Chapter One

1.1 Background to the Study

The cosmetics and skin care industry is a rapidly evolving sector that plays a significant role in global economies and in the everyday lives of individuals[1]. Historically, the use of cosmetics and skin care products can be traced back thousands of years to ancient civilizations such as Egypt, Greece, and China, where natural substances like oils, clays, and herbal extracts were utilized for beautification, hygiene, and therapeutic purposes[2]. The industry has expanded and is influenced by scientific advancements, changing society standards of beauty, and increasing consumer demand for health and wellness[3].

In the modern era, the cosmetics and skin care industry include a broad range of products including moisturizers, cleansers, sunscreens, anti-aging treatments, makeup, and personal care items. These products are designed to enhance appearance and promote skin health and overall well-being[4]. The global market value of the cosmetics industry has been steadily increasing, driven by factors such as urbanization, rising disposable incomes, increased consumer awareness about personal grooming, and the influence of social media and beauty influencers[5].

In recent years, consumer preferences have shifted to products that offer additional health benefits, are sourced from nature, and are manufactured using ethical practices. This has led to an increased demand for transparency in ingredient sourcing, production processes, and product claims[6]. The industry has seen innovation, with cutting-edge technologies such as biotechnology, nanotechnology, and personalized skincare solutions transforming traditional approaches to product development and marketing[7,8] .

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As the industry continues to grow, it faces challenges such as regulation, competition, and heightened scrutiny regarding the safety and environmental impact of ingredients and

packaging. These factors have encouraged manufacturers to seek alternatives to synthetic chemicals, turning towards more natural, sustainable, and biotechnologically-derived ingredients to meet evolving consumer expectations[9,10].

1.2 Importance of Natural and Biotechnological Ingredients

Natural and biotechnological ingredients have gained immense importance in the cosmetics and skin care industry, reflecting a broader shift towards sustainability, safety, and efficacy[10]. Natural ingredients, derived from plant, mineral, and animal sources, have traditionally been valued for their gentle nature, rich nutrient profiles, and perceived safety compared to synthetic compounds[12]. Consumers increasingly associate natural products with health, authenticity, and environmental responsibility, making them highly attractive in a market that emphasizes clean beauty and wellness[13].

The appeal of natural ingredients is not sentimental or marketing-driven; many natural substances possess potent bioactive properties such as antioxidant, anti-inflammatory, moisturizing, and antimicrobial activities[14]. For instance, botanical extracts like aloe vera, green tea, and chamomile are widely incorporated into skin care formulations for their proven therapeutic benefits. However, sourcing natural ingredients can pose challenges such as supply limitations, inconsistent quality, and environmental degradation, prompting the need for sustainable and innovative solutions[15].

This is where biotechnological ingredients have become increasingly important. Biotechnology involves the use of living organisms, such as bacteria, yeast, and plant cells, to produce active compounds for cosmetic applications[16]. Through processes like fermentation,

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tissue culture, and genetic engineering, biotechnology enables the production of high-purity, consistent, and sustainable ingredients without depleting natural resources. Examples include hyaluronic acid

produced via microbial fermentation, plant stem cell extracts, and bioengineered peptides that enhance skin repair and rejuvenation[17,18].

Biotechnological innovations not only offer environmental and ethical advantages but also open new possibilities for formulating highly effective and targeted skin care solutions[17]. They allow for the discovery and production of novel compounds that would be difficult or impossible to obtain through traditional extraction methods. Furthermore, biotechnological processes often require less land, water, and energy compared to traditional agriculture, making them a cornerstone of sustainable development in the cosmetics sector[12].

Natural and biotechnological ingredients is reshaping the cosmetics and skin care industry. It addresses consumer demands for safe, effective, and environmentally responsible products while also fostering scientific advancement and innovation.

1.3 Overview of Microbial Exopolysaccharides (EPS)

Microbial exopolysaccharides (EPS) are high-molecular-weight polymers composed mainly of sugar residues that are secreted by microorganisms into their surrounding environment. These complex biopolymers are produced by a wide range of microbes, including bacteria, fungi, and algae, either as capsular polysaccharides (closely associated with the cell surface) or as slime polysaccharides (released into the external medium)[1,2]. EPS play critical roles in microbial survival, offering protection against environmental stresses, aiding in surface adhesion, and facilitating biofilm formation[5].

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Structurally, EPS are highly diverse, consisting of homopolysaccharides (composed of one type of monosaccharide) or heteropolysaccharides (composed of several types of monosaccharides)[8]. Common sugar components include glucose, galactose, mannose, rhamnose, fucose, and uronic acids. This chemical diversity results in a wide range of physical,

chemical, and biological properties, making microbial EPS highly valuable for industrial and biomedical applications[7].

In the cosmetics and skin care industry, microbial EPS have attracted significant attention due to their exceptional biocompatibility, biodegradability, and multifunctionality. EPS can act as natural moisturizers, skin barrier enhancers, anti-aging agents, and soothing ingredients[8]. Their ability to form hydrating films on the skin surface helps in retaining moisture, improving skin texture, and providing a protective barrier against environmental pollutants and irritants. Additionally, certain EPS exhibit antioxidant, anti-inflammatory, and wound-healing properties, further enhancing their value in skincare formulations[8].

The sustainable production of EPS via biotechnology offers a significant advantage over the harvesting of plant or animal materials, aligning with the growing trend toward eco-friendly and ethical cosmetic products[9]. Microbial fermentation processes can be tightly controlled, ensuring consistent quality, high yield, and minimal environmental impact. Moreover, advances in metabolic engineering and synthetic biology have expanded the possibilities of tailoring EPS structures to enhance specific functionalities desirable in cosmetic applications[19].

Microbial exopolysaccharides represent a promising class of natural and sustainable ingredients with wide-ranging benefits for the cosmetics and skin care industry. Their

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multifunctionality, combined with environmentally friendly production methods, positions them as key components in the future development of innovative, safe, and effective skin care products[20].

1.4 Research Problem

Despite the growing interest in natural and biotechnological ingredients in the cosmetics and skin care industry, there remains a significant gap in the full exploration and utilization of microbial exopolysaccharides (EPS) as high-value cosmetic ingredients. While EPS have demonstrated remarkable moisturizing, anti-aging, antioxidant, and skin-protective properties in preliminary studies, their adoption in commercial formulations is still relatively limited compared to more conventional natural extracts and synthetic compounds[9, 10, 11].

The growing consumer demand for natural, biocompatible, and eco-friendly ingredients in the cosmetic industry has led to an increased interest in bio-based polymers[7]. Among these, microbial exopolysaccharides (EPS) have emerged as promising alternatives to synthetic additives due to their moisturizing, emulsifying, film-forming, and antioxidant properties[7]. Despite their demonstrated potential, the commercial use of microbial EPS in cosmetic formulations remains limited, particularly in sub-Saharan Africa, due to knowledge gaps in the biosynthesis, functional characterization, and application-specific performance of these biopolymers[8].

Furthermore, most commercially available EPS used in cosmetic formulations are derived from limited microbial sources such as *Xanthomonas campestris* (xanthan gum) and *Leuconostoc spp.* (dextran), leaving a vast array of potentially useful microbial strains underexplored[9]. There is also insufficient data on the comparative effectiveness of EPS from

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indigenous microbial isolates, especially those sourced from diverse ecological niches such as fermented foods, soil, and marine environments[9].

The absence of studies linking EPS biosynthetic potential with physicochemical characteristics and functional performance in cosmetic contexts poses a significant barrier to the development of innovative, locally sourced bio-ingredients. This research, therefore, seeks to address this gap by isolating and characterizing microbial exopolysaccharides with potential cosmetical

applications, providing a scientific basis for their development and use in the cosmetics industry.

1.5 General Aim and Objectives of the Study

To produce, optimize, and evaluate the application of microbial exopolysaccharide in the formulation of a clarifying skin gel.

The specific objectives are to

i. Isolate and identify potential exopolysaccharide producing microorganisms from various sources.

ii. Determine and optimize physicochemical conditions (such as pH, temperature, carbon sources) that influence the growth of the selected microorganisms and their exopolysaccharide production.

iii. Structurally characterize produced exopolysaccharide by techniques such as FTIR.

iv. Use exopolysaccharide in a base formulation for skin clarifying gel.

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v. Evaluate the final skincare product for quality parameters including stability, texture, and skin compatibility.

1.6 Research Questions/Hypotheses

The following research questions guide this research work;

1. What types of microorganisms from selected environmental or biological sources are capable of producing exopolysaccharides (EPS)?
2. How do varying physicochemical conditions (e.g., pH, temperature, and carbon source) affect the growth and EPS production of the selected microorganisms?
3. What are the structural and functional characteristics of the exopolysaccharides produced, as determined by FTIR and other relevant analytical techniques?
4. Can the extracted exopolysaccharide be effectively incorporated into a base formulation to develop a functional skin-clarifying gel?
5. How does the formulated skincare product perform in terms of stability, texture, and skin compatibility under standard evaluation protocols?

1.7 Significance of the Study

The significance of this study is in its contribution to advancing the understanding and application of microbial exopolysaccharides (EPS) as innovative, natural, and sustainable ingredients in the cosmetics and skin care industry. There is growing consumer demand for safe, eco-friendly, and effective cosmetic products, there is a pressing need to explore alternative bio-based materials that align with these expectations. This study addresses that need by shedding light on the potential of microbial EPS, which offer unique functional properties beneficial to skin health and cosmetic formulation[12].

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This research will contribute to the scientific community by expanding knowledge on the biological activities, production technologies, and formulation potentials of various microbial EPS. It will provide detailed insights into their moisturizing, antioxidant, anti-aging, and protective capabilities, paving the way for more research-driven innovations in natural skin care solutions.

The findings of this study will offer practical value to cosmetic manufacturers and product developers. By identifying the best-performing microbial EPS and understanding how to optimize their production and incorporation into products, companies can create more

effective, sustainable, and market-attractive skin care formulations. This can enhance product performance while also aligning with global trends toward clean beauty and environmental responsibility.

The study also holds significance for consumers by promoting safer and more ethical cosmetic options. Microbial EPS production via fermentation processes typically requires fewer chemical inputs and results in minimal environmental impact compared to the extraction of plant or animal-derived ingredients. Promoting microbial EPS thus supports sustainable practices while delivering high-performance products to end-users.

By also addressing the regulatory, safety, and consumer perception aspects of microbial EPS utilization, the study will help bridge the gap between scientific innovation and market adoption. It will contribute to the broader acceptance and understanding of microbial biotechnology in cosmetics, ultimately supporting industry growth, sustainability goals, and consumer trust. This study is significant because it advances scientific knowledge, supports sustainable innovation in cosmetics, benefits consumer health and choice, and contributes to

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environmental stewardship, all while responding to the evolving dynamics of the global cosmetic industry.

1.8 Operational Definition of Terms

Cosmetics - Products applied to the human body, particularly the skin, hair, nails, and lips, with the primary purpose of cleansing, beautifying, promoting attractiveness, or altering appearance without affecting the body's structure or functions.

Skin Care- A branch of cosmetics focused specifically on maintaining and improving the health, appearance, and function of the skin through the use of cleansers, moisturizers, serums, sunscreens, and treatment formulations.

Natural Ingredients - Substances derived from plant, mineral, microbial, or animal sources that have undergone limited processing and are incorporated into cosmetic formulations to provide functional or therapeutic benefits.

Biotechnological Ingredients - Ingredients produced using biological systems, including microorganisms, enzymes, and cell cultures, often through fermentation or genetic engineering processes, to yield high-purity, sustainable compounds for cosmetic use.

Microbial Exopolysaccharides (EPS) - High-molecular-weight polysaccharides secreted extracellularly by microorganisms (such as bacteria, fungi, and microalgae) during fermentation processes. In cosmetics, they are valued for their moisturizing, film-forming, antioxidant, and protective properties.

Fermentation - A biological process in which microorganisms convert substrates (such as sugars) into desired products (such as EPS) under controlled conditions, often used for sustainable production of cosmetic ingredients.

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Antioxidant Activity - The ability of a substance to neutralize free radicals and reduce oxidative stress, which helps prevent skin aging, inflammation, and damage caused by environmental factors.

Moisturization - The process of increasing or maintaining the water content of the skin's outermost layer (stratum corneum), leading to improved skin softness, elasticity, and overall appearance.

Clean Beauty - A consumer-driven movement that emphasizes the use of products formulated with safe, non-toxic, and environmentally sustainable ingredients, often avoiding synthetic chemicals perceived as harmful.

Sustainable Cosmetics - Cosmetic products developed with minimal environmental impact throughout their lifecycle including sourcing, production, packaging, and disposal often utilizing renewable resources like microbial EPS.

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Chapter Two

Literature Review

2.1 Exopolysaccharides: Definitions, Structures, and Types

Exopolysaccharides (EPSs) as described earlier are high-molecular-weight polymers predominantly composed of sugar residues, which are secreted by a wide range of microorganisms into their extracellular environment[1]. These complex carbohydrate molecules are either loosely attached to the microbial cell surface or released freely into the surrounding medium[1]. This means that they are not covalently bound to the cell surface, although in some cases, such as in capsular exopolysaccharides, they may form a protective layer around the microbial cell[2]. EPSs play pivotal roles in the microbial life cycle, influencing both ecological interactions and physiological responses. This is because EPS is often stimulated under stressful environmental conditions, such as nutrient limitation, desiccation, or the presence of toxic compounds, as they serve to protect the cell and facilitate survival [3]. They

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are primarily produced by bacteria, archaea, fungi, and certain microalgae, with bacterial exopolysaccharides being the most extensively studied due to their diverse functions and industrial applications[2]. In bacteria, EPSs contribute to the formation and stability of biofilms, enabling microorganisms to adhere to surfaces and to each other, creating a structured microbial community with enhanced resistance to environmental stresses and antimicrobial agents[3].

2.2 Components of Exopolysaccharides

The structure of exopolysaccharides is highly variable, depending on the producing organism and the environmental conditions during synthesis[4]. Chemically, EPSs consist of repeating units of monosaccharides such as glucose, galactose, mannose, rhamnose, and fructose. In addition to these common sugars, uronic acids (e.g., glucuronic acid), amino sugars (e.g., N acetylglucosamine), and non-carbohydrate substituents like acetate, phosphate, or sulfate groups may also be present, conferring unique chemical properties and biological activities to the EPS[4].

Structurally, exopolysaccharides can be broadly classified into homopolysaccharides and heteropolysaccharides. Homopolysaccharides are composed of a single type of monosaccharide, often linked in a linear or branched fashion[5]. Common examples include dextran (composed of glucose), levan (composed of fructose), and curdlan (a β-1,3-glucan). In contrast, heteropolysaccharides consist of two or more different types of monosaccharides arranged in repeating units of varying complexity. These polymers may exhibit branched or linear configurations and often have higher structural diversity than homopolysaccharides[5].

The physical properties of EPSs such as viscosity, solubility, and gel-forming ability are directly influenced by their molecular weight, degree of branching, and the nature of their

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constituent sugars. These characteristics not only determine their biological function but also influence their suitability for various industrial applications, including food, pharmaceuticals, agriculture, and biotechnology[6].

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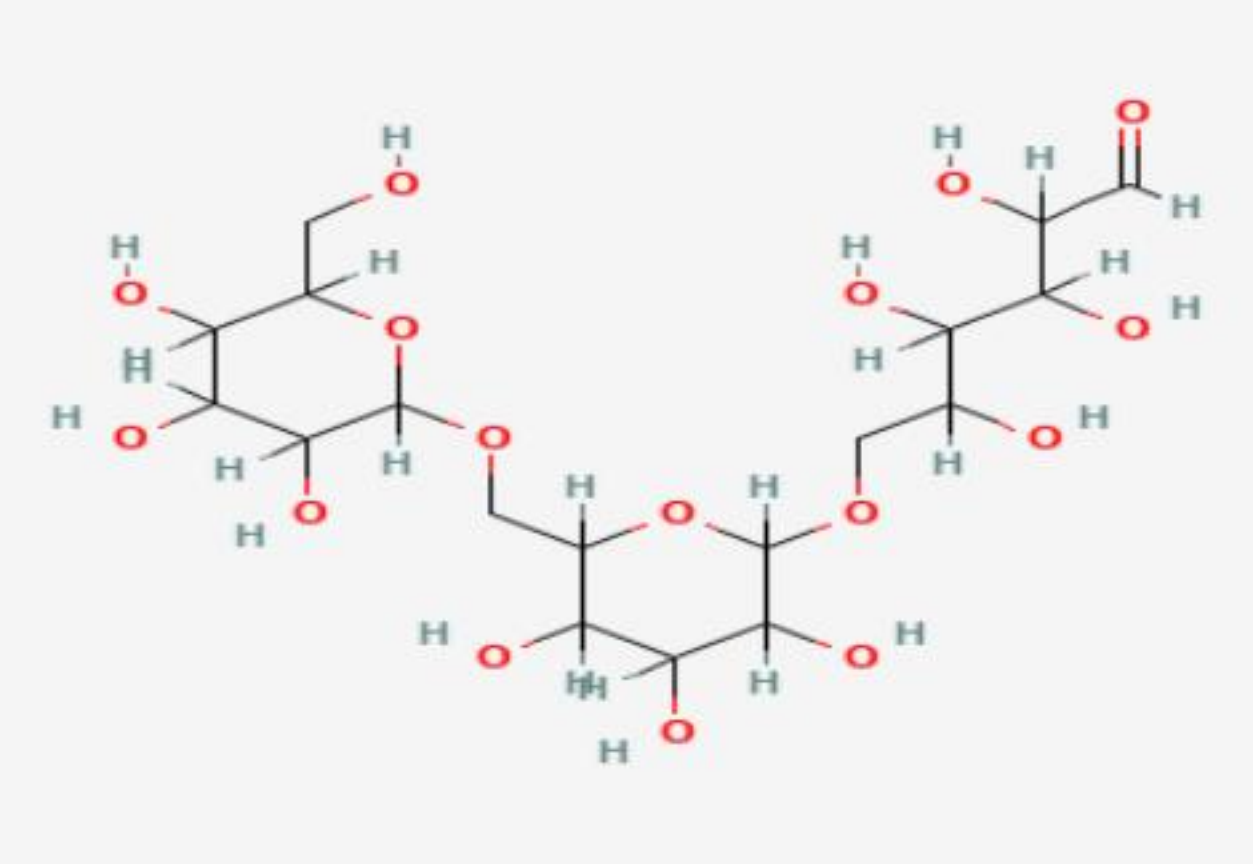

Figure 2.2: Polysaccharide Structure

Source[5]

2.3 Groups of Exopolysaccharides

Exopolysaccharides are categorized based on their chemical composition, function, and mode of association with the microbial cell and can be divided into the following groups;

2.3.1 Capsular Exopolysaccharides

Also known as capsular polysaccharides, these are tightly associated with the microbial cell surface, forming a capsule or slime layer. They provide physical protection against desiccation, phagocytosis, and antimicrobial agents. For pathogenic bacteria such as *Streptococcus pneumoniae* and *Neisseria meningitidis*, capsular EPSs are important virulence factors that help evade host immune responses[7].

2.3.2 Slime Exopolysaccharides

Unlike capsular EPSs, slime polysaccharides are loosely attached or completely released into the surrounding environment. They are integral components of the extracellular polymeric substance matrix in biofilms. Their primary role includes facilitating surface adhesion, trapping nutrients, and maintaining biofilm architecture. Slime EPSs contribute to the

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mechanical stability of the microbial community and offer resistance to environmental and chemical insults[8].

2.3.3 Neutral and Anionic Exopolysaccharides

Based on their net charge, EPSs can be neutral or anionic. Anionic EPSs contain uronic acids or inorganic substituents such as phosphates and sulfates, which give them a negative charge[9]. This feature is particularly important in metal ion binding, making them useful in bioremediation and wastewater treatment applications. Neutral EPSs, on the other hand, lack such charged groups and exhibit different solubility and binding characteristics[9].

2.3.4 Homopolysaccharides

Homopolysaccharides are a type of exopolysaccharides (EPSs) composed of only one kind of monosaccharide unit. Several notable examples highlight their diverse applications across various industries. Dextran, for instance, is synthesized by *Leuconostoc mesenteroides* and is widely used as a plasma volume expander in medicine as well as a food emulsifier. Another example is levan, which is made up of fructose units. Levan is recognized for its prebiotic properties and holds promising potential in both the medical and cosmetic fields. Curdlan, a β-1,3-glucan, is known for its gelling ability and is utilized in the food industry and also serves as a carrier in pharmaceutical formulations[10].

2.3.5 Heteropolysaccharides

Heteropolysaccharides are structurally more complex than homopolysaccharides, as they consist of different types of monosaccharide units. Several well-known heteropolysaccharides demonstrate their broad utility in various sectors. Gellan gum, produced by *Sphingomonas elodea*, serves as an effective gelling agent in both the food and pharmaceutical industries[11].

