration of NaCl increases, the growth of the isolates decreases. Hence the obtained isolates cannot grow on the higher concentration of NaCl .

Fig: 10. NaCl 0.5% concentration

Table 9: Effect of pH on the growth of lipase producing isolates

Sl. No.	Samples	pH1	pH2	pH3	pH4	pH5	pH6	pH7	pH8	pH9	pH10	pH11	pH12	pH13	pH14
1	JRD4	-	+	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + +	+ + + +	+ + + +	+ + +	+ +	-	-
2	JRD6	-	+ +	+ +	+ + +	+ + +	+ + +	+ + + +	+ + +	+ + +	+ + + +	+ + +	+	-	-
3	JRD9	-	+	+ +	+ + +	+ + +	+ + +	+ + + +	+ +	+ +	+ + + +	+ + +	+	-	-
4	**JRD11**	**-**	**+**	**+ +**	**+ + +**	**+ + +**	**+ + +**	**+ + + +**	**+ + + +**	**+ + + +**	**+ + + +**	**+ + +**	**+**	**-**	**-**
5	JRD24	-	+ +	+ +	+ + +	+ + +	+ + +	+ + + +	+ ++	+ +	+ + + +	+ + +	+	-	-
6	JRD39	-	+ + +	+ +	+ + +	+ + +	+ + +	+ + + +	+ +	+ + +	+ + + +	+ +	+	-	-
7	JRD44	-	+ +	+ +	+ +	+ +	+ +	+ + + +	+ +	+ + +	+ + + +	+ +	+	-	-

In effect of pH, the physiological characterization of effect of pH is determined by the use of pH for the growth of culture in petri dish. In each petri dish different concentration of pH is taken along with the media for the growth of culture from the respective isolates. The pH ranges from pH1, pH2, pH3, pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11, pH12, pH13 and pH14. All the 7 isolates are grown in different concentration of pH each and observed. In pH7 and pH10, all the 7 isolates show excellent growth with ++++ whereas in pH1, pH13 and pH14 none of the 7 isolates shows growth. In pH2 and pH12, all the 7 isolates show poor growth whereas in rest of the remaining pH i.e. pH3, pH4, pH5, pH6, pH8, pH9 and pH11 all the 7 isolates show very good (+++) to good (++) growth . Therefore, it is seen that as the concentration of pH increases, the growth of the isolates decreases due to high concentration of pH. Hence isolates does not grow in higher and lowest pH .

Fig: 11. Bacterial growth on pH 7

Fig:12. Bacterial growth on pH 8

Fig: 13. Bacterial growth on pH 9

Fig: 14. Bacterial growth on pH 10

4.8 Screening of isolates for the production of Lipase

Table 10: Quantitative screening of isolates for the production of lipase

Sl. No.	Isolates	Zone of Clearance (mm)
01	JRD4	20
02	JRD6	18
03	JRD9	22
04	**JRD11**	**28**
05	JRD24	26
06	JRD39	15
07	JRD44	24

Table 8, indicates the quantitative screening of the isolates of lipase production. Here among from the 45 isolates, 7 isolates were chosen for the quantitative test. The excellence zone of clearance was observed in JRD 11, and all other 6 isolates were observed as poor to very good zone of clearance.

Notes, poor(+), good (++), very good(+++), and excellence(++++).

4.9 Optimization of process conditions for optimized production of Lipase by JRD11

Table 11: Influence of still and shaking conditions on the production of lipase by JRD11

	Still condition	Shaking condition
Enzyme activity		
160rpm	0	95.02

Table 11 shows the comparison between influence of still and shaking condition in 160rpm.

Table 12: Influence of shaking condition on the production of lipase by JRD11

Shaking condition (rpm)	**120rpm**	**140rpm**	**160rpm**	**180rpm**	**200rpm**
Enzyme activity (U/ml)					
0	0	0	0	0	0
20	23.01	25.03	32.05	24.03	21.05
40	42.01	45.03	50.06	43.06	41.04
60	63.07	65.02	71.09	66.01	62.05
80	80.06	81.03	82.05	80.03	80.02
100	91.01	93.03	95.02	90.04	90.01