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Xanthan gum, synthesized by *Xanthomonas campestris*, is widely employed as a thickener and stabilizer in food products, cosmetics, and even in enhanced oil recovery processes. Another important example is alginate, which, although commonly derived from brown algae, is also produced by certain bacteria such as *Pseudomonas aeruginosa*. In microbial contexts, alginate contributes significantly to biofilm formation and confers resistance to antibiotics[12].

2.3.6 Functional Exopolysaccharides

Some EPSs exhibit unique biological activities such as antioxidant, immunomodulatory, antitumor, antiviral, or cholesterol-lowering effects. These functional EPSs are of considerable interest in nutraceuticals and pharmaceuticals. Their activity often depends on specific structural features such as monosaccharide composition, glycosidic linkages, molecular weight, and branching patterns[13].

Exopolysaccharides are structurally diverse extracellular polymers with a wide array of biological roles and industrial applications. They can be tailored by the producing microorganisms in response to environmental stimuli and serve critical functions in microbial ecology, including protection, adhesion, and communicationb[14]. The diversity in their chemical structures ranging from simple homopolysaccharides to complex heteropolysaccharides underpins their varied physicochemical properties and broad utility. As research continues to uncover novel EPSs and elucidate their structure-function relationships, these biopolymers promise even greater potential in biotechnology, environmental management, and health-related fields[14].

2.4 Microbial Sources of EPS (e.g., Bacteria, Fungi, Algae)

Exopolysaccharides (EPSs) are primarily synthesized and secreted by a wide variety of microorganisms across different taxonomic groups, including bacteria, fungi, and algae.

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These microbial sources differ significantly in their habitats, physiology, and metabolic capabilities, leading to the production of exopolysaccharides with distinct chemical structures and functional properties[15]. The diversity of EPS-producing microorganisms reflects the ecological necessity of these biopolymers in microbial adaptation, survival, and interaction within varied environments. The microbial origin of an EPS often determines its monosaccharide composition, molecular weight, degree of branching, and its physicochemical and biological properties, which in turn influence its industrial and environmental applications[15].

2.4.1 Bacterial Sources of Exopolysaccharides

Bacteria represent the most extensively studied and exploited microbial producers of exopolysaccharides. They are capable of synthesizing a wide variety of EPSs with differing structures and functionalities. Bacterial EPSs are often involved in biofilm formation, adhesion to surfaces, protection against desiccation, antimicrobial resistance, and interactions with host organisms in both pathogenic and symbiotic relationships[16] .

Some well-known bacterial EPS producers include

i. *Xanthomonas campestris*: This Gram-negative bacterium produces xanthan gum, one of the most commercially significant bacterial EPSs. Xanthan gum is a heteropolysaccharide composed of glucose, mannose, and glucuronic acid, with side chains that provide viscosity and stability over a wide range of temperatures and pH values. It is widely used in food, pharmaceuticals, and oil recovery[17].

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ii. *Leuconostoc mesenteroides*: Known for producing dextran, a homopolysaccharide composed of α-1,6-linked glucose units with occasional α-1,3 branches. Dextran is used in medical applications as a blood plasma volume expander and in the food industry as a thickening agent[15].

iii. *Acetobacter xylinum* (now *Komagataeibacter xylinus*): Produces bacterial cellulose, a pure form of cellulose with high tensile strength, water retention, and biocompatibility. Bacterial cellulose is applied in wound dressings, tissue engineering, and cosmetics[17].

iv. *Zoogloea ramigera:* Known for producing EPSs involved in bioflocculation during wastewater treatment processes. These EPSs aid in the aggregation of microbial cells and suspended solids[18].

v. *Lactobacillus spp.* and *Bifidobacterium spp*.: These probiotic bacteria produce EPSs that contribute to their ability to colonize the gut, modulate the immune system, and protect against gastrointestinal pathogens. Their EPSs are often heteropolysaccharides with health-promoting effects[14].

vi. *Pseudomonas aeruginosa*: A pathogenic bacterium that produces alginate, an anionic EPS that contributes to biofilm formation and antibiotic resistance, particularly in chronic infections such as those seen in cystic fibrosis patients[19].

Bacterial EPS production can be influenced by environmental factors such as carbon source availability, temperature, pH, and oxygen concentration. Because of their metabolic versatility and the relative ease of genetic manipulation, bacterial systems are extensively used in industrial biotechnology for the commercial production of EPSs[19].

2.4.2 Fungal Sources of Exopolysaccharides

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Fungi, especially filamentous fungi and yeasts, are also prolific producers of exopolysaccharides. Fungal EPSs are typically more complex and larger in molecular weight than those of bacteria and are known for their biological activity, including immunomodulatory, antitumor, and antioxidant effects. Fungi secrete these EPSs into their growth medium during submerged fermentation, and their production can be optimized by altering culture conditions[20].

Notable examples include:

i. *Ganoderma lucidum* (Reishi mushroom): This medicinal fungus produces β-glucans branched polysaccharides composed predominantly of glucose with β-1,3 and β-1,6 linkages. These β-glucans have potent immunostimulatory and anticancer properties[20].

ii. *Lentinula edodes* (Shiitake mushroom): Produces lentinan, another β-glucan with demonstrated antitumor and antiviral activities. Lentinan is used as a complementary therapy in cancer treatment[20].

iii. *Aureobasidium pullulans*: This yeast-like fungus synthesizes pullulan, a linear polysaccharide consisting of maltotriose units linked by α-1,6 bonds. Pullulan is biodegradable, non-toxic, and forms oxygen-impermeable films, making it suitable for use in edible coatings, drug delivery, and bioplastics[20].

iv. *Cryptococcus laurentii* and other basidiomycetous yeasts: These produce heteropolysaccharides with antioxidant and emulsifying properties, which may find applications in the food and cosmetic industries[20].

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v. *Aspergillus niger*: Known for producing galactomannans and other complex polysaccharides, which can have thickening or stabilizing functions in food processing[21].

Fungal EPSs are often associated with the fungal cell wall and may also play roles in pathogenesis or environmental resilience. Their high molecular complexity offers a rich resource for biotechnological exploration, especially in the fields of health and materials science[21].

2.4.3 Algal Sources of Exopolysaccharides

Microalgae and cyanobacteria (blue-green algae) are photosynthetic microorganisms capable of producing substantial amounts of EPSs, particularly in aquatic environments. These EPSs can either remain loosely attached to the cell surface or be secreted into the surrounding medium. Algal EPSs are often anionic in nature, due to the presence of uronic acids and sulfate groups, and are involved in flocculation, protection against UV radiation, and heavy metal sequestration.

i. Cyanobacteria (e.g., Nostoc, Anabaena, Microcystis): These microorganisms produce complex EPSs that help them form mats and protect against desiccation and environmental toxins. Nostoc spp. secrete gelatinous sheaths composed of heteropolysaccharides with antioxidant and immunostimulatory activities[24].

ii. *Porphyridium cruentum*: A red microalga known for secreting sulfated EPSs rich in xylose, galactose, and glucuronic acid. These polysaccharides have antiviral, anti-inflammatory, and antioxidant properties and have shown promise in cosmetic and pharmaceutical applications[24].

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iii. *Chlorella spp*.: Though best known as a protein-rich nutritional supplement, certain strains of *Chlorella* produce EPSs with emulsifying and thickening properties useful in food and cosmetic industries[23].

iv. *Botryococcus braunii:* A green alga that produces extracellular polysaccharides alongside hydrocarbons. Its EPSs aid in colony formation and environmental resilience[21].

The EPS production in algae is influenced by environmental conditions such as light intensity, nutrient availability, salinity, and temperature. Because algal EPSs are often produced in aquatic systems, they are of particular interest in marine biotechnology and environmental remediation, including as biosorbents for heavy metals and as bioflocculants in wastewater treatment [20].

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2.5 Biosynthesis of EPS by Microorganisms

The biosynthesis of exopolysaccharides (EPS) by microorganisms is a highly regulated and complex process involving the coordination of various cellular components and biochemical pathways[21]. This process is vital not only for the survival and adaptability of microorganisms but also for their ecological interactions and pathogenicity. EPS production allows microbes to form biofilms, protect themselves against environmental stresses, and interact with other organisms in both mutualistic and antagonistic relationships[21]. From an industrial and biotechnological point of view, understanding the biosynthesis of EPS is essential for manipulating microbial strains to optimize yield and tailor the functional properties of the polymers for various applications[22].

2.5.1 Overview of EPS Biosynthesis

The biosynthesis of microbial exopolysaccharides (EPS) typically follows a multi-step process. It begins with the uptake and activation of sugar precursors, which serve as the building blocks for the polysaccharide[22]. Once these precursors are activated, they undergo polymerization to form repeating sugar units. The resulting polysaccharide chain may then be modified through processes such as acetylation or phosphorylation to alter its structure and functional properties. Following these modifications, the polymer is exported across the cell membrane[23]. Finally, the EPS is assembled and released into the extracellular environment, where it can perform various biological functions.

Each of these stages is controlled by specific genes and enzymes, often clustered together in operons or gene clusters, and regulated in response to environmental stimuli such as nutrient levels, osmotic pressure, temperature, pH, and population density (quorum sensing) [23].

2.5.2 Sugar Precursor Synthesis and Activation

The biosynthesis of exopolysaccharides (EPS) begins with the formation of nucleotide sugar precursors, which are activated forms of monosaccharides that serve as the fundamental building blocks for the polysaccharide chain. These precursors include molecules such as UDP-glucose (uridine diphosphate glucose), GDP-mannose (guanosine diphosphate mannose), UDP-galactose, dTDP-rhamnose, and UDP-glucuronic acid [24]. They are derived from central metabolic pathways, primarily glycolysis and the pentose phosphate pathway. For instance, glucose-6-phosphate an intermediate in glycolysis is converted into UDP-glucose by the enzyme UDP-glucose pyrophosphorylase. The availability and regulation of

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these nucleotide sugars are crucial, as they directly influence the efficiency and rate at which EPS is produced by the microorganism [24].

2.5.3 Polymerization of Repeating Units

Once activated sugars are synthesized, they are transferred to specific carrier molecules—often undecaprenyl phosphate (C_{55}-P) embedded in the cytoplasmic membrane. Glycosyltransferases then catalyze the stepwise addition of sugar moieties to form repeating oligosaccharide units on the lipid carrier. These enzymes determine the sequence and structure of the repeating units by linking the sugar residues through specific glycosidic bonds[25].

There are two main mechanisms for polymerization

1. Wzy-dependent pathway: This is the most common pathway, especially in Gram-negative bacteria. After the repeating unit is assembled on the lipid carrier, it is flipped across the inner membrane by a flippase (Wzx), and then polymerized into a growing chain by a polymerase (Wzy). The chain length may be regulated by proteins such as Wzz[26].

2. Synthase-dependent pathway: Found in the synthesis of homopolysaccharides like cellulose and alginate. In this pathway, the polysaccharide chain is synthesized and extruded simultaneously through a transmembrane synthase complex. This is a simpler and more direct process than the Wzy-dependent route[26].

2.5.4 Post-Polymerization Modifications

Many exopolysaccharides (EPS) undergo chemical modifications either during or after the polymerization process, which significantly influence their final properties. These

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modifications can alter the solubility, charge, viscosity, and biological activity of the polysaccharide[27]. One common modification is acetylation, where acetyl groups are added to the hydroxyl groups of sugar residues. This occurs in polysaccharides like alginate and xanthan gum, and can impact both viscosity and immunogenicity[27].

Sulfation is another important modification, particularly prevalent in algal EPS, where the addition of sulfate groups contributes to a negative charge and imparts biological functions such as anticoagulant or antiviral activities[27]. Other modifications, such as phosphorylation and pyruvylation, add further structural complexity and enhance the functionality of EPS molecules. These chemical alterations are carried out by specific enzymes, such as transferases and other modifying enzymes, which operate either within the cytoplasm or at the periplasmic or extracellular side of the membrane[27].

2.5.5 Transport and Secretion

After polymerization and any required chemical modifications, the exopolysaccharide (EPS) must be transported across the cell envelope to reach the extracellular environment, where it performs its functions. This transport is facilitated by various specialized systems[28]. One such system is the ABC (ATP-binding cassette) transporter, which uses the energy from ATP hydrolysis to actively move EPS precursors or fully formed polymers across the cell membrane[28]. Another mechanism is the Wzx/Wzy-dependent system, which involves specific membrane proteins Wzx, responsible for flipping repeating sugar units across the inner membrane, and Wzy, which facilitates polymerization on the periplasmic side[29]. Additionally, some bacteria utilize secretion pores or trans-envelope complexes, such as the cellulose synthase complex found in *Komagataeibacter xylinus*, which directly extrudes cellulose fibrils into the surrounding environment[29].

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In Gram-negative bacteria, the transport process is more complex, as EPS must traverse both the inner and outer membranes. This requires coordinated machinery, including porins or specialized channels that facilitate polymer export. In contrast, Gram-positive bacteria and fungi, which lack an outer membrane, have a simpler secretion process, though it remains highly regulated to ensure proper EPS release and function[29].

2.5.6 Regulation of EPS Biosynthesis

EPS biosynthesis involves the expanding of lots of energy thus, it is tightly controlled at multiple levels genetic, enzymatic, and environmental. Regulation mechanisms include:

i. Transcriptional regulation: Genes encoding EPS biosynthetic enzymes are often organized in operons or gene clusters that respond to environmental signals. Transcriptional regulators such as sigma factors and response regulators of two-component systems modulate their expression[30].

ii. Quorum sensing: Many bacteria regulate EPS production in response to population density via quorum sensing molecules (e.g., acyl-homoserine lactones in Gram-negative bacteria), which synchronize EPS production and biofilm formation[30].

iii. Environmental stimuli: Nutrient limitation (especially nitrogen or phosphate), osmotic stress, or the presence of certain carbon sources (e.g., sucrose, glucose) can trigger EPS synthesis as a protective or adaptive response[30].

iv. Post-translational control: Enzyme activities involved in EPS synthesis can also be regulated by allosteric modulators or covalent modifications such as phosphorylation[31].

2.5.7 Bioenergetics and Metabolic Cost

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The production of EPS demands considerable energy and carbon resources. For every monosaccharide unit polymerized into an EPS chain, one or more molecules of ATP or UTP are consumed in the activation of the sugar precursors. This metabolic cost is justified by the ecological advantages conferred by EPS, such as enhanced survival under stress, surface adhesion, resistance to antimicrobials, and immune evasion[31].

Microorganisms often optimize EPS production based on metabolic status. For example, in nutrient-limited conditions, excess carbon may be diverted toward EPS production instead of biomass accumulation. This carbon overflow mechanism ensures survival while also promoting the formation of protective biofilms[32].

The biosynthesis of exopolysaccharides in microorganisms is a highly orchestrated process that involves precursor synthesis, polymerization, modification, secretion, and regulation. Each of these stages contributes to the structural and functional diversity of microbial EPS. Understanding the molecular mechanisms of EPS biosynthesis is not only fundamental to microbial physiology but also pivotal for biotechnological exploitation[33]. By manipulating biosynthetic pathways through metabolic engineering, synthetic biology, and fermentation optimization, it is possible to enhance EPS yields and customize their properties for a wide range of industrial, pharmaceutical, agricultural, and environmental applications. The dynamic nature of EPS biosynthesis, tightly linked with microbial adaptation and survival, underscores its evolutionary and functional significance across microbial life[32].

2.6 Functional Properties of Exopolysaccharides (EPS) Relevant to Skin Care

Microbial exopolysaccharides (EPS) have emerged as vital bioactive compounds in the cosmetic and dermatological industries due to their multifunctional properties that support skin health, aesthetics, and protection. Produced naturally by bacteria, fungi, algae, and other

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microorganisms, EPS are high-molecular-weight polysaccharides secreted into the surrounding environment[33]. Their unique chemical structures often comprising complex and branched sugar moieties confer a variety of physicochemical and biological properties that are particularly beneficial to skin care formulations. These include hydration, protection against oxidative stress, anti-aging effects, wound healing promotion, and soothing of irritated skin. The following subsections explore the major functional attributes of EPS that make them suitable for application in skin care products[34].

2.6.1 Moisturization

One of the most valued properties of EPS in skin care is their exceptional ability to retain moisture. Moisturization is essential to maintaining the skin's barrier integrity, elasticity, and suppleness. EPS act as natural humectants, meaning they attract and hold water molecules from the environment or underlying skin layers, thereby enhancing hydration at the surface level. Their high molecular weight and branched polymeric structure create a biofilm on the skin, which helps reduce transepidermal water loss (TEWL). This film-forming ability also improves the sensory feel of topical formulations, leaving a smooth and hydrated finish without greasiness[35].

For instance, EPS produced by marine bacteria such as *Alteromonas macleodii* have demonstrated strong water retention capacities due to their high content of uronic acids and sulfated sugars. These chemical groups can bind water molecules tightly, mimicking the skin's natural moisturizing factors (NMFs)[36]. Furthermore, these polysaccharides often exhibit rheological properties such as viscosity and elasticity that enhance the texture and stability of creams, lotions, and serums. Through these mechanisms, EPS contribute not only

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to immediate moisturization but also to long-lasting skin hydration, making them ideal for dry and sensitive skin care solutions[37].

2.6.2 Anti-aging and Anti-wrinkle Effects

Skin aging is characterized by the degradation of structural components such as collagen and elastin, leading to wrinkles, fine lines, loss of firmness, and sagging. EPS exert anti-aging and anti-wrinkle effects through multiple pathways[36]. First, their moisturizing effect helps plump the skin, which can immediately reduce the appearance of fine lines. More importantly, certain EPS have been shown to stimulate fibroblast activity, promoting the synthesis of collagen and other extracellular matrix components essential for skin elasticity and firmness[36].

In addition to structural support, EPS can inhibit the activity of matrix metalloproteinases (MMPs) enzymes that degrade collagen and elastin fibers in response to environmental stressors like UV exposure and pollution. By suppressing MMP activity, EPS help preserve the skin's architectural integrity and slow the visible signs of aging[37].

Moreover, EPS possess antioxidant properties, which are crucial in neutralizing free radicals that accelerate skin aging. For example, EPS from *Vibrio diabolicus* and certain cyanobacteria have demonstrated in vitro radical-scavenging activity and the ability to reduce oxidative stress markers in skin cells[37]. These actions help protect cellular components from oxidative damage,

support mitochondrial function, and improve overall skin vitality. Through these combined mechanisms, EPS function as potent natural alternatives to synthetic anti-aging agents, offering gentle yet effective rejuvenation benefits[37].

2.6.3 Skin Repair and Wound Healing

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Skin damage due to cuts, abrasions, inflammation, or dermatological procedures requires timely and effective repair to prevent infection and scarring. EPS play a significant role in accelerating wound healing and skin regeneration by modulating various biological activities involved in tissue repair. Some EPS stimulate keratinocyte proliferation and migration, essential steps in re-epithelialization the process by which new skin cells cover a wound[38].

EPS can also promote angiogenesis (formation of new blood vessels), thereby enhancing nutrient and oxygen supply to damaged tissues. Furthermore, they may influence the secretion of growth factors such as transforming growth factor-beta (TGF-β), which regulates cell proliferation, differentiation, and immune responses critical to healing[38].

In microbial biotechnology, EPS like those from *Lactobacillus plantarum* and *Bacillus subtilis* have been studied for their cytoprotective effects on skin cells exposed to oxidative or inflammatory stress[39]. These polysaccharides have shown the ability to reduce inflammatory cytokines, prevent cellular apoptosis, and support matrix remodeling key aspects of effective wound repair. In cosmetic applications, their use in formulations such as post-treatment gels, soothing masks, and healing balms offers both protective and regenerative effects, particularly beneficial for sensitive or damaged skin[39].

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2.6.4 UV Protection and Antioxidant Activity

Chronic exposure to ultraviolet (UV) radiation is one of the leading causes of photoaging, DNA damage, pigmentation disorders, and even skin cancer. EPS contribute to photoprotection through their antioxidant and UV-absorbing capabilities. Many EPS,

particularly those containing sulfate or carboxyl groups, can absorb and scatter UV rays, forming a protective barrier on the skin surface[39].

Beyond physical shielding, EPS combat the oxidative stress induced by UV radiation. UV light stimulates the production of reactive oxygen species (ROS) in skin cells, which can damage DNA, lipids, and proteins. EPS with antioxidant potential scavenge these ROS and enhance the skin's endogenous antioxidant defense systems, including enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx)[40].

For example, EPS from marine algae and thermophilic bacteria have been shown to significantly reduce UVB-induced apoptosis in human skin fibroblasts by maintaining mitochondrial membrane potential and inhibiting oxidative damage. Their integration into sunscreens and daily protective creams can thus offer a natural, biocompatible means of shielding the skin from photodamage and premature aging, complementing or replacing synthetic UV filters that may cause irritation or environmental harm[41].