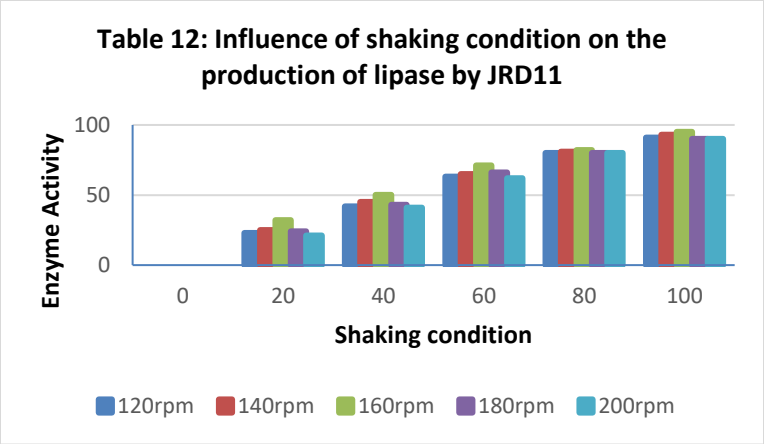


Fig: 15. Influence of shaking condition on the production of lipase by JRD11

The findings made it abundantly clear that shaking was necessary for the bacteria to create lipase because there was no lipase production in a stationary state. Production of the lipase enzyme was enhanced by agitation between 120 and 160 rpm.160rpm of agitation was the ideal speed for the bacteria to produce lipase. The synthesis of enzymes decreased when the agitation rate exceeded 160 rpm. larger oxygen transfer rates, larger surface areas in interface with media components, and improved oil substrate dispersibility during fermentation under agitated conditions may all be contributing factors to the rise in lipase synthesis (Veerapagu et al., 2013).

Table 13: Influence of incubation period on the production of lipase by JRD11

Inorbital shaker / **Time (in hours - h)**	**110rpm**	**130rpm**	**150rpm**	**170rpm**	**180rpm**
0hr	0	0	0	0	0
12hr	4.04	6.02	8.02	7.01	5.02
24hr	5.03	8.04	8.03	10.02	6.05
36hr	8.01	12.05	14.02	13.02	9.04
48hr	12.05	14.05	17.07	16.05	11.07
60hr	10.02	12.3	14.01	13.05	9.05
72hr	8.07	11.08	13.04	12.03	8.02

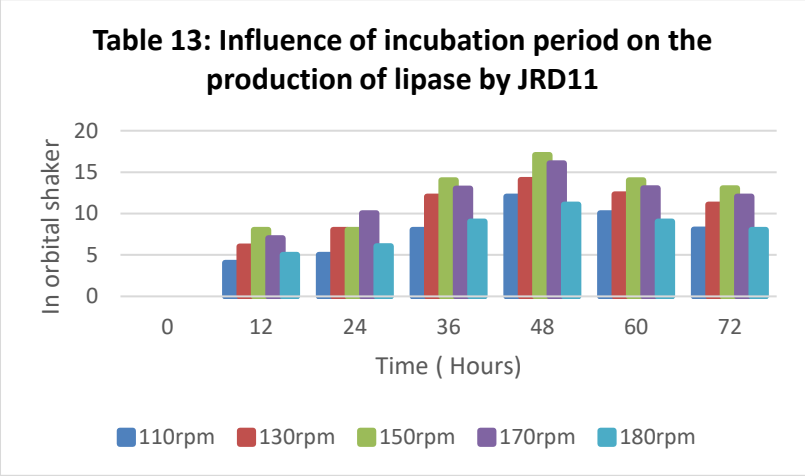


Fig: 16. Influence of incubation period on the production of lipase by JRD11

Inoculated with Tributyrin broth, the isolates were extracted after 8 hours. The early stationary phase was 48 hours when the enzyme activity peaked. After 48 hours, the enzyme activity gradually reduced (Sirisha et al., 2010)

Table 14: Influence of different temperature conditions on the production of lipase by JRD11

Temperature (in °C) -------→ **Time (in hours - h)**	**25°C**	**30 ° C**	**35 ° C**	**40 ° C**	**45° C**
0hr	0	0	0	0	0
12hr	5.05	7.01	9.01	8.02	6.01
24hr	6.04	9.03	9.02	11.01	7.04
36hr	9.02	13.04	15.01	14.01	10.03
48hr	12.03	14.03	17.05	16.03	11.05
60hr	11.01	13.2	15.02	14.06	10.06
72hr	9.06	12.09	14.05	13.04	9.01

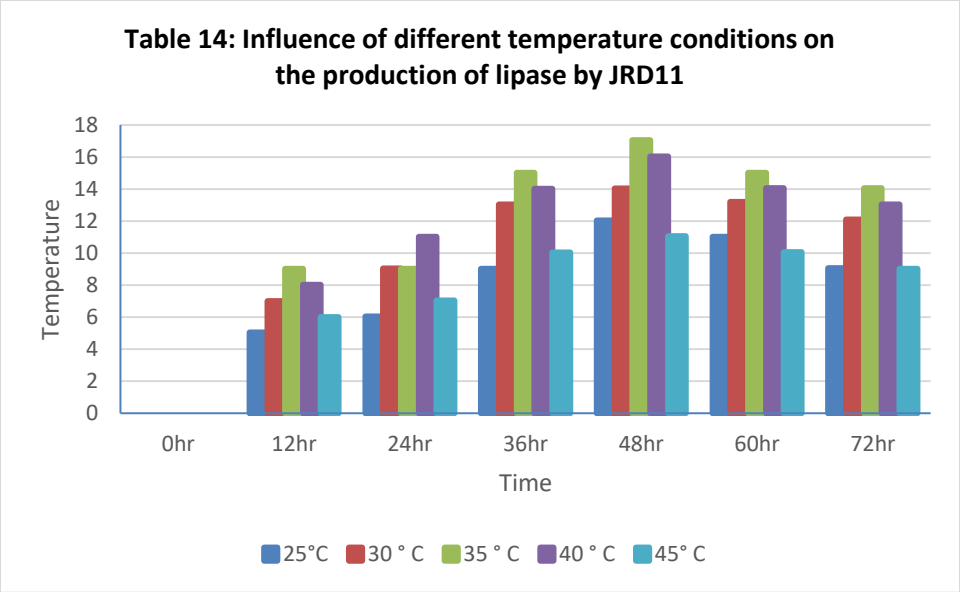


Fig: 17. Influence of different temperature conditions on the production of lipase by JRD11

Table 15: Influence of different pH conditions on the production of lipase by JRD11

pH----→ / **Time (in hours - h)**	**5**	**6**	**7**	**8**	**9**
0hr	0	0	0	0	0
12hr	4.03	7.02	9.01	6.8	5.09
24hr	8.06	11.09	13.07	10.03	9.01
36hr	9.01	13.01	16.01	13.02	10.01
48hr	11.04	15.03	20.03	15.05	14.02
60hr	10.01	13.04	17.03	16.01	11.06
72hr	9.09	12.01	15.03	14.06	10.01

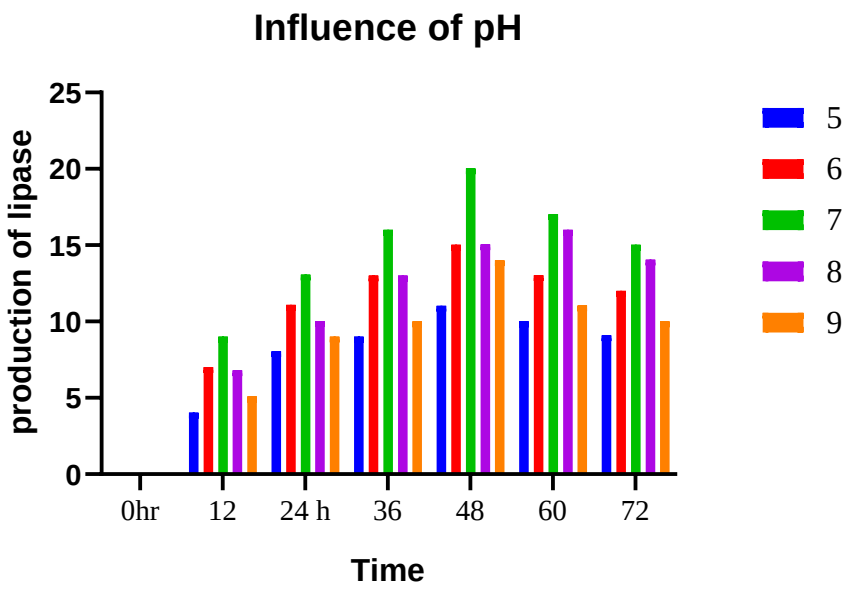


Fig: 18. Influence of different pH on the production of lipase by JRD11

Different pH fermentation media, such as 5, 6, 7, 8 and 9 were created and inoculated with bacterial cultures. In order to produce the most lipase enzyme possible, the lipase activity was evaluated, and the ideal pH was chosen (Jaiswal et al., 2017). The value of pH 7 on 48 hr is highest with the value of 20.03 an lowest at pH 5 on 12hr with the value of 4.03.