2.6.5 Anti-inflammatory and Soothing Properties

Inflammation is a common underlying factor in various skin conditions, including acne, eczema, dermatitis, and rosacea. EPS exhibit anti-inflammatory and soothing effects that make them highly valuable in the formulation of products for sensitive and reactive skin. These polysaccharides can modulate immune responses by inhibiting pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β)[42].

Certain EPS also interfere with the signaling pathways involved in inflammation, such as the NF-κB and MAPK pathways, leading to the downregulation of inflammatory gene expression. As a result, they reduce redness, swelling, and discomfort in irritated skin[43]. Additionally,

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their film-forming and moisture-retentive properties contribute to a calming and protective barrier, which soothes and shields the skin from further irritation caused by environmental aggressors, allergens, or cosmetic actives. EPS from lactic acid bacteria, for instance, have been incorporated into dermo-cosmetic products designed for atopic or hypersensitive skin, with proven effects in reducing itchiness and flare-ups[44].

The functional properties of microbial exopolysaccharides make them exceptional bioactives for advanced skin care applications. Through their moisturizing, anti-aging, wound healing, antioxidant, UV-protective, and anti-inflammatory actions, EPS provide comprehensive benefits that align with the demands of modern cosmeceuticals. As natural, biocompatible, and eco-friendly ingredients, they offer a safe alternative to synthetic polymers and actives, supporting sustainable and skin-friendly cosmetic innovations. The ongoing exploration of novel microbial sources and the optimization of EPS production techniques are expected to further expand their relevance and efficacy in the skin care industry[45].

2.7 Specific EPS Used in Cosmetic Products (e.g., hyaluronic acid, dextran, xanthan gum, levan)

In the cosmetic and personal care industry, microbial exopolysaccharides (EPS) have become increasingly valuable due to their biocompatibility, biodegradability, and multifunctional properties that align with both consumer preferences for natural products and scientific expectations for efficacy[46]. Among the myriad EPS produced by microorganisms, a few have gained prominence and widespread acceptance as safe, effective ingredients in skin care and cosmetic formulations. These include hyaluronic acid, dextran, xanthan gum, and levan. Each of these exopolysaccharides possesses unique physicochemical characteristics and biological

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activities that support a wide range of cosmetic applications, from hydration and anti-aging to film formation and product stabilization[47].

2.7.1 Hyaluronic Acid (HA)

Hyaluronic acid, also known as hyaluronan, is a high-molecular-weight polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine. Though traditionally derived from animal tissues, modern production of HA commonly utilizes microbial fermentation, particularly using *Streptococcus zooepidemicus* or *Bacillus subtilis*, offering a vegan-friendly and pathogen-free alternative[48].

HA is most celebrated for its exceptional water-binding capacity, capable of retaining up to 1,000 times its weight in water, making it one of the most effective moisturizers used in skin care today. It forms a viscoelastic film on the skin surface, helping to retain moisture, smoothen fine lines, and improve skin elasticity. Low-molecular-weight forms of HA penetrate deeper into the epidermis and contribute to dermal hydration and skin plumping, while high-molecular-weight HA forms a protective barrier that prevents transepidermal water loss (TEWL)[49].

Additionally, HA has demonstrated anti-inflammatory, wound-healing, and antioxidant properties, making it useful in products designed for sensitive, aging, or damaged skin. It is widely used in serums, moisturizers, sheet masks, injectable dermal fillers, and even hair care formulations for scalp hydration. The versatility and efficacy of hyaluronic acid ensure its continued dominance as a gold-standard EPS in cosmetic applications[49].

2.7.2 Dextran

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Dextran is a complex branched glucan predominantly composed of α-1,6-linked D-glucose units with varying degrees of α-1,3-branching. It is typically synthesized by *Leuconostoc mesenteroides* through the fermentation of sucrose. In the cosmetics industry, dextran is valued for its hydrophilicity, film-forming capacity, and skin-soothing effects[29]. Dextran's moisturizing action arises from its ability to attract and retain water, thereby enhancing skin hydration and reducing dryness. Furthermore, dextran has been shown to reduce inflammation and strengthen the skin barrier, making it suitable for formulations targeting sensitive, reactive, or inflamed skin conditions[30].

In addition to hydration and soothing properties, dextran oligosaccharides exhibit anti-aging potential by protecting skin cells from oxidative damage and improving skin tone and texture. When combined with other actives like peptides or niacinamide, dextran can enhance penetration and bioavailability, amplifying overall efficacy[32]. Dextran also plays a rheological role in cosmetics, helping to stabilize emulsions, enhance product spreadability, and contribute to the pleasant texture of creams and gels. Its use is seen in a wide range of products, including anti-aging creams, under-eye gels, facial serums, and after-sun care[23].

2.7.3 Xanthan Gum

Xanthan gum is a high-molecular-weight polysaccharide composed of glucose, mannose, and glucuronic acid, with a backbone similar to cellulose and side chains of trisaccharides. It is industrially produced by the fermentation of glucose or sucrose by the bacterium *Xanthomonas campestris* [28]. Although widely known as a natural thickener and stabilizer, xanthan gum also possesses film-forming, hydrating, and protective properties that benefit the skin. Its strong viscosity-enhancing capabilities make it invaluable in formulating serums, emulsions, and gels with desirable flow characteristics and consistency[18].

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On the skin, xanthan gum creates a soft, flexible film that helps lock in moisture, enhance the delivery of active ingredients, and shield the skin from external aggressors such as pollutants and allergens. Its film also contributes to the tactile feel of cosmetic products, imparting a smooth and luxurious texture[39]. Beyond functional attributes, xanthan gum is non-toxic, non-irritating, and compatible with a wide pH range and diverse active ingredients. It is often used in products targeting dry skin, sensitive skin, and even in formulations marketed as "clean" or "green" cosmetics due to its natural origin and excellent safety profile. It is also suitable for vegan and cruelty-free formulations[40].

2.7.4 Leva

Levan is a lesser-known but increasingly studied EPS, composed mainly of β-2,6-linked fructofuranosyl units, sometimes with β-2,1 branching. It is produced by certain bacteria such as *Bacillus subtilis, Zymomonas mobilis*, and species of Pseudomonas, typically through the enzymatic conversion of sucrose by levansucrase[41]. Levan is gaining interest in cosmetics due to its remarkable moisturizing, anti-inflammatory, and anti-aging properties. It is capable of forming a light, non-occlusive film on the skin, which hydrates the epidermis while allowing the skin to breathe. Its small molecular size and unique branched structure allow it to penetrate the upper layers of the skin and contribute to skin softening and elasticity enhancement[43].

Levan also exhibits antioxidant and UV-protective properties, helping to mitigate photoaging and oxidative stress in skin cells. Its gentle, soothing action makes it suitable for post-treatment care or products designed to address sunburn, rosacea, or chemical irritation[28]. Importantly, levan has demonstrated bioactive interactions with skin cells, such as enhancing

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fibroblast proliferation and stimulating the synthesis of collagen and elastin. These properties make it a promising candidate in anti-wrinkle, firming, and regenerative skin care products[22].

Commercial interest in levan is growing, especially as manufacturers seek biodegradable, non-sensitizing, and sustainable ingredients for high-performance skin care. Current applications include moisturizing serums, firming creams, facial masks, and bioactive delivery systems. The specific exopolysaccharides hyaluronic acid, dextran, xanthan gum, and levan represent a class of high-performance, bio-based ingredients that have transformed cosmetic science[29]. Each of these EPS offers a unique set of physical, chemical, and biological properties that contribute to skin hydration, protection, rejuvenation, and sensory enhancement. Their widespread use in skin care formulations not only reflects their proven efficacy but also their alignment with clean beauty trends, vegan formulations, and environmental sustainability[15].

As scientific research continues to uncover novel microbial strains and fermentation processes, the range and sophistication of EPS in cosmetics are expected to expand. This evolution underscores a promising future for microbial biopolymers in delivering innovative, safe, and effective skin care solutions tailored to diverse skin needs and consumer preferences[18].

2.8 Challenges and Limitations in Using Microbial EPS in Cosmetics

While microbial exopolysaccharides (EPS) have carved a prominent niche in the cosmetic industry due to their multifunctional benefits such as moisturization, anti-aging effects, film-forming abilities, and biocompatibility their adoption and optimal utilization are not without challenges[33]. Several scientific, technological, economic, and regulatory constraints hinder the large-scale application and broader market penetration of these biopolymers in cosmetics.

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These challenges span the entire value chain, from microbial strain selection and fermentation conditions to product formulation, regulatory acceptance, and consumer perception[36].

2.8 1. Production Challenges and Yield Optimization

One of the foremost limitations in using microbial EPS in cosmetics stems from low production yields and the complexity of biosynthesis[45]. The production of EPS is influenced by multiple factors, including microbial strain type, culture medium composition, fermentation parameters (pH, temperature, oxygen availability), and downstream processing methods. While some bacteria such as *Xanthomonas campestris* (for xanthan gum) or *Streptococcus zooepidemicus* (for hyaluronic acid) are well-characterized for industrial fermentation, others like levan-producing strains may require fine-tuning for optimal yield and consistency[37].

Moreover, EPS production can be an energy-intensive and time-consuming process, requiring extended fermentation times and specific nutrient inputs, which ultimately raises production costs. Batch-to-batch variability also poses a risk, especially in maintaining uniform molecular weight, viscosity, and purity critical parameters for consistent performance in cosmetic formulations[46].

2.8.2. Purification and Cost Constraints

After biosynthesis, purification of EPS to cosmetic-grade quality is a major hurdle. Many microbial EPS are produced alongside impurities such as cell debris, proteins, nucleic acids, and secondary metabolites[7]. Removing these contaminants involves complex downstream processes centrifugation, precipitation, filtration, dialysis, and drying which not only increase operational complexity but also contribute significantly to production costs[59].

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In addition, to compete with synthetic polymers or plant-derived alternatives, microbial EPS must meet strict quality standards while remaining economically viable[60]. However, high purification costs often limit the use of certain EPS to premium skincare products, leaving them inaccessible for broader consumer markets or mass-market brands[62].

2.8.3. Formulation and Stability Issues

Incorporating microbial EPS into cosmetic formulations presents several technical formulation challenges[40]. EPS such as xanthan gum, while excellent thickeners, can exhibit incompatibilities with other actives, particularly certain salts, surfactants, and preservatives, leading to changes in viscosity, precipitation, or phase separation. Moreover, the stability of EPS in emulsions, gels, or serums is heavily influenced by pH, ionic strength, and storage conditions[48].

Some EPS may also degrade or lose functional integrity over time, especially when exposed to high temperatures, UV light, or microbial contamination during storage. This necessitates the use of preservatives or co-stabilizers, which may not align with consumer expectations for "clean," "natural," or preservative-free formulations. Achieving long-term stability while preserving bioactivity remains a critical hurdle for formulators[7, 22].

2.8.4. Sensory and Aesthetic Limitations

Consumer acceptance of cosmetics is closely linked to aesthetic properties such as texture, spreadability, absorption, and skin feel. While some EPS like hyaluronic acid deliver excellent sensorial performance, others (e.g., xanthan gum) may impart undesirable stickiness, tackiness, or residue on the skin[1]. These sensory drawbacks can diminish user experience and negatively impact product perception, particularly in high-end skincare products where a luxurious skin feel is paramount. Moreover, color and odor issues may arise from impure

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EPS extracts, especially when derived from fermentation broths that contain pigmented or aromatic by-products. Achieving colorless, odorless EPS without extensive refining which adds to manufacturing costs and processing time[22].

2.8.5. Regulatory and Safety Hurdles

Another significant challenge lies in the regulatory approval and safety validation of microbial EPS for cosmetic use[1]. While ingredients like hyaluronic acid and xanthan gum have long-established safety profiles, new or less-known EPS (e.g., from novel microbial strains or genetically modified organisms) may require extensive toxicological testing, clinical evaluation, and regulatory submissions to be accepted for use[7] . In addition, the use of genetically engineered microorganisms (GEMs) to improve EPS yield or functionality is often viewed with skepticism by both regulators and consumers, raising concerns about GMO-derived ingredients, even when the final product contains no detectable genetic material [43].

2.8.6. Environmental and Sustainability Concerns

While microbial EPS are biodegradable and often positioned as eco-friendly alternatives to petroleum-based polymers, their production still consumes large volumes of water, energy, and feedstock sugars, raising concerns about environmental sustainability[60]. The use of food-grade raw materials (e.g., glucose, sucrose) for EPS production also competes with food supply chains, which may be ethically and economically contentious[61].

Additionally, the waste generated during fermentation and downstream purification—such as spent media and chemical reagents requires careful management to minimize environmental

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impact. Manufacturers must balance the environmental benefits of biodegradable EPS with the carbon footprint and resource demands of their production processes[62].

2.8.7. Limited Functional Diversity and Targeted Delivery

Despite their general benefits, many microbial EPS exhibit broad but nonspecific bioactivities, such as moisturization or film formation. There is a growing demand for EPS that can deliver targeted skin benefits, such as stimulating collagen synthesis, modulating skin microbiota, or enhancing penetration of actives[55]. However, only a limited number of EPS have been characterized for such bio-specific roles. Efforts to develop multifunctional or tailored EPS derivatives such as sulfated, acetylated, or conjugated forms require advanced biotechnological tools and often face additional safety and regulatory scrutiny[58]. Furthermore, controlled-release systems using EPS-based matrices remain in early stages of development, with scalability and stability being key concerns[51].

while microbial exopolysaccharides offer immense potential as natural, effective, and multifunctional ingredients in cosmetic products, their broader adoption is challenged by numerous scientific, technical, and commercial limitations[8]. These include low production yields, costly purification processes, formulation complexities, regulatory burdens, and environmental considerations. Overcoming these challenges requires interdisciplinary collaboration among microbiologists, biotechnologists, cosmetic chemists, regulatory experts, and product developers[6].

Advances in synthetic biology, fermentation technology, and formulation science may pave the way for the next generation of high-performance, sustainable, and consumer-friendly EPS-based cosmetic ingredients[7]. Yet, careful consideration of cost, efficacy, safety, and user

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experience remains essential to ensuring the successful integration of microbial EPS into mainstream skin care and cosmetic products[2].

2.9 Regulatory and Safety Considerations

The incorporation of microbial exopolysaccharides (EPS) into cosmetic products offers promising functional and commercial benefits, particularly due to their natural origin, biocompatibility, and diverse bioactivities[13]. However, for any bio-derived ingredient to be successfully adopted within the personal care and cosmetic industry, it must meet strict regulatory and safety standards. These regulations are designed to ensure consumer safety, product efficacy, environmental protection, and industry transparency[18]. Regulatory and safety considerations surrounding microbial EPS span several domains, including ingredient registration, safety assessments, labeling, permissible usage levels, manufacturing practices, and environmental impact evaluations[12].

2.9.1. Ingredient Registration and Regulatory Approval

Before a microbial EPS can be legally incorporated into a cosmetic product, it must be recognized as a safe cosmetic ingredient by the relevant regulatory authority in the target market. Regulatory frameworks differ across regions, but most countries maintain positive lists or ingredient inventories of approved substances[13]. Novel EPS especially those from genetically modified organisms (GMOs) or uncommon microbial sources may need to undergo pre-market notification or approval procedures. This involves submitting detailed safety data, toxicology reports, and information on production processes and potential impurities[44].

2.9.2. Safety Assessments

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In other to ensure the safety of microbial exopolysaccharides (EPS) for human use, manufacturers must conduct thorough toxicological evaluations. These assessments are designed to identify any potential health risks associated with exposure to the EPS[45]. One important aspect of this evaluation is dermal toxicity testing, which determines whether the substance may cause skin irritation, corrosion, or allergic reactions[61]. Eye irritation tests are also crucial, especially when the EPS is intended for use in products such as facial cleansers, masks, or eye creams[62].

Depending on the intended application and expected exposure levels, both acute and chronic toxicity tests are performed to evaluate the effects of short-term and long-term exposure. In addition, mutagenicity and genotoxicity studies are conducted to determine whether the EPS has the potential to cause genetic mutations or damage to DNA[22]. For products with potential for systemic absorption, reproductive and developmental toxicity assessments are carried out to evaluate any risks to fertility or embryonic development. These comprehensive evaluations are essential to confirm the safety of microbial EPS in consumer and medical products[23].

Many of these tests are now conducted using alternative methods that comply with animal testing bans in the EU and other regions. These include in vitro assays (e.g., reconstructed human epidermis models) and computational toxicology approaches using quantitative structure–activity relationships (QSAR)[31]. For EPS with established use, such as hyaluronic acid, dextran, or xanthan gum, existing toxicological monographs and historical safety data often suffice. However, for novel EPS, especially those chemically modified (e.g., sulfated, cross-linked, or acetylated), fresh data are usually required[33].

2.9.3. Microbiological and Chemical Purity

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Another critical aspect of ensuring the safety of microbial exopolysaccharides (EPS) involves addressing microbiological contamination and chemical purity. Because EPS are produced through microbial fermentation, it is essential that the final product undergo rigorous purification to eliminate any potential contaminants[22]. This includes the removal of pathogenic microorganisms or spores that may pose a health risk if present.

Particular attention must be given to endotoxins, especially those originating from Gram-negative bacteria, as these can trigger harmful immune responses in humans. Additionally, residual components from the fermentation process, such as unused media or trace amounts of antibiotics, must be thoroughly eliminated[18]. Lastly, any heavy metals or solvent residues introduced during downstream processing must be carefully removed to ensure the final EPS product is chemically pure and safe for its intended application[23].

Cosmetic-grade EPS must meet strict microbial limits, often defined by pharmacopeial standards or cosmetic GMP guidelines. For example, the United States Pharmacopeia (USP) and the European Pharmacopoeia (Ph. Eur.) set upper limits for total aerobic microbial count (TAMC), total yeast and mold count (TYMC), and the absence of specific pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Moreover, heavy metal contamination (e.g., lead, arsenic, mercury) is tightly regulated in most jurisdictions[28, 31]. Manufacturers must conduct batch-wise quality control and provide certificates of analysis (COA) to assure regulators and formulators of ingredient safety and consistency[22].

2.9.4. Allergenicity and Sensitization Potential

Despite being natural biopolymers, microbial EPS can sometimes elicit allergic reactions or skin sensitization, particularly in individuals with compromised skin barriers or sensitivities to microbial byproducts. While most EPS like hyaluronic acid are non-immunogenic and

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well-tolerated, polysaccharides with high molecular weight or those containing residual protein contaminants may pose a risk[22].

Regulators often require Human Repeat Insult Patch Testing (HRIPT) or in vitro sensitization tests to assess allergenic potential. In some cases, ingredients must carry labeling declarations indicating their source or processing method especially if derived using animal-based enzymes or GMOs to alert sensitive consumers[30].

2.9.5. Environmental and Ecotoxicological Considerations

There is increasing global emphasis on sustainability and green chemistry, the environmental impact of cosmetic ingredients including microbial EPS is a growing concern. Regulatory agencies and certification bodies assess not only the biodegradability of the EPS but also the ecotoxicity of any residual waste or processing by-products[44]. Some EPS fermentation processes generate wastewater, CO_2 emissions, and solid waste. Manufacturers are therefore encouraged to adopt eco-friendly production methods, use renewable substrates, and implement circular bioeconomy principles[50].

2.9.6. Regulatory Compliance and Documentation

Bringing microbial exopolysaccharides (EPS) to market requires manufacturers to compile comprehensive technical dossiers that provide detailed documentation on various aspects of the product[6]. This includes a clear description of the ingredient's identity and its microbial source, as well as a thorough outline of the manufacturing and purification processes used to obtain the final product. The dossier must also detail the physicochemical properties of the EPS, such as molecular weight, pH, and solubility[38].

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In addition, stability and shelf-life data are essential to demonstrate the product's performance over time. Manufacturers must also include toxicological and safety assessments to ensure the EPS is safe for its intended use, supported by microbiological and chemical test results to verify purity and quality[25]. Where relevant, documentation should also include a certificate of origin and information about the product's GMO status, particularly for regulatory compliance and market transparency[31].

These dossiers support compliance with cosmetic notification systems like the EU Cosmetic Product Notification Portal (CPNP) and similar systems in other jurisdictions. Additionally, proper INCI (International Nomenclature of Cosmetic Ingredients) naming is essential for labeling transparency and regulatory acceptance[28]. Failure to comply with regulatory or safety standards can result in product recalls, market bans, or reputational damage, underscoring the importance of thorough documentation and ongoing compliance monitoring[25].

Regulatory and safety considerations form a foundational pillar for the integration of microbial EPS into cosmetic products. Ensuring compliance requires a comprehensive understanding of international and regional guidelines, rigorous safety testing, transparent ingredient documentation, and adherence to good manufacturing practices[24]. While EPS derived from well-studied sources like *Streptococcus*, *Xanthomonas*, or *Leuconostoc* may face fewer regulatory barriers, the rise of novel microbial EPS and biotechnological modifications will necessitate ongoing vigilance, safety validation, and regulatory innovation[43].

With the growing consumer demand for sustainable and biocompatible ingredients, microbial EPS are well-positioned to thrive but only if their safety and quality can be assured through

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robust scientific and regulatory frameworks[33]. As the industry evolves, collaborative efforts between researchers, formulators, regulatory authorities, and ingredient manufacturers will be key to unlocking the full potential of EPS in cosmetics[31].

2.10 Exoploysachharides in Marine and Terrestrial Environments

Exopolysaccharides (EPS) are biopolymers serve a wide range of ecological and functional roles, including the protection of microbial cells against environmental stress, facilitation of biofilm formation, nutrient acquisition, and intercellular communication[63]. EPS can be derived from both marine and terrestrial microorganisms, with their properties and applications influenced greatly by their origin. The exploration of EPS from these two environments has expanded in recent years, particularly due to their growing use in biomedical, pharmaceutical, food, and cosmetic industries[46].

Marine microorganisms live in unique and often extreme habitats such as deep-sea hydrothermal vents, polar ice, salt marshes, and open oceans. As a result, marine-derived EPS tend to have distinctive structural and functional features that reflect adaptations to such challenging conditions. For example, marine microbial EPS often contain unusual or rare sugar residues such as fucose, rhamnose, and uronic acids, and are frequently sulfated or acetylated [42]. These modifications are not only essential for their protective functions in the marine environment but also contribute to their unique bioactivities such as antioxidant, anti-inflammatory, antimicrobial, and anti-aging effects. Common marine EPS-producing bacterial genera include *Pseudoalteromonas*, *Alteromonas*, *Vibrio*, *Halomonas*, and *Colwellia*. These organisms are known to produce bioactive EPS with high anionic charge densities and excellent water retention and gelling properties[45].

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In contrast, terrestrial microbial EPS are primarily derived from soil bacteria, plant root-associated microbes, and freshwater microorganisms. These EPS are generally composed of more common monosaccharides such as glucose, mannose, galactose, and N-acetylglucosamine, and they are typically neutral or only mildly acidic[31]. While they may lack some of the exotic structural features of marine EPS, terrestrial EPS still play crucial ecological roles. For example, they help in soil aggregation, moisture retention, and root colonization, and are important in forming protective biofilms in terrestrial habitats. Prominent EPS-producing terrestrial genera include *Bacillus*, *Rhizobium*, *Lactobacillus*, *Streptomyces*, and *Pseudomonas*[32].

From a structural perspective, marine EPS exhibit greater chemical complexity compared to their terrestrial counterparts. This complexity is attributed to their diverse monosaccharide compositions and frequent post-polymerization modifications like sulfation, methylation, and acetylation. Such features make marine EPS especially attractive for high-value applications in cosmeceuticals and pharmaceuticals[38]. For example, sulfated EPS from *Pseudoalteromonas* species have demonstrated the ability to boost collagen synthesis and reduce skin wrinkles, making them suitable for anti-aging formulations. Another marine EPS from *Vibrio diabolicus* has shown promising results in promoting skin regeneration and hydration. These bioactivities are supported by the polymers' ability to form stable films, retain moisture, and act as antioxidants, thereby protecting skin cells from oxidative stress and environmental damage[39].

Terrestrial EPS, on the other hand, have already found widespread industrial use due to their ease of production and established safety profiles. Xanthan gum, gellan gum, and dextran are well-known terrestrial EPS used extensively as thickeners, stabilizers, and emulsifiers in food,

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pharmaceuticals, and cosmetics[40]. Although they may not possess the broad bioactivity of marine EPS, they are economically viable and widely accepted in regulatory frameworks.

The biosynthesis of EPS in both marine and terrestrial microbes generally follows a similar sequence of steps: the intracellular synthesis of sugar nucleotide precursors, assembly of repeating units, polymerization, post-synthetic modification, and secretion through membrane-associated transport systems such as ATP-binding cassette (ABC) transporters or Wzx/Wzy-dependent pathways [41]. However, the regulation of EPS biosynthesis in marine microorganisms is often more complex due to the variable and extreme conditions of their habitats. Additionally, many marine bacteria possess unique genetic arrangements and regulatory mechanisms that allow them to modify their EPS structures in response to environmental stressors[39].

One of the most promising aspects of marine EPS lies in their potential applications in high-end cosmetics and therapeutics. Marine EPS are increasingly used in moisturizers, serums, facial masks, and anti-aging products due to their bioadhesive properties and ability to retain water and enhance skin barrier function[22]. Their antioxidant and anti-inflammatory effects further enhance their value in skincare, especially for products targeting sensitive or aging skin. Meanwhile, terrestrial EPS continue to play vital roles in the development of oral drug delivery systems, vaccines, and blood plasma substitutes. Their established use in food products also makes them suitable for functional foods and prebiotics[23].

Despite their many advantages, marine EPS face significant challenges that limit their commercial exploitation. These include the low yield of EPS in natural conditions, difficulties in cultivation of marine strains, high costs of downstream processing, and the complexity of structural characterization[24]. Advances in biotechnology, such as synthetic

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biology, metabolic engineering, and omics technologies, are being employed to overcome these limitations. For instance, genetically engineered microbes can be developed to produce marine-like EPS at higher yields under controlled fermentation conditions. Moreover, metagenomic approaches are revealing new marine microbial strains with potential for novel EPS production[26].

Both marine and terrestrial microbial exopolysaccharides offer immense potential for diverse industrial and biomedical applications. While terrestrial EPS dominate current markets due to their ease of production and regulatory acceptance, marine EPS are rapidly gaining traction owing to their superior bioactivity and unique structural attributes[37]. Research and development in microbial biotechnology, coupled with sustainable bioprocessing techniques, are expected to expand the utilization of both marine and terrestrial EPS in the future. As industries move toward natural and eco-friendly ingredients, the demand for bioactive polysaccharides from microbial sources is poised to grow significantly.

2.11 Exopolysacharides from Fungi

Exopolysaccharides (EPSs) produced by yeasts have garnered significant attention in recent years due to their unique structural properties and diverse applications across various industries, including cosmetics, pharmaceuticals, and food technology[39]. Unlike bacterial EPSs, yeast-derived EPSs often exhibit distinct physicochemical characteristics, which can be attributed to the unique9metabolic pathways and environmental adaptations of yeast species[39].

One notable area of research focuses on Antarctic yeasts, which thrive in extreme cold environments. These psychrophilic yeasts have evolved to produce EPSs that confer protection against harsh conditions, such as low temperatures and high salinity. Studies have shown that EPSs from Antarctic yeasts like *Cryptococcus flavus* possess high viscosity and

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stability, making them suitable for incorporation into cosmetic formulations[46]. For instance, creams containing these EPSs have demonstrated improved moisturizing properties and enhanced skin barrier functions, which are essential for skincare products[26].

Beyond their physical properties, yeast EPSs also exhibit various physiological functions. Research has indicated that these biopolymers can modulate immune responses, exhibit antioxidant activities, and even possess antitumor properties. These bioactivities are attributed to the complex sugar compositions and branching patterns of yeast EPSs, which interact with cellular receptors and influence biological pathways. These findings suggest potential applications of yeast EPSs in developing functional foods and therapeutic agents[27].

In the realm of cosmetics, the incorporation of yeast-derived EPSs offers several advantages. Their natural origin aligns with the growing consumer demand for bio-based and sustainable ingredients. Moreover, their ability to form protective films on the skin, retain moisture, and deliver bioactive compounds enhances the efficacy of cosmetic products. The use of yeast EPSs in formulations can lead to products that not only improve skin aesthetics but also promote skin health[30].

Furthermore, the production of yeast EPSs is considered environmentally friendly. Yeasts can be cultivated using renewable resources, and the downstream processing of EPSs often requires fewer chemicals compared to synthetic polymers[14]. This sustainable production process, combined with the functional benefits of yeast EPSs, positions them as valuable ingredients in the development of eco-conscious products[12].

In conclusion, yeast-derived exopolysaccharides represent a promising class of biopolymers with multifaceted applications[28]. Their unique structural features, coupled with beneficial physiological activities, make them suitable for use in cosmetics, pharmaceuticals, and food

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industries. Ongoing research and development efforts continue to uncover new potentials of these biopolymers, paving the way for innovative and sustainable product formulations[22].

2.12 Future Prospects for Exopolysaccharides

Microbial exopolysaccharides (EPS) are high-molecular-weight polymers secreted by microorganisms into their surrounding environment[32]. These biopolymers have garnered significant attention due to their diverse structural properties and functional capabilities, which make them suitable for various applications in medicine, cosmetics, and biotechnology[36].

In the medical field, EPS have been explored for their potential in drug delivery systems. Their ability to form hydrogels and encapsulate therapeutic agents allows for controlled and sustained release of drugs, enhancing efficacy and reducing side effects[28]. Furthermore, EPS can be functionalized to target specific tissues or cells, improving the precision of drug delivery.

Beyond drug delivery, EPS have shown promise as vaccine adjuvants. Their immunomodulatory properties can enhance the body's immune response to antigens, making vaccines more effective. Additionally, certain EPS possess inherent antiviral and antibacterial activities, which could be harnessed in developing new antimicrobial therapies[71]. Diagnostic imaging is another area where EPS are being investigated. By conjugating EPS with imaging agents, researchers aim to develop novel contrast materials that are biocompatible and provide enhanced imaging capabilities for techniques like MRI and CT scans[46].

In the cosmetics industry, EPS derived from marine microorganisms have been identified as valuable ingredients due to their moisturizing, anti-aging, and protective properties. These EPS can form films on the skin, retaining moisture and providing a barrier against

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environmental stressors. Their antioxidant activities help in combating oxidative stress, a major contributor to skin aging[35].

Cyanobacteria, a group of photosynthetic microorganisms, produce EPS with unique bioactive compounds. These compounds have been found to offer photoprotective effects, shielding the skin from harmful UV radiation. Incorporating such EPS into skincare products can enhance their protective capabilities and appeal to consumers seeking natural and effective solutions[72]. Beyond medical and cosmetic uses, EPS have potential in various biotechnological applications. Their rheological properties make them suitable as thickeners, stabilizers, and emulsifiers in the food industry. In environmental biotechnology, EPS can aid in bioremediation processes by binding heavy metals and pollutants, facilitating their removal from contaminated sites[73].

The development of EPS-based composites is an emerging area of interest. By combining EPS with inorganic materials, researchers aim to create novel biomaterials with enhanced mechanical properties for applications in tissue engineering and regenerative medicine. These composites can serve as scaffolds that support cell growth and tissue formation, offering solutions for repairing or replacing damaged tissues[35].

While the potential applications of microbial EPS are vast, several challenges need to be addressed to fully realize their benefits. These include optimizing production processes to increase yield and reduce costs, ensuring consistency and purity of EPS products, and conducting comprehensive safety assessments for their use in various applications[32]. Advancements in genetic engineering and fermentation technologies hold promise for overcoming these challenges. By manipulating microbial strains and optimizing growth conditions, it is possible to enhance EPS production and tailor their properties for specific

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applications. Continued interdisciplinary research and collaboration between microbiologists, chemists, and engineers will be crucial in advancing the development and application of microbial EPS[30].

Microbial exopolysaccharides represent a versatile and promising class of biopolymers with applications spanning medicine, cosmetics, and industry[23]. Ongoing research and technological advancements are expected to unlock new potentials for these natural compounds, contributing to innovations that benefit health, beauty, and the environment.

2.13 Microorganisms Used in Cosmetic Industry

Microorganisms have become central to the advancement of the cosmetics industry, offering diverse bioactive compounds that enhance both the functionality and safety of products. With increasing demand for sustainable and naturally derived cosmetic ingredients, microbial biotechnology now plays a pivotal role in skin care, anti-aging, pigmentation, UV protection, and product preservation. Current research, demonstrates the scientific and commercial potential of microbial applications in cosmetics[67, 68, 69,.]

Accordingly systems metabolic engineering has revolutionized the use of microorganisms for producing high-value cosmetic compounds. This advanced biotechnology combines synthetic biology, systems biology, and evolutionary engineering to modify microbial strains such as *Escherichia coli*, *Saccharomyces cerevisiae*, and *Corynebacterium glutamicum*[64.] These engineered microbes are now capable of producing compounds like hyaluronic acid, coenzyme Q10, and essential amino acids. For example, hyaluronic acid, widely valued for its moisturizing and anti-aging effects, is produced by engineered strains of *Bacillus subtilis* and *Streptococcus zooepidemicus*. Similarly, coenzyme Q10, a potent antioxidant used to

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prevent skin aging, is synthesized using *Rhodobacter sphaeroides* and *Agrobacterium tumefaciens*[64].

Microbial pigments are another essential group of bioactive agents in cosmetics. As synthetic dyes fall out of favor due to toxicity concerns, biopigments from microorganisms have become desirable alternatives. Studies highlights the significance of microbial pigments such as carotenoids, melanins, and violacein. Carotenoids produced by *Blakeslea trispora* and *Rhodotorula glutinis* not only add color but also offer antioxidant and UV-protective benefits[69]. Melanin from microbes like *Bacillus thuringiensis* and *Streptomyces* provide natural sun protection and are useful in anti-aging products. Violacein, derived from *Chromobacterium violaceum*, possesses antimicrobial and anti-inflammatory properties, making it suitable for sensitive skin formulations[69].

Marine microorganisms also offer unique compounds for cosmetic use, especially due to their adaptation to extreme environments. Researchers point out that marine microbes such as *Pseudoalteromonas* species produce exopolysaccharides that hydrate and repair the skin. Additionally, marine enzymes like collagenases and lipases help in skin exfoliation and rejuvenation, while mycosporine-like amino acids (MAAs) from cyanobacteria serve as natural sunscreens due to their UV-absorbing properties[17].

A new frontier in cosmetic science involves the interaction between cosmetics and the skin microbiota. Studies have emphasize the importance of fermented cosmetic ingredients derived from microbes like *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*. These microbes produce metabolites such as peptides, lactic acid, and ceramides during fermentation, which improve skin hydration, elasticity, and barrier function[69]. Moreover, they help modulate the skin's microbiome, supporting the growth of beneficial bacteria while

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suppressing pathogens. For instance, *Lactobacillus plantarum* has been found to reduce inflammation and enhance overall skin health when used in cosmetic formulations[69].

Microbial-derived antimicrobials also contribute to the safety and longevity of cosmetic products. A study notes that compounds such as bacteriocins from lactic acid bacteria and lipopeptides from *Bacillus subtilis* can act as natural preservatives. These bioactives protect against microbial contamination and help maintain skin health by preventing the growth of harmful organisms like *Staphylococcus aureus* and *Candida albicans*[70].

Despite these benefits, the incorporation of microorganisms in cosmetics also presents safety concerns. Microbial contamination of cosmetics, particularly with opportunistic pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, can occur due to poor manufacturing practices or inadequate preservation[65]. Furthermore, microbial interaction with ingredients like hydroquinone and heavy metals can exacerbate toxicity. Hence, thorough microbial risk assessments and quality control are necessary to ensure consumer safety[65].

Microorganisms offer a rich and versatile source of functional ingredients for the cosmetic industry. From engineered compounds and natural pigments to fermented extracts and marine-derived enzymes, microbial applications are shaping the future of cosmetics[65]. These innovations respond to increasing consumer demand for natural, eco-friendly, and microbiome-conscious products. However, maintaining rigorous safety protocols remains essential to maximizing benefits and minimizing risks associated with microbial use in cosmetics[65]

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2.14 Skin Microbiota and Cosmetics which can Damage the Skin

The skin microbiota is a complex ecosystem composed of bacteria, fungi, viruses, and other microorganisms plays a vital role in maintaining skin health, supporting immune defense, and

preserving the skin's barrier function. In recent years, scientific attention has turned toward understanding how cosmetics can both support and disrupt this delicate microbial community[32]. While some products enhance skin health through probiotics, biopigments, or fermented ingredients, others pose risks due to poor formulation, contamination, or the presence of harsh chemicals. This duality reflects an urgent need for greater awareness and regulation within the cosmetics industry[32].

There exists a symbiotic relationship between skin microbiota and human skin physiology. Their study reveals that certain metabolites produced by commensal skin microbes, such as lactic acid and short-chain fatty acids, contribute to maintaining an acidic pH and inhibiting pathogen colonization[69]. When cosmetics disrupt the natural microbiome either through preservatives, emulsifiers, or antimicrobial agents this can lead to dysbiosis[69]. Dysbiosis is characterized by a loss of microbial diversity or overgrowth of pathogenic species, which has been linked to conditions like acne, atopic dermatitis, and increased susceptibility to infections[69].

Conversely, the incorporation of probiotics and fermented ingredients in cosmetics has shown promise in preserving and even enhancing the skin's microbial ecosystem. Probiotics such as *Lactobacillus* and *Bifidobacterium* species can compete with pathogens, strengthen the skin barrier, and modulate local immune responses[69]. Fermented cosmetic products containing microbial metabolites can reduce inflammation and oxidative stress, contributing to overall skin wellness[69].

However, not all cosmetic products are beneficial. Studies have been conducted to investigate the safety and quality of widely used topical cosmetics and found that many products contained potentially harmful substances[70]. Studies have identified heavy metals, unlisted

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preservatives, and microbial contaminants in several formulations. These toxic elements not only pose a direct chemical hazard to the skin but can also disturb the skin microbiota, fostering an environment conducive to infection or chronic inflammation. Their findings underscore the need for stringent quality control and transparency in labeling to protect consumer health[70].

In addition, the growing trend of including probiotics in cosmetic and personal care products. While this approach is theoretically beneficial for the skin microbiome, the authors highlight challenges such as the viability of live bacteria in commercial formulations and the lack of regulatory frameworks[73]. Improper storage conditions or inadequate formulation can render probiotics inactive, or worse, promote contamination. The commercialization of "probiotic cosmetics" thus presents both opportunities and risks, necessitating robust clinical validation and regulatory oversight[73]

Sun exposure and UV radiation are additional external stressors that damage the skin and its microbiota. Research has shown that the skin microbiota, when supported by topical probiotics, can offer protective effects against UV-induced oxidative stress[72]. Their findings suggest that microbial metabolites can modulate antioxidant defenses and inflammatory responses, mitigating the harmful effects of UV radiation. However, many sunscreens and anti-aging products contain synthetic UV filters or reactive compounds that may impair microbial viability on the skin, inadvertently compromising the skin's natural defenses[72]

Biopigments of microbial origin offer a promising alternative to synthetic dyes, which are often toxic to both the skin and its microbiome. A study describes the benefits of pigments such as carotenoids, violacein, and melanin, which are not only biodegradable and non-toxic but also possess antioxidant and antimicrobial properties[72]. Yet, the indiscriminate use of

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synthetic colorants and preservatives in conventional cosmetics remains widespread, posing cumulative risks to skin health. These synthetic compounds can act as irritants or allergens, altering the skin's pH and suppressing beneficial microbial communities[72].

Studies further elaborate on the production of microbial pigments, highlighting their role as bioactive compounds with UV-protective and anti-inflammatory potential[71]. They note that when these natural pigments are incorporated into cosmetic formulations, they offer both aesthetic and functional benefits without the environmental and biological toxicity associated with petrochemical derivatives. Despite their advantages, microbial pigments are still underutilized in mainstream cosmetics, largely due to production cost and regulatory hurdles[71]

The relationship between skin microbiota and cosmetics is a double-edged sword. While innovative formulations involving probiotics, fermented ingredients, and microbial pigments offer significant promise in supporting skin health, the continued use of harsh chemicals, synthetic preservatives, and contaminated products can have detrimental effects. Damage to the skin microbiota may manifest in both acute and chronic dermatological issues, underscoring the importance of microbiome-conscious cosmetic design. Future directions in cosmetic science must prioritize biocompatibility, microbial safety, and rigorous testing to ensure that products not only beautify but also protect and nourish the skin[73].

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Chapter Three

Materials and Methods

3.1 Collection of Sample Material

In other to carry out this research, five different samples were obtained from five different markets in Ibadan, Oyo state Nigeria. Palm wine was bought from Toll Gate Area of Ibadan, Soursop was collected from Oje market in Ibadan. Grape was bought from Oje, Challenge and Molete market. Maize gruel (Ogi) was collected from challenge, Orita Challenge and Felele areas of Ibadan. Locust beans (Iru), was bought from Challenge, Orita Challenge and Felele. All samples were asceptically collected into sterile containers and promptly transported into the laboratory.

3.2 Materials, Equipment, Reagents and Media

3.2.1 Materials

Cotton wool, petri dishes (disposable), aluminium foil, conical flasks (100ml, 250ml, 500ml, 1000ml), McCartney bottles, syringe (10ml, 5ml) and needle, beakers, measuring cylinder, weighing balance, inoculating loop, spirit lamp, paper tape, marker, filter paper (Whatman no 1), microscopic slide, pipette, pipette tips, bijou bottles.