Table 16: Influence of different Carbon Source on the production of lipase by JRD11

Carbon source / Time(hr)	Tween 20	Tween80	Olive Oil	Glucose	Fructose
0hr	0	0	0	0	0
12hr	9.06	15. 06	18.08	4.08	6.06
24hr	10.08	17.05	19.05	5.07	7.08
36hr	11.05	19.14	20.05	6.04	8.06
48hr	8.03	14.04	17.01	3.02	5.03
60hr	7.05	13.18	16.10	2.06	4.02
72hr	6.04	12.06	15.05	1.08	3.08

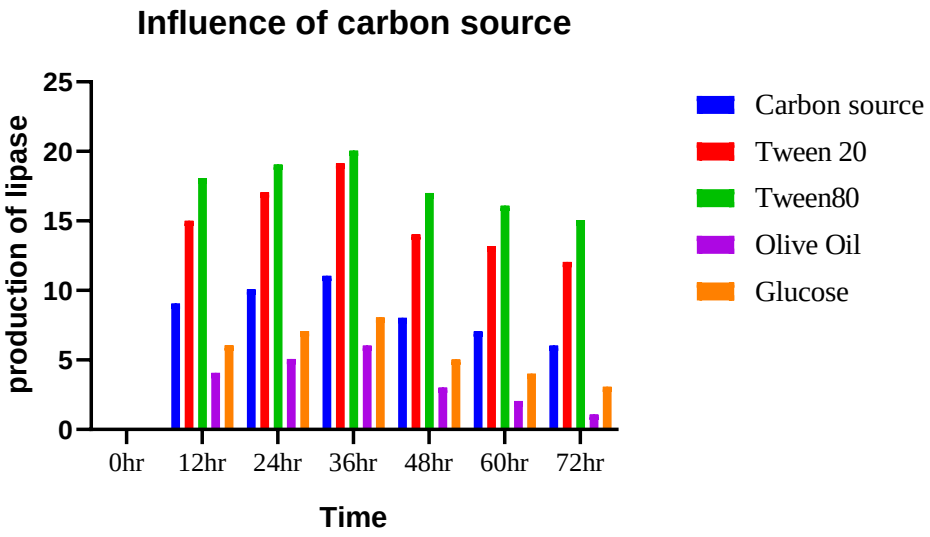


Fig:19. Influence of different carbon source on the production of lipase by JRD11

Table 9.2 shows influence of carbon source. Tween20, Tween80, olive oil, glucose, and fructose were some of the carbon sources chosen since they might stimulate the formation of lipase (Ilesanmi et al., 2020). Each type of carbon source was present in the nutrient broth where the bacterial culture was injected. Cultures were cultured at 30 °C for 12 hours, 24hr, 36hr, 48hr, 60hr and 72 hr . The highest value observed is in olive oil in 36hr (20.05) and second highest in tween 80 in 36hr (19.14). The lowest value is in glucose with the value of 1.08 at 72hr.

Table 17: Influence of different Nitrogen Source on the production of lipase by JRD11

Nitrogen source	Yeast Extract	Peptone	Caesin	Ammonium nitrate	Potassium Nitrate
Time(hr)					
0hr	0	0	0	0	0
12hr	9.04	7.05	6.01	3.05	2.08
24hr	11.06	9.05	8.06	4.06	3.03
36hr	13.07	11.06	9.05	5.07	4.06
48hr	17.06	15.04	12.05	6.05	5.08
60hr	15.05	14.06	10.08	8.01	7.03
72hr	13.01	14.02	8.05	6.03	5.06