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3.2.2 Equipments

Incubator (Imperial iii), Autoclave (Model-ym50), Electronic weighing balance (Mettler Toledo, FA2104A), Hot air oven (NYC-101) and Water bath (Grant, SBB Aqua 12 plus), CO_2 Incubator (Multition INFORS), Centrifuge (90-1, U-Clear).

3.2.3 Media

Nutrient Agar (NA), YEPD (Yeast Extract Peptone Dextrose), YEGA (Yeast Extract Glucose Agar), EPS (Exopolysaccharide producing Agar), De Man Rogosa and Sharpe Agar (MRS)

3.2.4 Reagents

Ethanol, Sterile distilled water, Deionized water, Methylated spirit, Isopropanol, Iodine, Methylene blue, Safranin, Crystal violet.

3.3 Methods

3.3.1 Sterilization of Glassware

All glassware was thoroughly washed with liquid soap, rinsed with tap water followed by distilled water, air dried and then sterilized in a hot oven at 300 ^{o}C for 3 hours. Conical flasks and culture media were sterilized by autoclaving at 121 ^{o}C for 15 minutes. The inoculating wire loop was sterilized by flaming until red-hot in a spirit lamp at regular interval.

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3.3.2 Culture Media

Nutrient Agar (NA), YEPD (Yeast Extract Peptone Dextrose), YEGA (Yeast Extract Glucose Agar), EPS (Exopolysaccharide producing Agar), De Man Rogosa and Sharpe Agar (MRS) were used for isolation of bacteria and yeast used for the study.

3.3.2.1 Preparation of Media

3.3.2.1.1 Nutrient Agar (NA)

Nutrient agar was prepared according to Titan Biotech Limited instruction's. Specifically, 28g of NA powder was dissolved in 1000ml of distilled water and mixed thoroughly until completely uniform dissolved. The mixture was transferred into a conical flask, plugged with cotton wool, and covered with aluminium foil. It was then sterilized in an autoclave at 121°C for 15 minutes, cooled to 40°C and poured into sterile petri dishes.

3.3.2.1.2 MRS Agar

MRS Agar was prepared according to Chaitanya Agro Biotech PVT Limited instruction's. A total of 62g of MRS agar powder was dissolved in 1000ml of distilled water. It was thoroughly mixed until the solution became homogenous. The mixture was transferred into a conical flask, sealed with cotton wool, and covered with aluminium foil. Sterilization was carried out in an autoclave at 121°C for 15 minutes at 15 psi. After cooling to about 40°C, the medium was poured into sterile petri dishes.

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3.3.2.1.3 YEGA (Yeast Extract Glucose Agar)

YEGA agar was prepared by dissolving 0.5% (w/v) yeast extract, 1% glucose (w/v), and agar-agar 15% (w/v) in 100ml distilled water[3]. The suspension was mixed until completely homogenous. The conical flask containing the medium was plugged with cotton wool and

covered with aluminium foil. The medium was homogenized in the water bath and sterilized in an autoclave at 121 °C for 15 minutes at 15psi, cooled to 40 °C after which amoxicillin 5mg/mL (w/v) was added and then poured into sterile petri dishes.

3.3.2.1.4 YEPD Agar (Yeast Extract Peptone Dextrose)

YEPD agar was prepared by dissolving 0.08g yeast extract, 10g glucose, 0.12g peptone, 0.1g $MgSO_4.7H_2O$, 1g KH_2PO_4, 0.2g NaCl and 15g agar-agar in 1000mL distilled water[3]. The suspension was mixed until completely homogenous. The conical flask containing the medium was plugged with cotton wool and covered with aluminium foil. The flask was sterilized in an autoclave at 121 °C for 15 minutes, cooled to 30 °C and poured into sterile petri dishes.

3.3.2.1.5 EPS Agar (Exopolysaccharide Producing Agar)

EPS agar was prepared by dissolving 0.4g/L yeast extract, 50g/L glucose, 0.6g/L peptone, 0.5g/L $MgSO_4.7H_2O$, 5g/L KH_2PO_4, 1g/L NaCl and agar-agar in 200ml distilled water[4]. The suspension was mixed until completely homogenous. The conical flask containing the medium was plugged with cotton wool and covered with aluminium foil. The flask was sterilized in an autoclave at 121 °C for 15 minutes at 15 psi, cooled to 40 °C and poured into sterile petri dishes.

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3.4 Isolation and Identification Techniques

3.4.1 Isolation from Palmwine, Maize Gruel and Locust Bean

One mL of palmwine, and 1gm of maize gruel, locust beans from each location were inoculated into 9ml of freshly prepared Nutrient Broth (NB). They were incubated for 24 hours at 35ºC. 100µL of the medium was then plated using the spread plate method on De man, Rogosa and Sharpe (MRS), YEPD, YEGA. The MRS agar was supplemented with 40µg/mL of fluconazole to prevent fungal growth, while YEPD and YEGA were supplemented with 50µg/mL of amoxicillin to prevent bacteria growth. Sub cultures were carried out on colonies that appeared shiny or mucoid in appearance. Once pure cultures were obtained, the cultures were inoculated into nutrient broth, incubated at 35ºC for 24 hours after which 20% sterile glycerol was added and the culture was kept at -20ºC.

3.4.2 Isolation from Grape Juice and Soursop

Isolation from soursop and grape was done by extracting the juice from each and allowing them to undergo fermentation for a maximum of 36 hours. Serial dilution was then carried out in distilled water to a dilution factor of 5. 100 µL of dilution factors -2 and -5 were each plated using the spread plate method on MRS, YEGA and YEPD supplemented with antifungal and antibacterial reagents as described above. Incubation was carried out at 35 ºC for 24 hours. Isolates with shiny or mucoid colonies were sub cultured until pure cultures were obtained. Pure cultures were kept as described above.

3.5 Gram Staining of Bacterial Isolates

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Gram staining was carried out on the isolates on MRS and Nutrient agar to differentiate the bacteria isolates into Gram-positive and Gram-negative based on cell wall characteristics. A clean glass slide was prepared to make a smear of the bacteria culture. The slide was air-dried and heat-fixed by passing it gently over a flame. After which the slides were flooded with the primary stain and allowed to stand for 60 seconds before being gently rinsed with distilled water. Subsequently, iodine solution was applied for 60 seconds to serve as a mordant, after which the slide was rinsed. Decolorization was then performed by flooding the smear with ethanol for 10–15 seconds, followed by immediate rinsing with distilled water to avoid over-decolorization. Counterstaining was carried out using safranin for 60 seconds, after which the slide was rinsed and allowed to air-dry. The Gram reaction of the cells was thereafter determined by examining the stained preparation under the oil immersion objective (100x) of the light microscope.

3.6 Simple Stain of Yeast Isolates

Simple Stain was carried out on isolates on YEPD and YEGA agar to observe the morphology of the yeast cells. A loopful of the yeast culture was transferred onto a clean glass slide and spread to make a thin smear. The smear was air-dried and heat-fixed by gently passing the slide through a flame. The fixed smear was stained with methylene blue and allowed to sit for 1-2 minutes. Excess stain was rinsed off gently with distilled water, and the slide was allowed to air-dry. The stained slide was then observed under a light microscope using the oil immersion objective (100x).

3.7 EPS Production

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All the isolates that produced shiny, mucoid colonies were presume to produce exopolysaccharides. These isolates were inoculated into 100 mL of EPS producing broth. The culture was incubated for 15 days with continuous agitation at 150 rpm in room temperature. The cultures were later centrifuged at 4000 rpm for 30 minutes and the supernatant was transferred to a new tube. Twice the volume of ice cold n-propanol was added and EPS was allowed to precipitate overnight after which it was centrifuged at 4000 rpm for 30 minutes[5]. The pellets were precipitated with isopropanol for decolorization. The pellets were gathered in a petri dish, dried, weighed and dissolved in 1ml of sterile distilled water.

3.8 Optimization Studies

3.8.1 Exopolysaccharide Production

Isolates with the highest EPS production were chosen for optimization studies. These were the yeast isolates. The exopolysaccharide production was optimized using three parameters (carbon source type, varying concentration of carbon source, and temperature). Glucose, fructose, sucrose, galactose, served as carbon source at different concentrations (4%, 6% and 8%). The growth of yeast isolates were observed at different temperatures (25, 30 and 35^{o}C). Isolated yeast strains were inoculated in basal media with minor modifications (Yeast extract 0.4g/L, peptone – 0.06g/L, K_2HPO_4 - 0.5g/L, Nacl – 0.1g/L, 0.5g/L $MgSO_4.7H_2O$, distilled water 100ml) and then incubated for 15days[3].

3.8.2 EPS Extraction and Purification

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For the evaluation of EPS production, the selected sample G1 was collected after 15 days of incubation. The EPS was obtained by addition of equal volumes of ethanol and n-propanol. The mixture was vortexed for 5 minutes and then the precipitate was left overnight at room temperature. The supernatant was then centrifuged for 20 minutes at 4000 rpm and precipitation was gathered in a petri dish and then dried in oven at 40^{o}C[3].

3.9 Determination of pH Level for Optimal Production on Highest EPS Producing Isolate

The sugar source (glucose) identified as the carbon source that supported the highest exopolysaccharide (EPS) production, was selected for further experiments. It was utilized at a concentration of 6%, which corresponded to the level that yielded maximum EPS output. The fermentation process was conducted at varying pH values of 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5. Incubation was carried out at 30 °C, the temperature determined to be optimal for EPS production, for a duration of 15 days under agitation at 150 rpm.

3.10 FTIR Analysis

Fourier Transform Infrared (FTIR) Spectroscopy was used to identify the functional groups present in the sample. The analysis was carried out using an FTIR spectrophotometer. A small quantity of the sample (1-2 mg) was mixed thoroughly with dried potassium bromide (KBr) and compressed into a thin pellet using a hydraulic press. The KBr pellet was then placed in the sample holder of the FTIR spectrophotometer. The spectrum was recorded over the range of 4000 to 400cm^{-1} at a resolution of 4cm^{-1}. The characteristics peaks obtained were used to identify the functional groups present in the sample by comparing them with standard reference spectra.

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3.11 Production of Clarifying Gel

3.11.1 Materials

The materials used for the preparation of the clarifying gel included hibiscus extract, xanthan gum, glycerin, Germa Plus (as preservative), and exopolysaccharide solution.

3.11.2 Preparation of Hibiscus Extract

Dried hibiscus flowers (4 g) were weighed and immersed in 200 mL of distilled water. The mixture was allowed to stand, after which it was filtered through filter paper (Whatman no 1) to obtain a clear extract. The filtrate served as the hibiscus extract for the formulation.

3.11.3 Formulation of Clarifying Gel

The hibiscus extract was transferred into a clean beaker, and 1 g of Germa Plus was added as preservative. The solution was mixed thoroughly to ensure uniform distribution. In a separate container, 17 g of glycerin was combined with 1.5 g of xanthan gum until a homogeneous blend was obtained. This mixture was then incorporated into the hibiscus extract and stirred until uniform. The formulation was covered and left to stand for one hour to allow proper gel formation.

3.11.4 Incorporation of Exopolysaccharide Solution

After one hour, the prepared clarifying gel was divided into two portions. One portion was supplemented with the exopolysaccharide solution, while the other portion, without exopolysaccharide, served as the control formulation.

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3.11.5 Skin Patch Test

A skin patch test was conducted on test participants to evaluate the compatibility, stability, and texture of the formulated gels. The test was used to assess any adverse skin reactions and to compare the physical properties of the formulations with and without exopolysaccharide.

3.12 Molecular Characterization of Isolate with Highest EPS Production

Genomic DNA was extracted from the cultures received using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented in Table 3.1. The PCR products were run on a gel and gel extracted with the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Catalogue No. D4001). The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3- 100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample.

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Table 3.1: Primer Sequences

Name of Primer	Sequence (5' to 3')
TUBUF2 (FORWARD)	CGGTAACAACTGGGCCAAGG
TUBUR1 (REVERSE)	CCTGGTACTGCTGGTACTCAG

Author's Laboratory Work, 2025

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Chapter Four

Results and Discussion of Findings

4.1 Results

Tables 4.1 shows the colonies and the morphology obtained from grape samples cultured on YEPD agar. Colonies from Challenge (H1–H4) were whitish to creamy in color, with smooth to shiny surfaces. The forms observed were circular and elongate, and the elevations ranged from raised to flat. Colonies from Oje market (I1–I4) were creamy to whitish, with mostly smooth surfaces, although a rough surface was observed in I2. The colonies were circular and elongate, with elevations ranging from raised to flat. Colonies obtained from Soka (C1–C4) were whitish, with smooth or dry surfaces. The forms observed were circular and elongate, while the elevations were either raised or flat.

Table 4.2 shows the colonies and the morphology obtained from the samples cultured on YEGA agar. Colonies from soursop obtained at Oje market (G1–G4) were whitish to creamy in colour, with smooth to shiny surfaces. The colonies were circular to elongate in form, with elevations that were either flat or raised. Colonies from maize gruel obtained at Challenge (SO3 1–2) were creamy to whitish, with rough surfaces. The forms observed were elongate and the elevations were flat. Colonies from locust beans obtained at Challenge (MO2 1–2) were whitish in colour, with rough surfaces. They were elongate in form and flat in elevation. Colonies from palm wine obtained at Tollgate (P1W1–2) were whitish, with rough surfaces. The colonies were circular in form and flat in elevation.

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Table 4.1: Colonial Description of Isolates obtained from Grape Samples on YEPD (Yeast Extract Peptone Dextrose) Agar

Isolate	Color	Surface	Appearance	Elevation
H1	**Whitish**	**Shiny**	**Circular**	**Raised**
H2	**Whitish**	**Shiny**	**Elongate**	**Flat**
H3	Creamy	Smooth	Elongate	Flat
H4	Creamy	Smooth	Elongate	Raised
I1	Creamy	Smooth	Circular	Raised
I2	Whitish	Rough	Circular	Raised
I3	Creamy	Smooth	Elongate	Flat
14	Creamy	Smooth	Elongate	Flat
C1	Whitish	Smooth	Elongate	Raised
C2	Whitish	Dry	Circular	Flat
C3	Whitish	Smooth	Circular	Flat
C4	Whitish	Dry	Circular	Flat

Source: Author's Field Work, 2025

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Table 4.2 Colonial Description of Isolates obtained from SourSop, Maize Gruel, Locust Beans, Palmwine on YEGA (Yeast Extract Glucose Agar)

Isolate	Color	Surface	Appearance	Elevation
G1	**Whitish**	**Shiny**	**Circular**	**Raised**
G2	**Whitish**	**Shiny**	**Circular**	**Flat**
G3	Creamy	Shiny	Circular	Raised
G4	Whitish	Smooth	Elongate	Raised
S03 1	Creamy	Rough	Elongate	Flat
SO3 2	Whitish	Rough	Elongate	Flat
MO2 1	Whitish	Rough	Elongate	Flat
MO2 2	Whitish	Rough	Elongate	Flat
F1	Whitish	Rough	Elongate	Flat
F2	Whitish	Rough	Elongate	Flat
YEG PW	**Whitish**	**Shiny**	**Circular**	**Flat**
P1W2	Whitish	Rough	Circular	Flat

Source: Author's Field work, 2025

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Table 4.3 shows the colonies and their morphology obtained from the samples cultured on MRS agar. Colonies from maize gruel obtained at Challenge (M1–M2) were creamy in colour with rough surfaces. The colonies were circular in form and raised in elevation. Colonies from locust beans obtained at Orita Challenge (N1–N2) were whitish with dry surfaces. They were circular in form, with elevations that were either flat or raised. Colonies from palm wine obtained at Tollgate (MRS PW) were creamy with dry surfaces. The colonies were circular in form and flat in elevation. Colonies from maize gruel obtained at Orita Challenge (SO2 1–2) were whitish in colour with rough surfaces. They were circular in form and flat in elevation. Colonies from maize gruel obtained at Challenge (Z1–Z2) were white in colour with rough surfaces. Both colonies were circular in form, with Z1 raised and Z2 flat in elevation. Colonies from maize gruel obtained at Felele (Y1) were creamy in colour with dry surfaces. They were circular in form and flat in elevation.

Table 4.4 shows the colonies and their morphology obtained from the samples cultured on Nutrient agar. Colonies from maize gruel obtained at Challenge (P1–P2) were whitish in colour with rough surfaces. The colonies were circular in form and flat in elevation. Colonies from maize gruel obtained at Orita Challenge (O1–O2) were creamy with smooth surfaces. They were circular in form and flat in elevation. Colonies from maize gruel obtained at Felele (R1–R2) were white with smooth surfaces. They were circular in form and flat in elevation. Colonies from palm wine obtained at Tollgate (CH1–CH2) were creamy in colour with smooth surfaces. The colonies were circular in form and flat in elevation. Colonies from locust beans obtained at Challenge (MO2 1–2) were creamy with rough surfaces. They were elongate in form and raised in elevation. Colonies from locust beans obtained at Orita Challenge (F1–F2) were creamy with rough surfaces. They were elongate in form, with F1 raised and F2 flat in elevation.

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Table 4.5 shows the colonies and morphology of the shiny isolates selected and cultured on EPS agar. Colonies from grape (H1–H2) were white in colour with shiny surfaces. The colonies were circular in form and raised in elevation. Colonies from soursop (G1–G2) were white with shiny surfaces. They were circular in form, with G1 raised and G2 flat in elevation. Colonies from palm wine (YEG PW) were white with shiny surfaces. The colonies were circular in form and raised in elevation.

Table 4.6 shows the Gram staining results of the bacteria isolates obtained from the different samples. Colonies from maize gruel (SO2 1) appeared as Gram-positive bacilli. Colonies from palm wine (MRS PW) were also observed as Gram-positive bacilli. Colonies from locust beans (M1 and N1) were Gram positive, with M1 appearing as cocci and N1 as cocci. Isolates from locust beans (D1 and E2) were Gram-positive bacilli. Colonies from maize gruel (Z1) were also Gram-positive bacilli. Colonies from palm wine (CH1 and CH2) appeared as Gram-positive bacilli.

Table 4.7 shows the simple stain results of yeast colonies obtained from the different isolates. Colonies from soursop (G1–G2) appeared oval to round in shape. G1 cells were observed singly in clusters, while G2 cells were mostly arranged in clusters. Colonies from grape (H1–H2) were oval to round in form. H1 appeared singly and scattered, whereas H2 showed budding cells. Colonies from palm wine (YEG PW) were oval in shape with budding forms and were observed in groups.

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4.3 Colonial Description of Isolates obtained from Locust Beans, Maize Gruel and Palm wine on MRS Agar

Isolate	Color	Surface	Appearance	Elevation
M1	Creamy	Rough	Circular	Raised
M2	Creamy	Rough	Circular	Raised
L1	Whitish	Rough	Circular	Raised
L2	Whitish	Smooth	Circular	Flat
N1	Whitish	Dry	Circular	Flat
N2	Whitish	Dry	Circular	Raised
MRS PW	Creamy	Dry	Circular	Flat
SO2 1	Whitish	Rough	Circular	Flat
SO2 2	Whitish	Rough	Circular	Flat
Z1	White	Rough	Circular	Raised
Z2	White	Rough	Circular	Flat
Y1	Creamy	Dry	Circular	Flat
Y2	White	Dry	Circular	Raised

Source: Author's Field Work, 2025

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4.4 Colonial Description of Isolates obtained from Locust Beans, Maize Gruel, Palmwine

on Nutrient Agar

Isolate	Color	Surface	Appearance	Elevation
P1	Whitish	Rough	Circular	Flat
P2	Whitish	Rough	Circular	Flat
O1	Creamy	Smooth	Circular	Flat
O2	Creamy	Smooth	Circular	Flat
R1	White	Smooth	Circular	Flat
R2	White	Smooth	Circular	Flat
CH 1	Creamy	Smooth	Circular	Flat
CH2	Creamy	Smooth	Circular	Flat
MO2 1	Creamy	Rough	Elongate	Raised
M02 2	Creamy	Rough	Elongate	Raised
F1	Creamy	Rough	Elongate	Raised
F2	Creamy	Rough	Elongate	Flat

Source: Author's Field Work, 2025

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Table 4.5: Colonial Description of Isolates obtained from Grape, Soursop and Palmwine on EPS (Exopolysaccharides) Agar

Isolates	Color	Surface	Appearance	Elevation
H1	White	Shiny	Circular	Raised
H2	White	Shiny	Circular	Raised
G1	White	Shiny	Circular	Raised
G2	White	Shiny	Circular	Flat
YEG PW	White	Shiny	Circular	Raised

Source: Author's Field Work, 2025

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Table 4.6: Gram Staining Results on Isolates

S/N	Isolates Code	Gram Reaction
1	SO2 1	Gram (+ve) Bacilli
2	MRS PW	Gram (+ve) Bacilli
3	M1	Gram (+ve) Cocci
4	N1	Gram (+ve) Cocci
5	D1	Gram (+ve) Bacilli
6	E2	Gram (+ve) Bacilli
7	Z1	Gram (+ve) Bacilli
8	CH1	Gram (+ve) Bacilli
9	CH2	Gram (+ve) Bacilli

Source: Author's Field Work, 2025

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Table 4.7 shows the simple stain results of yeast colonies obtained from the different isolates. Colonies from soursop (G1–G2) appeared oval to round in shape. G1 cells were observed singly in clusters, while G2 cells were mostly arranged in clusters. Colonies from grape (H1–H2) were oval to round in form. H1 appeared singly and scattered, whereas H2 showed budding cells. Colonies from palm wine (YEG PW) were oval in shape with budding forms and were observed in groups.

Table 4.8 shows the yield of exopolysaccharides obtained from the different yeast isolates. Colonies from soursop (G1 and G2) produced the highest amounts of exopolysaccharides, with G1 yielding 9.1 g/L and G2 yielding 7.1 g/L. Colonies from grape (H1–H2) produced lower amounts, with H2 yielding 1.5 g/L and H1 producing the least yield of 0.8 g/L. Palm wine isolate (YEG PW) produced 0.9 g/L of exopolysaccharides. Overall, soursop isolates produced the highest yields of exopolysaccharides, followed by grape and palm wine isolates.

Figure 4.1, 4.2, 4.3 Shows the effect of different sugars (galactose, sucrose, fructose, and glucose) at varying concentrations (4%, 6%, and 8%) and incubation temperatures (25 °C, 30 °C, and 35 °C) on exopolysaccharide (EPS) production by isolate G1.

At 4% sugar concentration, glucose supported the highest EPS production (2.4g) at 25 °C, while other sugars yielded comparatively lower values.

At 6% sugar concentration, a marked increase in EPS yield was observed across all sugars. The highest EPS yield overall was obtained with glucose at 30 °C (8.9g), followed by fructose at 3.5 °C (7.0g) and galactose at 30 °C (7.0g). Sucrose also showed appreciable yields, with maximum production at 25 °C and 35 °C (6.7g).

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At 8% sugar concentration, EPS yields generally declined compared to 6%. The highest yield under this condition was recorded with glucose at 30 °C (3.4g), while fructose gave the least (0.1g) at 35 °C).

Overall, the results indicate that 6% sugar concentration was optimal for EPS production by isolate G1, with glucose at 30 °C yielding the maximum EPS (8.9g)

Table 4.9 Shows the pH optimization study which revealed that EPS production by isolate G1 at 30 °C and 6% glucose. This were the conditions that had the highest EPS yield in previous optimization studies. EPS production of 14.12 g/L was observed at pH 5.5, indicating it as the optimum pH for EPS production. Lower yields were recorded at more acidic (pH 4.0–5.0) and slightly acid/close to neutral conditions (pH 6.0–6.5).

Figure 4.4 Shows the FTIR spectrum of the exopolysaccharide (EPS) extract showing characteristic functional groups.

Table 4.10 Shows a FTIR (Fourier Transform Infrared Spectroscopy) results and it points to a carbohydrate-rich, acidic polysaccharide (uronic-acid–containing) with α-glycosidic linkages, carrying a bit of peptide/protein i.e., a typical EPS/pectate-like polymer rather than a single small molecule.

13284 + 1045 cm^{-1} → strong –OH and C–O/C–O–C "carbohydrate" bands → polysaccharide backbone.

i. 845 cm^{-1} → anomeric C–H out-of-plane consistent with α-glycosidic linkages.

ii. 1405 cm^{-1} (COO^-) without a clear 1735–1745 cm^{-1} ester C=O band → de-esterified/ionic carboxylates → uronic acids (pectate/alginate-like) rather than neutral sugars or fully methyl-esterified pectin.

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iii. 1654 & 1540 cm^{-1} (amide I/II) → protein/peptide component, common in EPS or protein–polysaccharide complexes (could also be residual protein).

iv. 919 cm^{-1} → sugar ring/ glycosidic vibrations, supporting a polysaccharide assignment.

Note: A volunteer applied it on a skin surface wound, she reported it stung a bit when she applied it, but by the following morning, the wound had dried up completely.

Table 4.11 Shows the results of the molecular characterization of yeasts isolates. All the isolates showed ≥99% identity with known yeast species, with query coverage of 100% and E-values of 0.0, confirming the accuracy of the identification.

Isolate H1 produced a sequence of 490 bp, which showed 99.8% similarity to *Candida tropicalis* (Accession No. KY102456.1). Isolate YEG PW 1 generated a sequence of 805 bp with 100% similarity to *Saccharomyces cerevisiae* (Accession No. LC576548.1). Similarly, isolate G1 had a 602 bp sequence that matched *Kurtzmaniella quercitrusa* with 100% identity (Accession No. PP589185.1), while isolate G2 (558 bp) showed 100% similarity to *Lodderomyces elongisporus* (Accession No. JN606251.1).

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Table 4.7: Simple Stain Results on Isolates

Isolates	Cell Shape	Arrangement	Staining
G1	Oval to Round	Singly and in clusters	Purple (+)
G2	Round	Mostly in clusters	Purple (+)
H1	Oval	Singly scattered	Purple (+)
H2	Oval to Round	Budding Observed	Purple (+)
YEG PW	Oval with budding cells	In Groups	Purple (+)

Source: Author's Field Work, 2025

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Table 4.8: EPS Production Results for Isolates that Produced Mucoid Colonies

Isolates	EPS Yield (g/L)
G1	9.1
G2	7.1
H1	0.8
H2	1.5
YEG PW	0.9

Source: Author's Field Work, 2025

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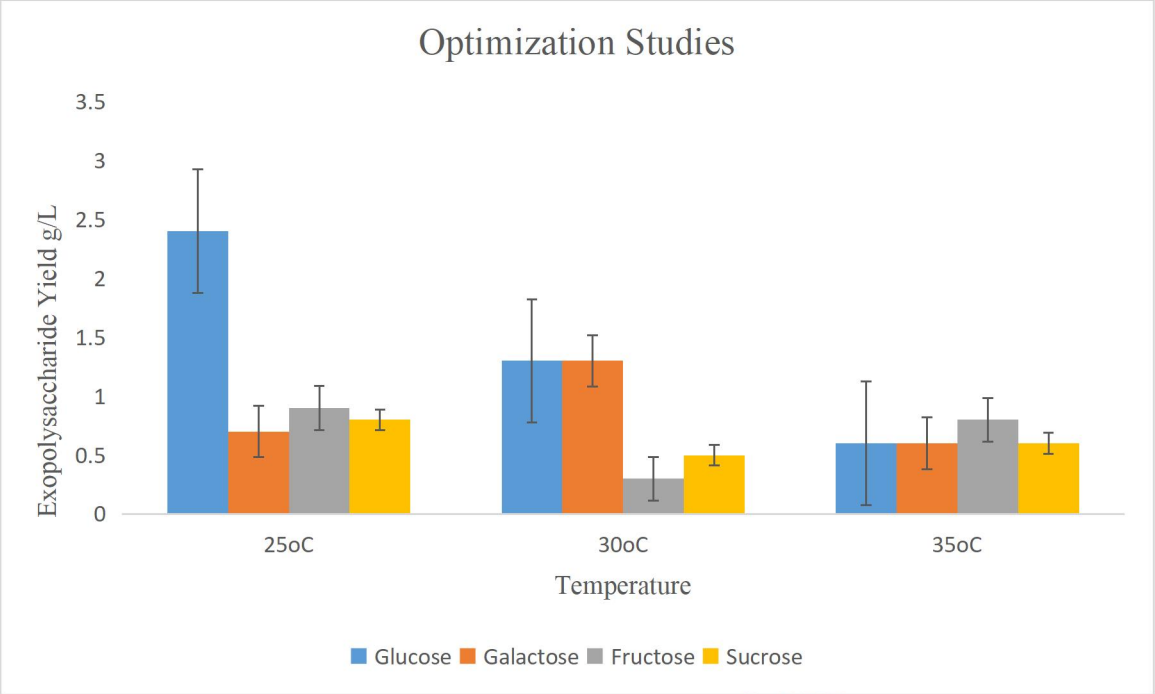

Figure 1: Exopolysaccharide Production at 4% Sugar Concentrations over a Temperature range of 25 °C to 35 °C.

Source: Author's Field Work, 2025

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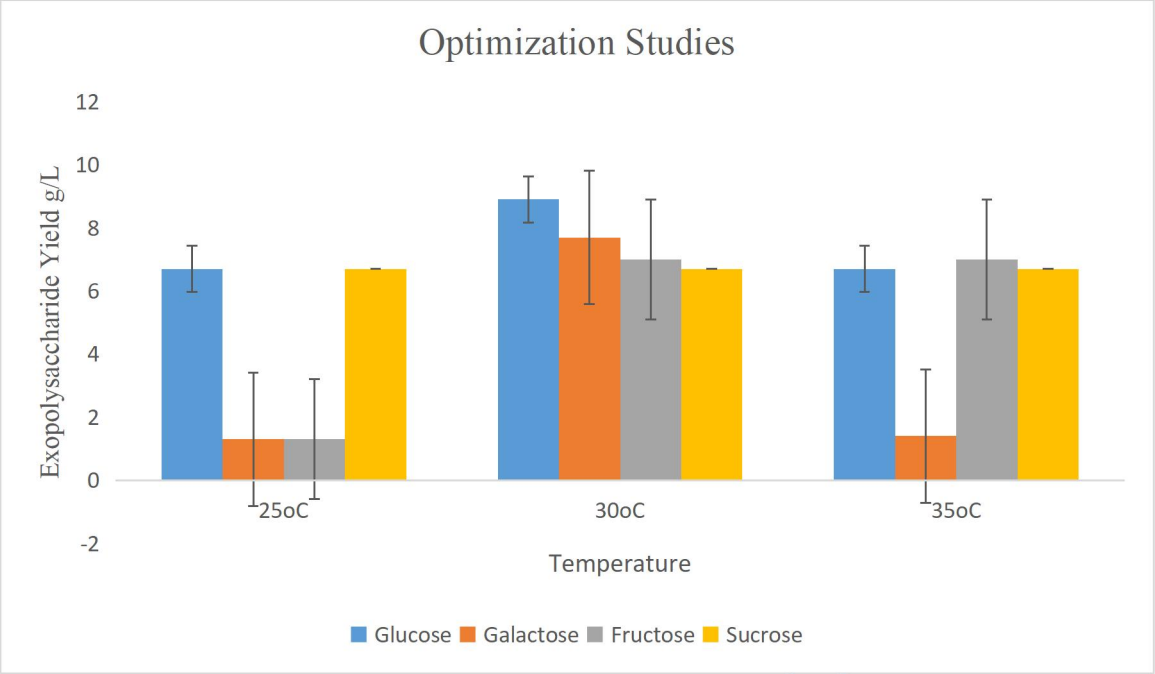

Figure 2: Exopolysaccharide Production at 6% Sugar Concentrations over a Temperature range of 25 ^{o}C to 35 ^{o}C.

Source: Author's Field Work, 2025

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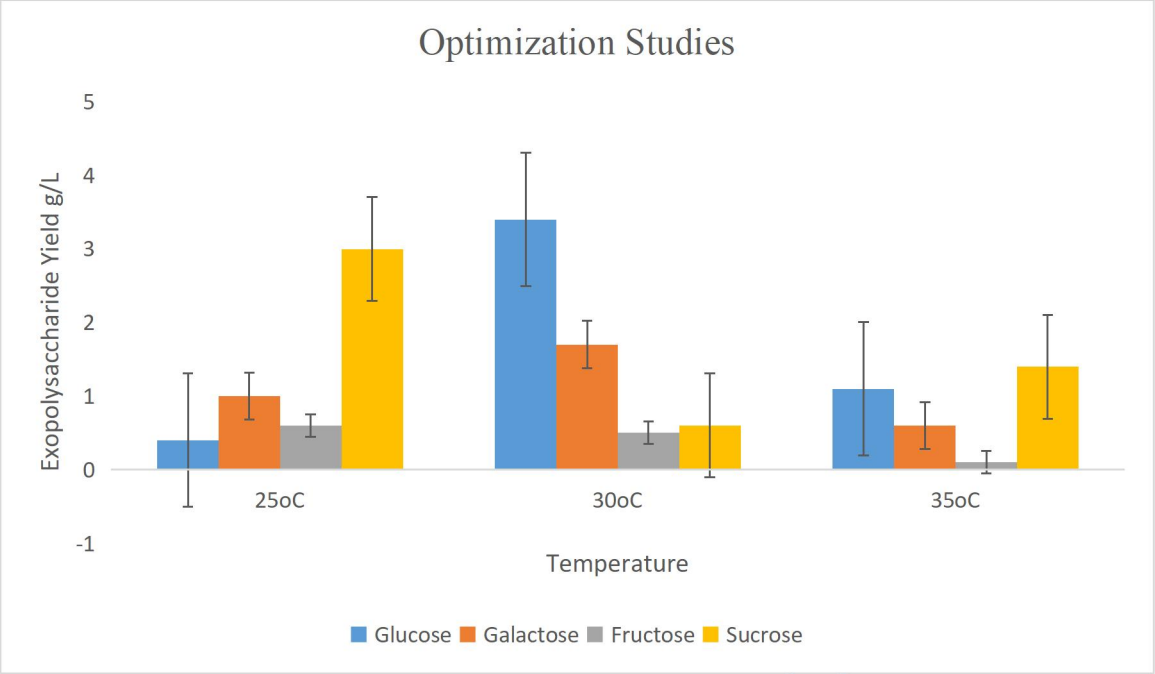

Figure 3: Exopolysaccharide Production at 8% Sugar Concentrations over a Temperature range of 25 ^{o}C to 35 ^{o}C.

Source: Author's Field Work, 2025

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4.9 EPS production at varying pH for Isolate G1

pH	EPS Yield (g/L)
4.0	7.1
4.5	8.1
5.0	8.1
5.5	14.12
6.0	9.1
6.5	10.0

Source: Author's Field Work, 2025

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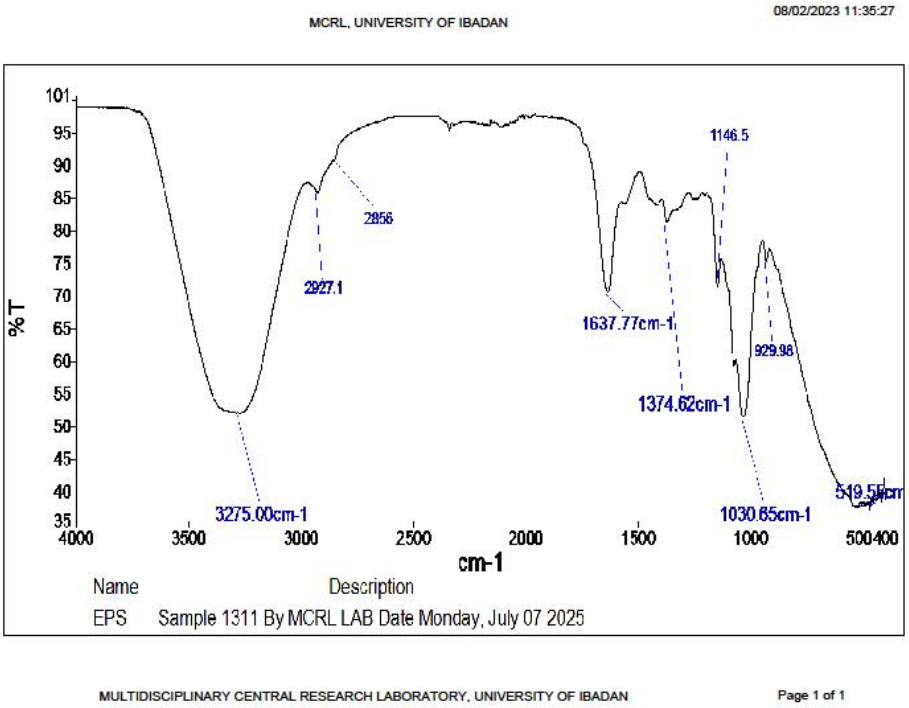
Fig 4.4: FTIR Spectrum of Exopolysaccharides (EPS)



Source: Author's Field Work, 2025

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Table 4.10 FTIR (Fourier Transform Infrared Spectroscopy)

Wave number (cm^{-1})	Range Value (cm^{-1})	Assignment	Vibrational Mode
3284	3200-32600	O-H stretch (strong, broad)	Hydrogen bond
2925	2850-2960	C-H stretch	Sp^2 Assymetric stretch
1654	1630-1680 1600-1680	C=O or C=C stretch	Ketones or alkenes
1540	1510-1580	N-H bend + C-N stretch	Peptides
1405	1380-1420	COO or C-H bending	Carboxylate salt, Alkane CH_2 Bending
1239 ester)	1200-1275	C-O stretch	(carboxylic acid,
1045	1000-1150	C-O-C or C-OH stretch	Ethers or alcohols
919	910-950	C-H wag (out-of-plane bending)	Monosubstitued alkene or sugars
845 compounds	800-880	C-H out of plane bend	Aromatic
611	600-650	Skeletal vibrations	Polysaccharide or Halogenated compound

Source: Author's Field Work, 2025

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Table 4.11: Sequence Prediction Report

Sample ID	Organism	Sample Length (bp)	% Identity	Accension no. of Blast hit	E-value	Highest Query coverage (%)
H1	*Candida tropicalis*	490	99.80%	KY102456.1	0.0	100%
YEG PW 1	*Saccharomyces cerevisiae*	805	100.00%	LC576548.1	0.0	100%
G1	*Kurtzmaniella quercitrusa*	602	100.00%	PP589185.1	0.0	100%
G2	*Lodderomyces elongisporus*	558	100.00%	JN606251.1	0.0	100%

>*Candida tropicalis*

TTCCGTAGGTGAACCTGCGGAAGGATCATTACTGATTTGCTTAATTGCACCACAT
GTGTTTTTATTGAACAAATTTCTTTGGTGGCGGGAGCAATCCTACCGCCAGAGG
TTATAACTAAACCAAACTTTTTATTTACAGTCAAACTTGATTTATTATTACAATAG
TCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGA
AATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCCC
TCAAACCCCCGGGTTTGGTGTTGAGCAATACGCTAGGTTTGTTTGAAAGAATTTA
ACGTGGAAACTTATTTTAAGCGACTTAGGTTTATCCAAAAACGCTTATTTTGCTA
GTGGCCACCACAATTTATTTCATAACTTGACCTCAAATCCAGGTAGG

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>Saccharomyces cerevisiae

TTTAATAATTTTGAAAATGGATTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTAC
TGGGCAAGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCG
GTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCAAACGGTGAGAGATTTCTG
TGCTTTTGTTATAGGACAATTAAAACCGTTTCAATACAACACACTGTGGAGTTTT
CATATCTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCAGAGGTAAC
AAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAACAAGAATTTTCGT
AACTGGAAATTTTAAAATATTAAAAACTTTCAACAACGGATCTCTTGGTTCTCGC
ATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCCGT
GAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGC
CTGTTTGAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTT
TGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTTTTCCAAAGAGAG
GTTTCTCTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTTTACCAAC
TGCGGCTAATCTTTTTTATACTGAGCGTATTGGAACGTTATCGATAAGAAGAGAG
CGTCTAGGCGAACAATGTTCTTAAAGTTTGACCTCAAATCAGGTAGGAGTACCCG
CTGAACTTAAGCATATCAATAAGCGGAGGAA

>Kurtzmaniella quercitrusa

TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAA
CTGCGCGGCGAAAAACCTTACACACAGTGATTTCTTTCTTTGAAAACATTGCTTT
GGTCTGGCGCAAGTTGGGCCAAAGGTTTATTAAACTTCAATTTTATATTGAACTG
TTATTTTAACTAAAGTCAATTTGTTGATTAAATTCAAAAATCTTCAAAACTTTCAA
CAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAATTGCGATAAGT
AATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
TTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAATCTTCGG
ATTTGGTTTTGAGTGATACTCTTAGTCGAACTAGGCGTTTGCTTGAAAAGTATTG
GCAAGAGTGGTACTTTAGGTGCTAAACTGTTTCAATGTATTAGGTTTATCCAACT
CGTTGAATCTGGTTAGTTACTTTAGGGTGCTTAGGCTCGGCCTTACAACAACAAA
CAAAGTTTGACCTCAAATCAGGTAGGATTACCCGCTGAACTTAAGCATATC

>Lodderomyces elongisporus

CTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAATTTTGAGAATTGTGCTTA
ACTGCACTTTTCTTATCTACACACGTGTTTTTGTTTTATTCTTAAAACTTGCTTTGG
CAGTGGCTGCTTAATTGCTCTGCTGCCAGAGGATAAACTCAACCTAAATTTTTTA
TTTTAAACTAGTCAACTGATTATATTTATTAATAGTCAAAACTTTCAACAACGGA
TCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGA
ATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTA
TTCCGGAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGG
TGATGAGCAATACGCCAGGTTTGCTTGAAAGTTAGGAGGAGTATTTATAACAATG
TATTAGGTCTAACCACTCCATTGTGCTTAATAAAAGCTCCAATCTATATTTCAAA
CTTCGACCTCAAATCAGGTAGGATTACCCGCTGAACTTAAGCATATCAATAAGCG
GAGGAAA

Source: Author's Field Work, 2025

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Figure 4.5: Phylogenetic Tree shows the Relationship of Isolate G1 with Closely Related Yeast Species Based on rRNA Gene Sequence Analysis.