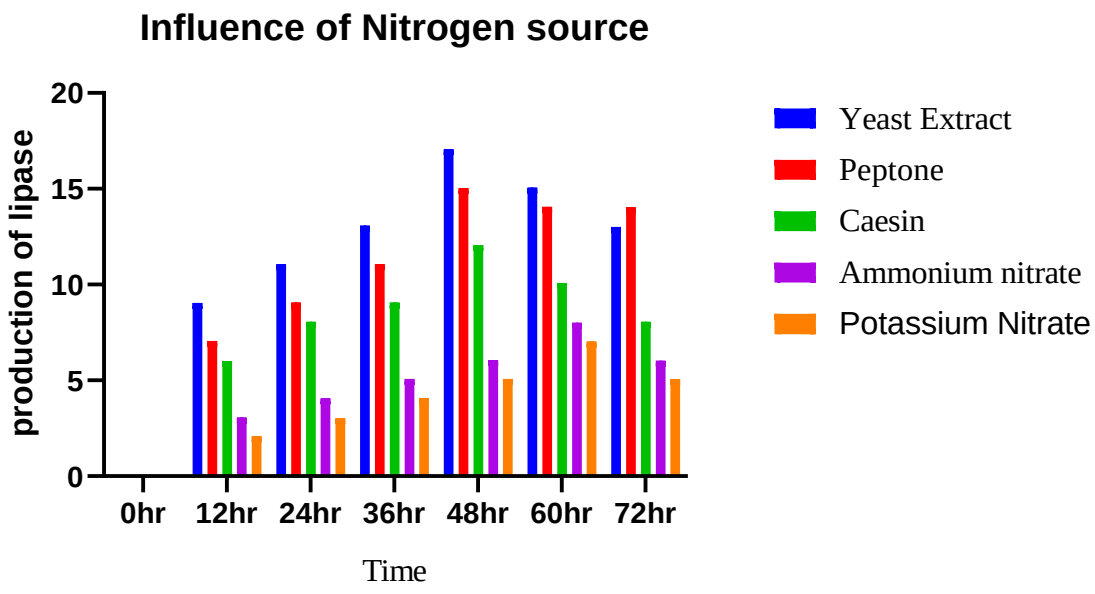


Fig: 20. Influence of different nitrogen source on the production of lipase by JRD11

Table 9.4 shows influence of nitrogen source. The production media were supplemented with the various nitrogen sources, such as yeast extract, peptone, caesin, ammonium nitrate and potassium nitrate (Jaiswal et al., 2017). The value is highest in 48hr of yeast extract (17.06) and lowest in 12hr of potassium nitrate (5.08).

4.1Molecular characterization

Sample JRD11 is found to be Pseudomonas aeruginosa based on nucleotide homology, & phylogenetic analysis.

>RES_3937_F

TGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAA
GCACCGGCTAACTTCGTGCC
AGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCGCGTAGGTGGTTCAGC
AAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAG
CTAGAGTACGGTAGAGGGT
GGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTG
GCGAAGGCGACCACCTGGAC
TGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCCGTAAACGA
TGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGT
CGACCGCCTGGGGAGTACG
GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
TGTGGTTTAATTCGAAGCAA
CGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGT
GCCTTCGGGAACTCAGACAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG

>RES_3937_R

TGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGACTGATACTGACACTG
AGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGTCGACTAGCCGT
TGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGA
GTACGGCCGCAAGGTTAAA
ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTA
CCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC

AGACACAGGTGCTGCATGG
CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCC
TTGTCCTTAGTTACCAGCAC
CTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAGTCATCATGGCC
CTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCC
GCGAGGTGGAGCTAATCCC ATAAAACC

>RES-3937_contig

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AGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCGCGTAGGTGGTTCAGC
AAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAG
CTAGAGTACGGTAGAGGGT
GGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTG
GCGAAGGCGACCACCTGGAC
TGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCCGTAAACGA
TGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGT
CGACCGCCTGGGGAGTACG
GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
TGTGGTTTAATTCGAAGCAA
CGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGT
GCCTTCGGGAACTCAGACAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTA
ACGAGCGCAACCCTTGTCCT
TAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCA
AGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAA
GGGTTGCCAAGCCGCGAGG TGGAGCTAATCCCATAAAACC

Chapter – V
Summary and Conclusions

CHAPTER 5: SUMMARY

In summary, lipase enzymes are important for soil ecosystems. They are created by a variety of soil-dwelling microorganisms, such as bacteria and fungi. In plant waste and other organic materials in the soil, lipids and other complex organic molecules, such as lipids, are broken down by lipase enzymes. Triglycerides and other lipid molecules are hydrolyzed by lipases in soil to produce glycerol and free fatty acids. By converting complex organic chemicals into simpler forms that may be easily used by other species in the soil food web, this process releases energy that has been stored in them. In soil ecosystems, lipases help in nutrient cycling and the breakdown of organic materials.