The phylogenetic tree constructed from ITS/16S rRNA gene sequences demonstrates the evolutionary placement of the *Kurtzmaniella quercitrusa* isolate from Lead City relative to other global strains. The Lead City strain clusters closely with *K. quercitrusa* isolate 2 (GenBank: KT175173.1), forming a moderately supported subclade. This suggests a close genetic relationship and potentially similar ecological or geographic origins. The subclade containing the Lead City isolate is further associated with *K. quercitrusa* strains PAR14, AUMC 10250 CTTCCG, and additional reference cultures from NIBR and other repositories, indicating conserved sequence features within this clade.

The overall clustering pattern supports the identification of the Lead City isolate as *Kurtzmaniella quercitrusa*, with no major divergence from known representatives of the species. Its position within a well-supported *K. quercitrusa* clade distinct from the outgroup species *Candida tropicalis* (MG720231.1) reinforces the taxonomic placement and genetic consistency of the isolate. These findings suggest that the Lead City strain shares significant evolutionary lineage with other *K. quercitrusa* strains, possibly reflecting common traits relevant for biotechnological or fermentative applications.

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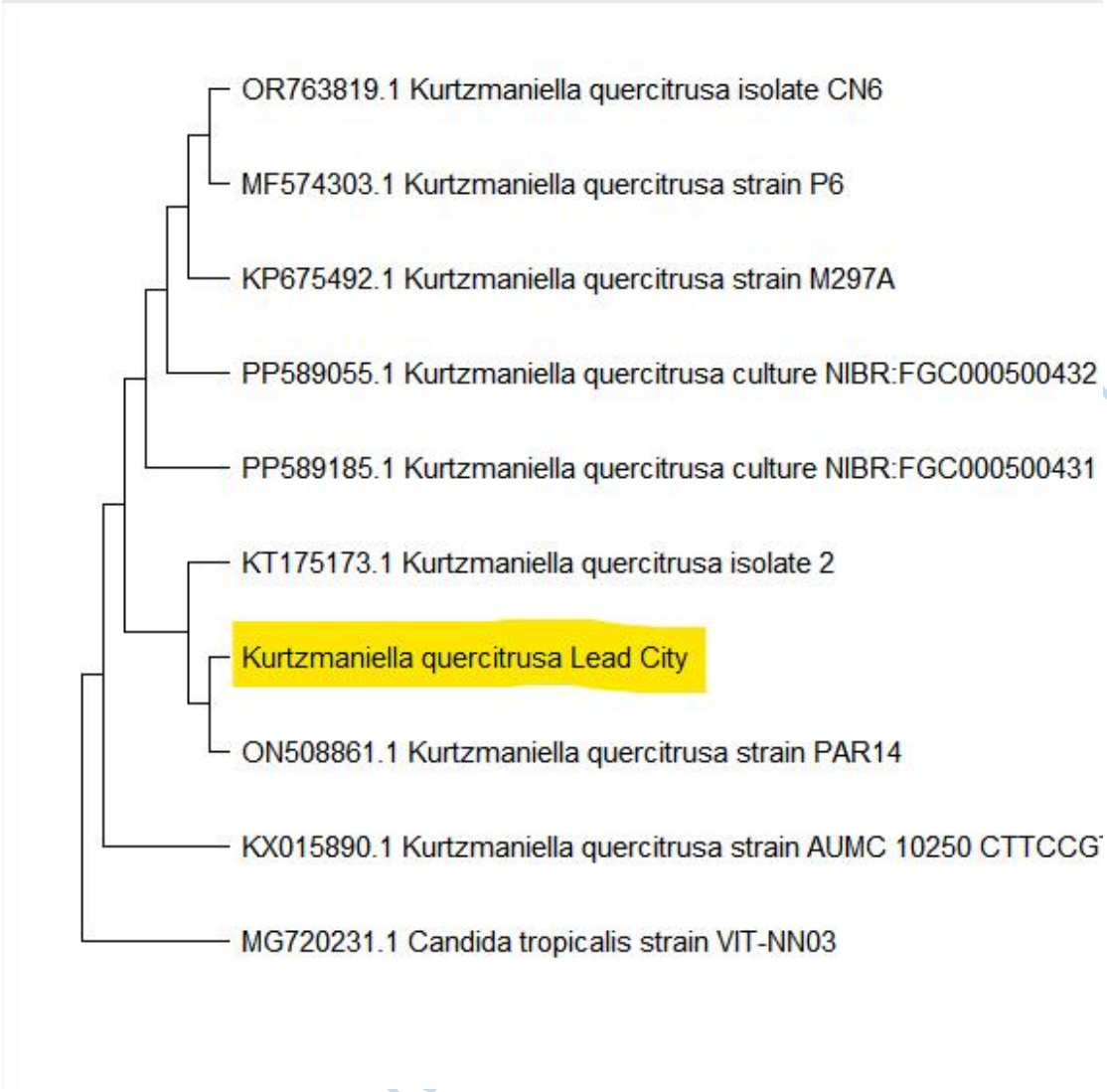

Figure 4.5: Phylogenetic Tree shows the Relationship of Isolate G1 with Closely Related Yeast Species Based on rRNA Gene Sequence Analysis.

Source: Author's Field Work, 2025

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Figure 4.6: Phylogenetic Tree shows the Relationship of *Candida tropicalis* H1 (Lead City isolate) with Closely Related Yeast Species Based on 18S rRNA Gene Sequence Analysis.

The phylogenetic tree constructed from 18S rRNA gene sequences demonstrates the evolutionary placement of the *Candida tropicalis* H1 isolate from Lead City relative to other global strains. The Lead City isolate clusters closely with *C. tropicalis* isolate C10 (GenBank: PP808753.1) and *C. tropicalis* isolate A4 (GenBank: PP808735.1), forming a strongly supported subclade with bootstrap values of 90 and 78, respectively. This indicates a high degree of genetic relatedness and suggests potential conservation of functional traits.

The Lead City isolate is further associated with *C. tropicalis* strain CBL Cd-3 (GenBank: EU924133.1) and *C. tropicalis* isolate 287 (GenBank: KU950724.1), indicating shared sequence identity across multiple reference strains.

The overall clustering pattern supports the identification of the Lead City isolate as *Candida tropicalis*, with no significant divergence from known representatives of the species. Its placement within a well-supported *C. tropicalis* clade distinct from the outgroup species *Kurtzmaniella quercitrusa* (GenBank: PP589185.1) reinforces the taxonomic classification and evolutionary stability of the isolate. These findings suggest that the Lead City isolate shares a strong evolutionary lineage with other *C. tropicalis* strains, potentially reflecting traits of clinical, biotechnological, or environmental significance.

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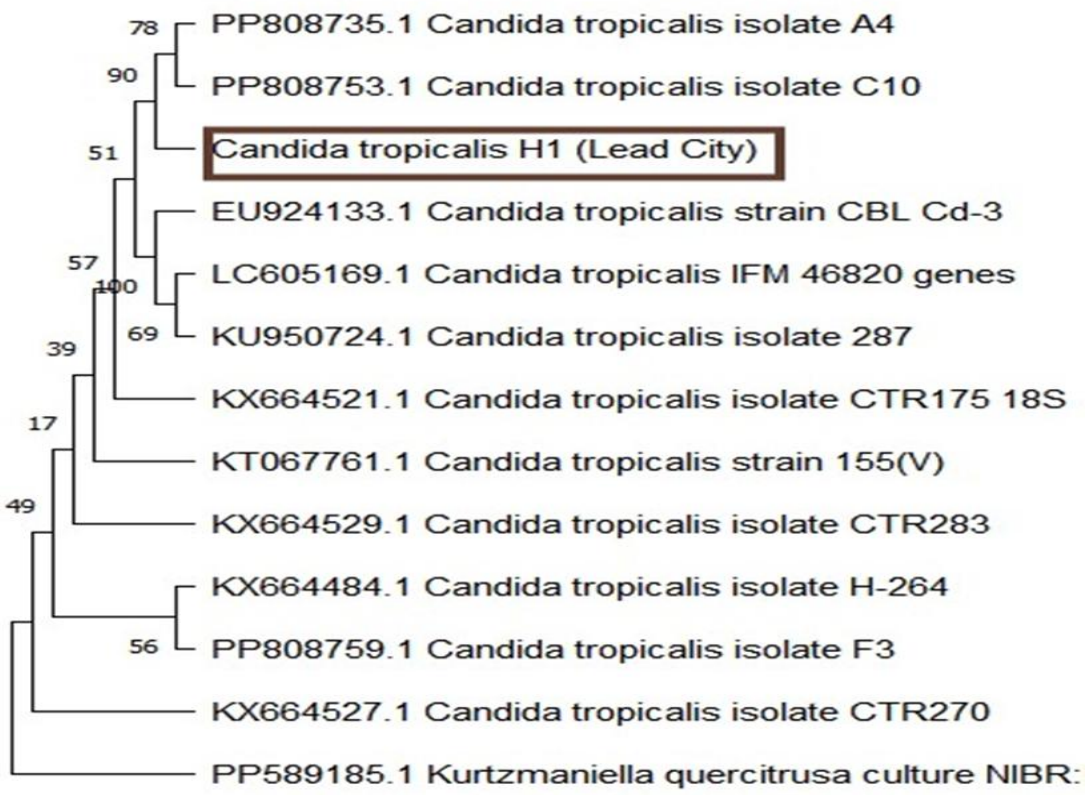

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Figure 4.6: Phylogenetic Tree shows the Relationship of Isolate H1 with Closely Related Yeast Species Based on rRNA Gene Sequence Analysis.

Source: Author's Field Work, 2025

Figure 4.7: Phylogenetic Tree shows the Relationship of *Saccharomyces cerevisiae* (Lead City isolate) with Closely Related Yeast Species Based on 18S rRNA Gene Sequence Analysis.

The phylogenetic tree constructed from 18S rRNA gene sequences demonstrates the evolutionary placement of the *Saccharomyces cerevisiae* isolate obtained from Lead City relative to other global strains. The Lead City strain clusters closely with *S. cerevisiae* isolate YN3 (GenBank: KJ502662.1), forming a moderately supported subclade (bootstrap value 21). This indicates a close genetic relationship and suggests possible similarities in ecological adaptation or evolutionary history.

The Lead City isolate is further associated with *S. cerevisiae* type strain CBS405 (GenBank: AM262830.1) and *S. cerevisiae* strain Chicha 05 (GenBank: KC183726.1), highlighting conserved sequence features within this lineage.

The overall clustering pattern supports the identification of the Lead City isolate as *Saccharomyces cerevisiae*, with no major divergence from known representatives of the species. Its placement within a well-supported *S. cerevisiae* clade distinct from the outgroup (*Candida tropicalis* strain CBL Cd-3, GenBank: EU924133.1) reinforces the taxonomic identity and genetic consistency of the isolate. These findings suggest that the Lead City strain shares significant evolutionary lineage with other *S. cerevisiae* strains, reflecting conserved traits relevant to fermentation, biotechnology, and industrial applications.

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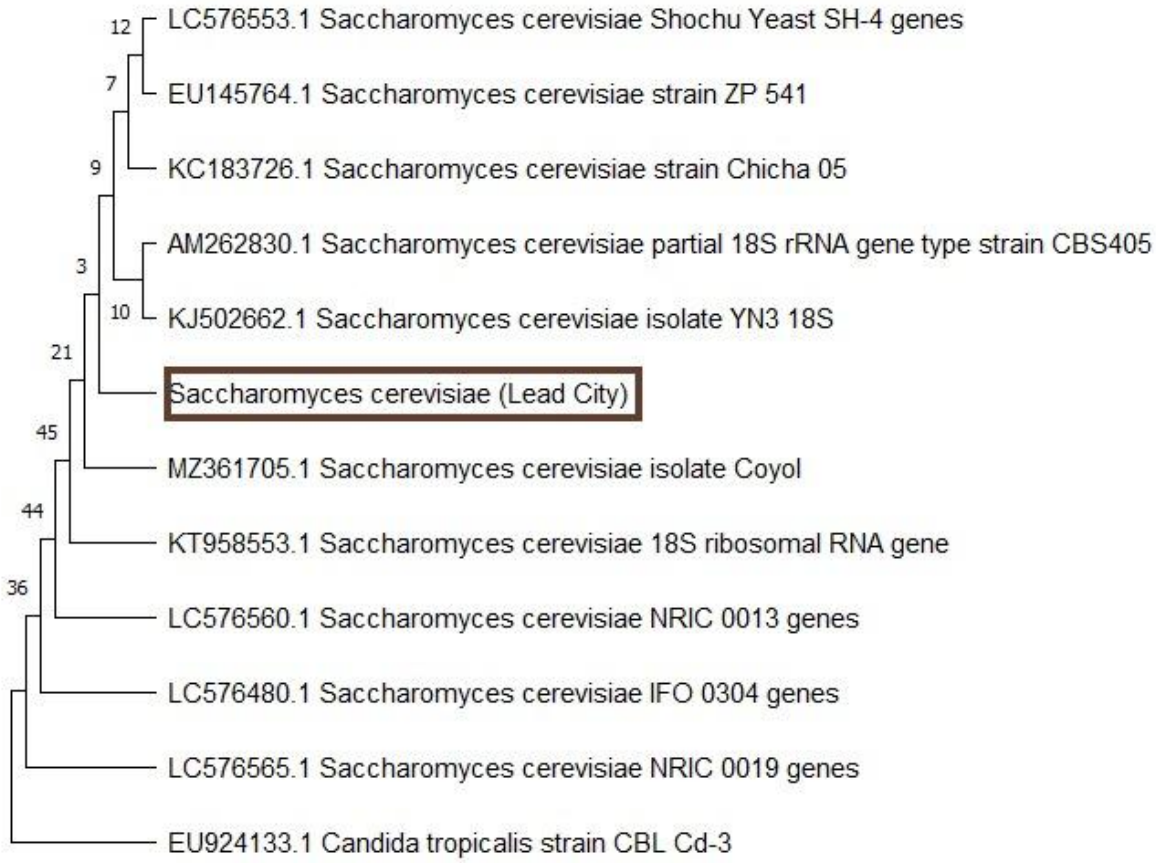

Figure 4.7: Phylogenetic Tree shows the Relationship of Isolate YEG PW 1 with Closely Related Yeast Species Based on rRNA Gene Sequence Analysis.

Source: Author's Field Work, 2025

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Table 4.12 shows a total of three participants tested both formulations of clarifying gel. One formulation contained hibiscus extract and glycerin (Formulation A), while the other contained hibiscus extract, glycerin, and exopolysaccharides (Formulation B). No adverse skin reactions were observed in any of the application of either formulation.

Table 4.13 shows the stability results of the clarifying gel by three participants. The observations revealed that the gel maintained its physical characteristics with no changes in color, texture, or consistency throughout the study period, indicating good product stability.

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Table 4.12: Observed Skin Reactions following Application of Clarifying Gels

Participant ID	Formulation A (Hibiscus + Glycerin)	Formulation B (Hibiscus + Glycerin + Exopolysaccharide)	Observed Reaction
P1	No Irritation, No Redness	No Irritation, No Redness	Moisturized well
P2	No Irritation, No Redness	No Irritation, No Reaction	Moisturized well
P3	No Visible Reaction	Skin remained normal	Moisturized well

Source: Author's Field Work, 2025

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4.13: Stability Observation of Clarifying Gel

Participant ID	Stability Observation
P1	No change in color/texture
P2	No watery consistency observed
P3	Texture remain unchanged

Source: Author's Field Work, 2025

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4.2 Discussion of Findings

The extraction, purification, and initial characterization of exopolysaccharides (EPS) derived from five traditionally fermented products palm wine, grape, soursop, maize gruel (ogi), and locust beans (iru) were critically evaluated in this study. These substrates, widely consumed within Nigerian communities, are characterized by diverse microbial populations that contribute to their metabolic richness and health-promoting properties. Such microbial consortia are known to produce a variety of biomolecules, among which EPS are of particular importance due to their dual nutritional and industrial relevance[1]

Morphological characterization revealed that creamy-white, smooth-surfaced Gram-positive colonies were prevalent across all studied substrates, including grape, soursop, maize gruel, palm wine, and fermented locust beans. Several isolates, including G1, G2, H1, H2, and YEG P.W, demonstrated consistent exopolysaccharide (EPS) production, with colonies displaying shiny, raised, and circular morphologies on selective media. These features are typical of EPS-producing microorganisms, a pattern previously associated with Lactic Acid Bacteria and Bacillus species[2,3]. The presence of such isolates in locally fermented foods supports the view that traditional substrates can be valuable sources of microbial EPS producers [4,5]. Results from this study will also seem to suggest that EPS-producing microorganisms are more associated with fruits or fermented foods with high fibre content. This is due to the fact that in the study, isolates from soursop and grape produced comparatively higher exopolysaccharide yields than those from palm wine, 'iru' or 'ogi', a trend which from literature seem to be attributable to the higher fiber content of these fruits. This observation aligns with earlier reports where fruit-based substrates produced higher expolysaccharide yields compared to cereal-based substrates[6]. Often in cereal based food preparations, the fibres are often separated from the starch before fermentation. This would thus exclude the

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complex carbohydrates such as cellulose, hemicellulose, pectin and some of the resistant starch that can be degraded via various enzymatic pathways that would release sugars that can be channeled into EPS production. Fibre rich food matrices are also structurally complex and microbes require the production of sticky EPS for them to be able to successfully colonize them and form stable microbial communities[11].

Yeasts are increasingly recognized as valuable sources of exopolysaccharides (EPS), though they remain less studied compared to bacterial producers. Several genera, including *Saccharomyces*, *Candida*, *Cryptococcus*, and *Pichia*, have been reported to synthesize EPS with diverse biological and industrial functions. For instance, *Saccharomyces cerevisiae* produces β-glucans and mannoproteins with immunomodulatory and antioxidant properties[7]. *Candida kefyr* and *C. utilis* have been associated with mannan and glucan production for emulsifying and stabilizing purposes[8] while *Cryptococcus laurentii* and *Pichia anomala* synthesize EPS with film-forming, viscosity-enhancing, and antimicrobial activities[7]. In this study, yeasts such as *Kurtzmaniella quercitrusa*, *Lodderomyces elongisporus*, *Saccharomyces cerevisiae* and *Candida tropicalis* were found to be relatively high EPS producers. Exopolysaccharide production in *Candida tropicalis* is generally regarded as an adaptive mechanism that enables the organism to withstand environmental stress. The polysaccharide layer secreted around the cells functions as a protective barrier, reducing the effects of osmotic imbalance, toxic metabolites, and other unfavorable conditions encountered during growth. In addition, EPS contributes to adhesion and biofilm formation, which facilitate colonization and persistence in nutrient-rich yet competitive environments. It has also been reported that under nutrient-limiting conditions, EPS may serve as a reserve source of carbon and energy, thereby supporting long-term survival. Furthermore, certain fractions of yeast-derived EPS possess antioxidant activity, providing additional protection against oxidative

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stress generated during metabolism. In the present study, the high EPS yield observed from *C. tropicalis* at the optimized glucose concentration, pH, and incubation conditions suggests that production was stimulated by a combination of osmotic and metabolic stress, factors previously shown to enhance polysaccharide biosynthesis in yeasts[8]. There is currently no documented evidence of EPS production by *Kurtzmaniella quercitrusa*. This makes the present work one of the first to identify this species as a potential EPS producer, thereby contributing novel insight into microbial diversity in fermentation ecosystems. The observed EPS production by this isolate may be linked to its adaptive mechanisms for survival in acidic, nutrient-variable environments such as soursop fermentation[8].

The decision to investigate the EPS producing abilities of *C. tropicalis* in this study was based on the curiosity in the field of pathobiotechnology. Pathobiotechnology is a field that exploits the use of pathogens or pathogen – derived factors for beneficial applications in the field of biotechnology, food and medicine. Even though the EPS producing ability of *C. tropicalis* was measured, it was not used in the production of the clarifying gel as there was not enough time to determine the exact composition of its EPS and to ascertain the possibility of its safety for use.

Under standard cultivation conditions, *Kurtzmaniella quercitrusa* and *Lodderomyces elongisporus* (both from soursop) demonstrated the highest baseline EPS production, with yields of 9.1 g and 7.1 g, respectively compares favorably with reports from previous studies. *Lactobacillus plantarum* from kimchi produced 1.5–2.0 g/L[7] while lactic acid bacteria strains have been documented to yield 1.0–2.5 g/L under sucrose-rich conditions[7]. Similarly, fruit-based substrates such as grape and pineapple supported yields exceeding 1.2 g/L[8]. The significantly high yield recorded in this study suggests that soursop isolates may represent a

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promising new source of high-yielding exopolysaccharide producers, surpassing many previously characterized microbial strains.