Temperature, moisture content, pH, and the accessibility of substrates are some of the variables that have an impact on the lipase enzyme activity in soil. Different lipase enzyme varieties may have different temperature and pH optimum ranges, enabling them to function at their best in particular soil conditions.

Overall, soil lipase enzymes support soil fertility, nutrient cycling, and organic matter breakdown. They play a crucial role in the availability and cycling of nutrients as well as the general health and productivity of soil ecosystems.

Chapter – VI

Conclusions

CHAPTER 6: CONCLUSIONS

In conclusion the goal of the current investigation was to separate and characterize microorganisms that produce lipase from distinct samples. Based on their ability to grow on media with lipid as the only source of carbon and their zone of hydrolysis, seven bacterial isolates were chosen. An isolate that produced the most lipase in the supernatant after being screened using quantitative estimates was chosen and further investigated. The bacterium was recognized as Pseudomonas aeruginosa after being biochemically characterized using "Bergey's Manual of Determinative Bacteriology" and by molecular characterization.

In nutritive and tributyrin broth media, growth curves were generated, and the phase of enzyme synthesis was identified. The isolation first seemed promising and could be a good source of lipase, but further characterisation of the growth and nutritional conditions is needed.

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APPENDIX

A. Nutrient Media Agar

Nutrient Agar	5.6g
Agar Agar	2g
Distilled water	200ml

B. **Tween 80 Media (**O.I. Ilesanmi, A.E. Adekunle and J.A. Omolaiye et al., 2020)

Peptone	10g/L
Agar agar	20g/L
NaCl	5g/L
$CaCl_2.2H_2O$	0.1g/L
Tween80	10ml
Distilled water	1000 ml

C. Staining and reagents

D. Gram staining (Aneja, 2018)

Crystal violet (primary stain)

Gram's iodine solution (the mordant)

Acetone/ethanol (the decolorizer)

Safranin (secondary stain)

E. Capsule staining (Aneja, 2018)

Crystal violet (85% dye content)

Nigrosine 10gm

F. Flagella staining (Aneja, 2018)

Flagella mordant

Ziehl's carbol fuchsin

Carbol fuchsin

1ml distilled water

Dichromate solution

95% alcohol

G. Biochemical tests and chemical

H. Urease Test (Aneja 2018)

Peptone	1.0g
Sodium chloride	5.0g
Potassium monohydrogen (or dihydrogen) phosphate	2.0g
Agar	20.0g
Distilled water	1000ml
Phenol red (0.2% solution)	6.0ml
Glucose	1.0g
Urea	100.0ml

I. Catalase test (Aneja 2018)

Trypticase	15.0g
Phytone	5.0g
Sodium chloride	5.0g
Agar	15.0g
Distilled water	1000.0ml

J. Indole test (Aneja 2018)

Peptone	10g
Distilled water	1000ml
Kovac's reagent	1ml

K. Methyl-Red and Voges-Proskauer tests (Aneja 2018)

Peptone	7.0g
Dextrose/Glucose	5.0g
Potassium phosphate	5.0g
Distilled water	1000.0ml
Methyl red indicator	1 drop each tube

L. Citrate test (Aneja 2018)

Ammonium dihydrogen phosphate	1.0g
Dipotassium phosphate	1.0g
Sodium chloride	5.0g
Sodium citrate	2.0g
Magnesium sulphate	0.2g
Agar	15.0g
Bromothymol blue	0.8g
Distilled water	1000.0ml

M. Gelatin test (Aneja 2018)

Gelatin agar medium	60ml
Mercuric chloride	20ml

N. Starch hydrolysis (Aneja 2018)

Meat extract	3.0
Peptic digest of animal tissue	5.0
Starch soluble	2.0
Agar	15.0
Distilled water	1000.0ml

O. Sugar fermentation (Aneja 2018)

Tripticase/peptone	10.0g
Glucose/Sucrose/Lactose	5.0g
Sodium Chloride	15.0g
Phenol red	0.018g
Distilled water	1000.0ml
pH	7.3

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