Optimization tests revealed that sugar type, concentration, and incubation temperature significantly influenced EPS yield. *Kurtzmaniella quecitrusa* produced up to 8.9 g/L of EPS when grown on glucose at 6% concentration and 30°C, with similarly high yields obtained on fructose and sucose at comparable conditions. These outcomes are in line with earlier reports that moderate sugar concentrations and mesophilic to thermophilic ranges (30–37°C) favor microbial metabolism and EPS biosynthesis[9]. Furthermore, glucose and sucrose have been identified as preferred monosaccharides due to their efficient uptake and metabolic conversion, making them ideal carbon sources for EPS production[8] A decline in yield was observed at 8% sugar concentration, possibly due to osmotic stress or metabolic inhibition, a phenomenon previously reported in related studies[8,9].

The effect of pH on EPS yield further confirmed the importance of environmental conditions in optimizing production. *Kurtzmaniella quercitrusa* achieved its highest yield (14.12 g) at pH 5.5 suggesting a preference for near acidic conditions. Similar findings have shown that slightly acidic to neutral pH ranges (5.5–6.5) support optimal enzymatic activity and polysaccharide elongation in several microbial strains[10,11]. These results demonstrate the potential for adjusting cultivation parameters to maximize EPS output, particularly for cosmetic applications where pH stability is crucial.

The high-yielding EPS isolates, especially G1 and G2, exhibited properties desirable for dermatological and cosmetic use. Their non-pathogenic origins, natural substrate sources, and significant yield enhance their suitability for consumer applications. EPS molecules are known to retain moisture and form protective films, both of which are essential for

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maintaining skin hydration and barrier function[10]. Moreover, EPS has been linked with antioxidant and anti-aging effects, partly through mechanisms associated with the skin–gut axis [9]. These functional attributes highlight the potential of the EPS identified in this study for incorporation into cosmetic formulations such as moisturizers and serums.

In addition to EPS yield optimization, the stability of the formulated clarifying gel was also assessed. Observations from three participants showed no changes in color, texture, or consistency during the study period, indicating that the gel remained physically stable. Stability is a critical quality attribute for cosmetic and dermatological formulations, as it ensures product safety, consumer acceptability, and shelf-life. Similar reports have shown that herbal or plant-based gels maintain their structural integrity under normal conditions when properly formulated[7,8]. In this study, the absence of phase separation, watery consistency, or textural change confirms the compatibility of the incorporated ingredients and validates the gel's robustness for potential topical application.

These findings are in line with earlier studies that reported stability in hibiscus-based gels and other plant extract formulations[8] The result further supports the suitability of hibiscus extract and glycerin combinations in gel formulations, consistent with prior research highlighting their moisturizing and non-irritant properties[10]. Consequently, the clarifying gel developed in this study demonstrates both stability and functional attributes that make it promising for cosmetic application.

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Chapter Five

Conclusion

5.1 Summary of Findings

The study was carried out to produce, optimize, and evaluate the application of microbial exopolysaccharide (EPS) in the formulation of a clarifying skin gel. The study was guided by the following objectives: the isolation and identification of potential EPS-producing microorganisms from various sources, the optimization of physicochemical conditions (such as pH, temperature, and carbon sources) influencing microbial growth and EPS production, the structural characterization of the EPS using FTIR, the incorporation of the EPS into a base formulation to develop a clarifying skin gel, and the evaluation of the final product for quality parameters including stability, texture, and skin compatibility.

The study revealed that potential EPS-producing microorganisms were successfully isolated and identified, with the most efficient strain selected for further studies. Optimization experiments showed that EPS yield was significantly influenced by pH, temperature, and carbon source, with maximum production observed under moderately acidic conditions and glucose as the preferred substrate. Structural analysis using FTIR confirmed the presence of functional groups characteristic of polysaccharides. The formulated clarifying gel exhibited desirable cosmetic qualities, including smooth texture and ease of application. Stability tests across participants indicated no observable changes in color, texture, or consistency, while skin compatibility evaluation revealed no irritation or adverse reactions.

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5.2 Conclusion

Microbial exopolysaccharides (EPS) derived from fruit-based and fermented substrates offer a lot of potential for application in skincare and cosmetic formulations, as this work has successfully demonstrated. The findings support the notion that both naturally occurring and conventionally fermented materials are excellent sources of EPS-producing bacteria. Furthermore, it was shown that the yield and efficiency of EPS generation were significantly influenced by environmental conditions, such as the kind of carbon source (sugar), incubation temperature, and pH levels. Among the isolates studied, the best EPS-producing strain, *Kurtzmaniella quercitrusa*, exhibited yield and performance characteristics that are comparable to and sometimes even superior to those reported for well-known EPS-producing bacteria in the corpus of recent literature. This illustrates its suitability for upcoming biotechnological developments.

Furthermore, it was shown that microbial EPS had a number of benefits connected to skincare, including enhanced skin barrier function, moisture retention, protection from environmental stressors, and potential anti-aging effects. These functional properties promote the utility of microbial EPS as bioactive ingredients in topical treatments. All things considered, the study backs up the feasibility of applying microbial biotechnology to produce environmentally benign and locally sourced cosmetic basic components. By using local resources and eco-friendly production methods, microbial EPS show potential as alternatives to synthetic polymers and surfactants often used in the cosmetics industry. As a result, this study significantly advances both the broader objective of developing ecologically friendly personal care products and the expanding field of sustainable cosmetic science.

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Importantly, the incorporation of the produced EPS into a clarifying skin gel formulation demonstrate promising results. The gel maintained stability during observation, with no changes in texture, color, or consistency, and was well tolerated on application, showing no signs of irritation or adverse reactions. These outcomes confirm the compatibility of EPS as a functional ingredient in cosmetic gel formulations and highlight its role in enhancing product stability and skin-friendliness.

5.3 Recommendation

Several recommendations are put forth in light of the study's findings to direct future research and possible commercial uses. First, it is advised that the EPS obtained from isolates G1 and G2 be used to create prototype skincare formulations, such as creams, gels, and face masks, as they showed encouraging physicochemical and safety profiles. Partnerships between the biotechnological and cosmetics sectors should be formed to enable the scale-up of EPS production under ideal fermentation conditions in order to support commercial viability.

The molecular weight, monosaccharide composition, and functional group distribution of the EPS should also be ascertained by means of sophisticated biochemical and structural analyses, such as Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Gas Chromatography–Mass Spectrometry (GC-MS). These data would provide critical insights into their structure-function relationships. It is also important to evaluate the rheological behavior and formulation stability of these biopolymers within different cosmetic matrices.

Dermatological evaluations and cytotoxicity tests should be conducted to verify skin compatibility because safety assessments are still of utmost importance. Additionally, to support the usage of EPS as active components in skincare products, both in vitro and in vivo

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studies are required to confirm the EPS's moisturizing, anti-inflammatory, and anti-aging activity.

5.4 Contribution to Knowledge

This study has contributed to knowledge by demonstrating that microbial exopolysaccharides (EPS) isolated from local sources can be successfully applied in the formulation of a clarifying skin gel. The work established that EPS from *Kurtzmaniella quercitrusa* produced a stable gel with good texture and skin compatibility, highlighting its potential as a natural alternative to synthetic polymers in cosmetic formulations. It also provided baseline information on the physicochemical conditions that influence EPS production, which can serve as a reference point for future optimization studies.

5.5 Suggested Areas for Further Studies

Further studies are suggested to build on the findings of this research. Long-term stability studies of the clarifying gel under different environmental conditions should be carried out to establish product shelf life. In addition, large-scale skin compatibility and dermatological assessments are required to confirm safety across wider populations. It is also recommended that future work explore the incorporation of EPS into other cosmetic formulations, such as creams and serums, to broaden its applications. Finally, more detailed structural characterization of the EPS will provide insights into its functional properties and strengthen its use as a bioactive cosmetic ingredient.

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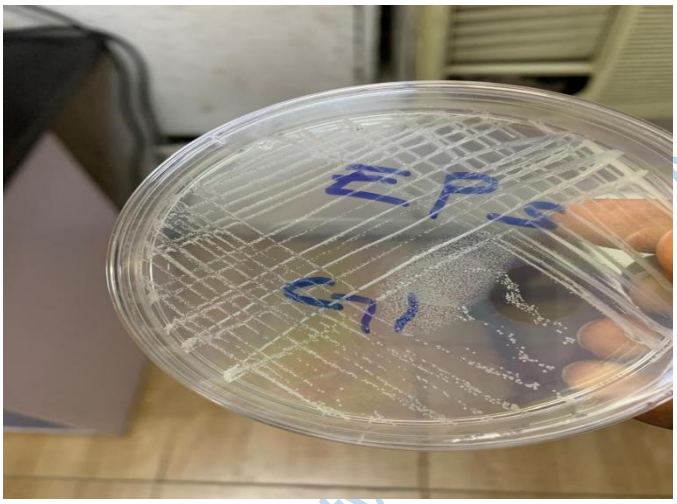
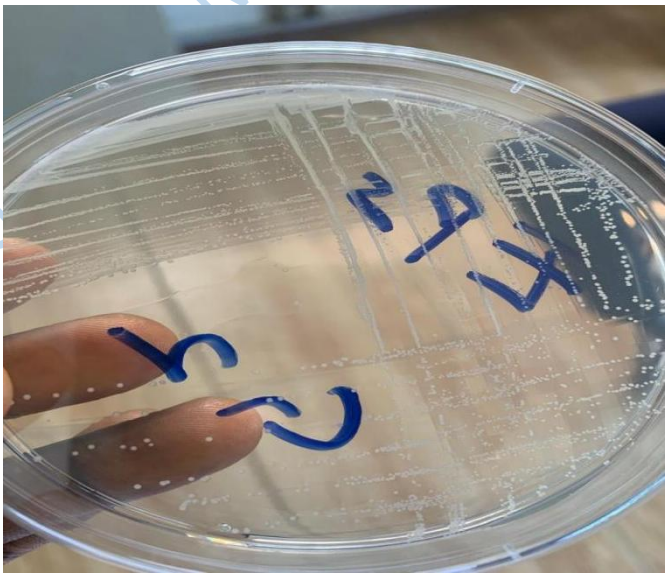
Appendices

Appendix 1: Plate Culture of EPS Isolates



Kurtzmaniella quecitrusa



Lodderomyces elongisporus

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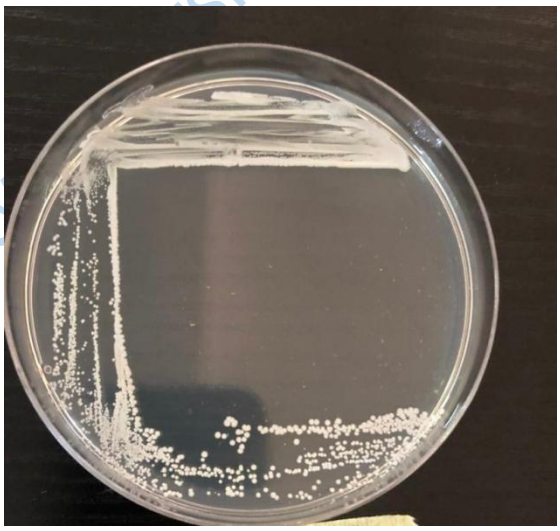
Source: Author's Field Work, 2025

Appendix 2: Plates Culture on EPS Isolates

Saccharomyces cerevisiae



Candida tropicalis

Source: Author's Field Work, 2025

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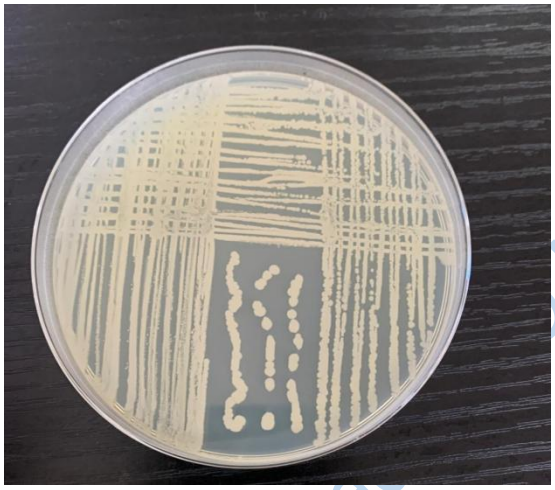
Appendix 3: Plates Culture on Nutrient Agar



CH1

F1

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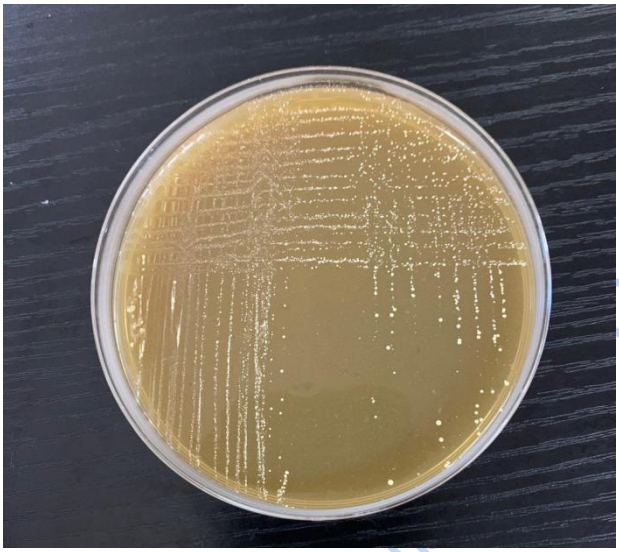
Source: Author's Field Work, 2025

Appendix 4: Plates Culture on MRS Agar



SO2

MRS PW

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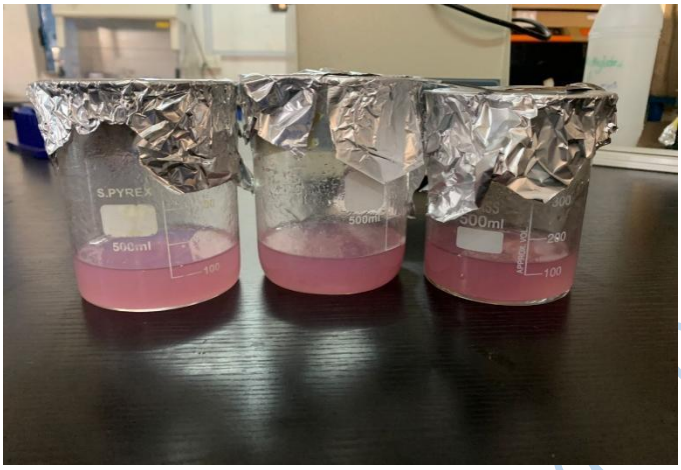
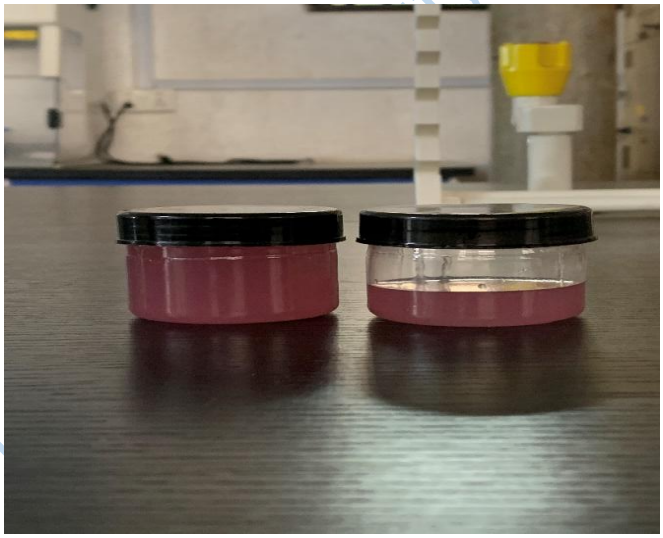
Source: Author's Field Work, 2025

Appendix 5: Clarifying Skin Gel





Source: Author's Field Work, 2025

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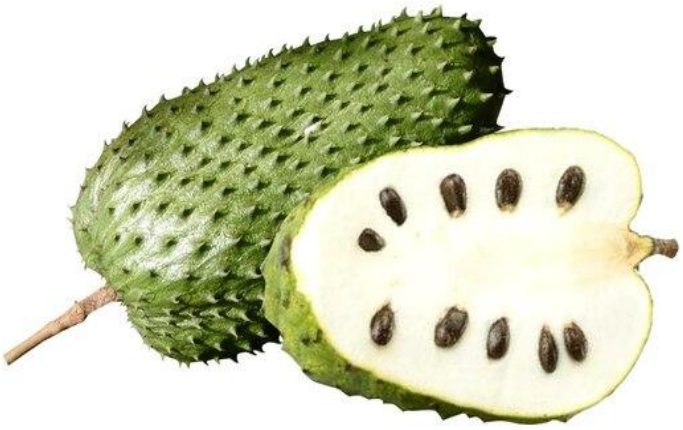
Appendix 6: Sample Used



Soursop

Source: Author's Field Work, 2025

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Appendix 7: Sample Used

Grape

Source: Author's Field Work, 2025

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Appendix 8: Sample Used

Palmwine

Source: Author's Field Work, 2025

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Appendix 9: Sample Used

Maize Gruel

Source: Author's Field Work, 2025

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Appendix 10: Sample Used

Fermented *Parkia biglobosa* (Iru)

Source: Author's Field Work, 2025

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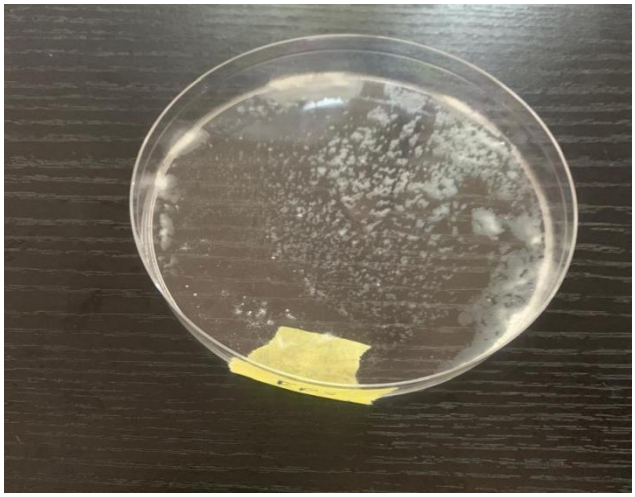
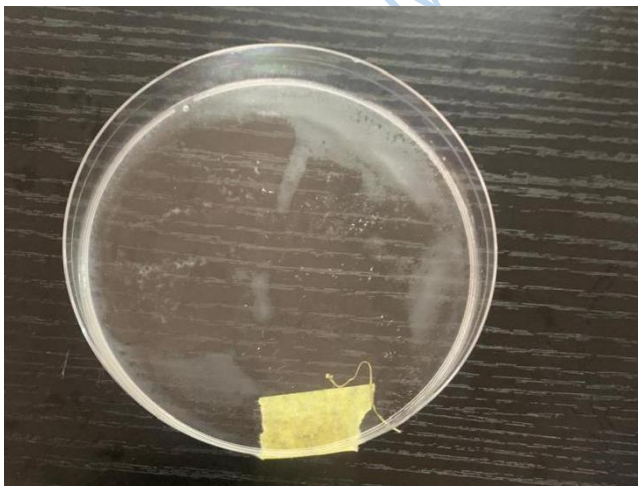
Appendix 11: Exopolysaccharide Plates



Kurtzmaniella quercitrusa Exopolysaccharide



Lodderomyces elongisporus Exopolysaccharide

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Source: Author's Field Work, 2025

Bio-Data

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Name of next of kin	Aboderin Babatunde

B. Educational Background

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M.Sc. in Industrial Microbiology Lead City University Ibadan, Oyo State	(In View)
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SSCE At-Taoheed International College, Ogbomoso, Oyo State.	(2008-2014)
First School Leaving Certificate Kings International Schools, Ogbomoso, Oyo State.	(2003-2008)

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C. Working Experience with Dates.

Pleasant Jay Events, Ibadan, Oyo State. Event Coordinator	(2023-Till Date)
Federal Ministry of Petroleum Resources, Herbert Macaulay, Central Business District, Abuja, FCT. (NYSC) Gas Department.	(2022)
Lautech Teaching Hospital, Ogbomoso, Oyo State. Assistant Technologist	(2019)

D Awards and Fellowships

Nil

E Membership of Academic/Professional Bodies

Nil

F Publications

Nil

G Referees

Available on Request

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Signature **Date**

University Compliance Certificate

This is to certify that, this Thesis written by **Sakirat Yetunde ABODERIN** with Matric No. **LCU/PG/005434**, in the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead city University, Ibadan is in full compliance with the approved University format and style.

Signature **Date**

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