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Isolation of Exopolysaccharide-Producing Bacteria from Curd and Extraction of Exopolysaccharides for Industrial Use

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ABSTRACT:
This research paper investigates the isolation of exopolysaccharide (EPS)-producing bacteria from curd samples and the subsequent extraction of EPS for industrial utilization. Exopolysaccharides are high molecular weight polysaccharides synthesized by microorganisms, exhibiting diverse functional properties with significant industrial applications. Curd, a fermented dairy product, harbors a rich microbial community, making it an attractive source for isolating EPS-producing bacteria. The isolation process involves selective enrichment and screening of bacterial isolates for EPS production, followed by characterization of morphological, biochemical, and molecular properties. Various extraction methods, including physical, chemical, and enzymatic techniques, are employed to solubilize and recover EPS from bacterial biomass. Purification techniques such as filtration, chromatography, and membrane separation facilitate the removal of impurities and cellular debris, resulting in concentrated EPS solutions with desirable functional properties. Evaluation of EPS functional properties reveals their potential applications in food, pharmaceuticals, cosmetics, and biomedicine, including thickening, stabilizing, emulsifying, and regenerative properties. The diversity of EPS-producing bacteria isolated from curd underscores the rich microbial diversity present in fermented dairy products, offering new opportunities for discovering novel microbial resources with industrial relevance. Molecular identification techniques provide insights into the taxonomic diversity and evolutionary relationships of EPS-producing bacteria, guiding strain selection and further characterization for industrial applications. Overall, the isolation of EPS-producing bacteria from curd and the extraction of EPS offer promising avenues for biotechnological innovation and industrial utilization, contributing to the development of functional ingredients, biomaterials, and

biotechnological solutions across diverse industries.

Keywords: Exopolysaccharides, Curd, Bacteria, Industrial Applications, Extraction.

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I.Introduction

Exopolysaccharides (EPS) represent a class of high molecular weight polysaccharides produced by a wide range of microorganisms, including bacteria, archaea, and fungi, under various environmental conditions [1]. These polymers play crucial roles in microbial physiology and ecology and have garnered increasing attention due to their diverse functional properties and potential applications in various industries.

Overview of Exopolysaccharides

Exopolysaccharides are complex macromolecules composed of repeating sugar units joined by glycosidic bonds. They can be linear or branched and may contain other chemical moieties, such as proteins or lipids, contributing to their structural diversity and functional versatility.

Table 1: Summary of Research Parameters

Parameter	Scope	Key Finding	Aspect	Process
Isolate ID	Identification of EPS-producing isolates from curd samples	Diversity of EPS-producing bacteria in curd samples	Microbial ecology	Selective enrichment and screening
EPS Composition	Chemical composition of EPS produced by each isolate	Glucose, fructose, galactose; Glucose, mannose, xylose; Glucose, rhamnose, glucuronic acid; Galactose, N-acetylglucosamine, mannose; Glucose, galactose, arabinose	Biochemical analysis	Gas chromatography, mass spectrometry
EPS Yield	Quantification of EPS yield for each isolate	8.2 g/L; 7.5 g/L; 9.1 g/L; 6.8 g/L; 8.5 g/L	Production efficiency	Gravimetric analysis, spectrophotometry
Molecular Analysis	Identification of isolates using molecular techniques	16S rRNA gene sequencing; Whole-genome sequencing; MALDI-TOF mass spectrometry; Fourier-transform infrared spectroscopy; Nuclear magnetic resonance spectroscopy	Taxonomic classification, structural analysis	Polymerase chain reaction, sequencing
EPS Functional	Evaluation of	Thickening, stabilizing;	Industrial	Rheological

Properties	functional properties of EPS	Emulsifying, film-forming; Rheological modification; Antimicrobial, antioxidant; Immunomodulatory, prebiotic	application s	assays, emulsification tests
Extraction Method	Techniques used for EPS extraction	Acid precipitation, enzymatic extraction, heat treatment, alkaline extraction, solvent extraction	Efficiency, scalability	Centrifugation, filtration, chromatograph y
Purification Technique	Methods employed for EPS purification	Filtration, chromatography, membrane separation, precipitation, dialysis	Purity, removal of impurities	Ultrafiltration, size-exclusion chromatograph y
Evaluation Assay	Assessment of EPS functional properties	Rheological behavior, emulsifying capacity, film-forming ability, bioactive properties	Application -specific characterist ics	Oscillatory rheometry, antimicrobial assays

EPS are synthesized intracellularly by microbial cells and then secreted into the extracellular environment, where they form a protective matrix known as the extracellular polymeric substance (EPS) or biofilm. This matrix provides structural integrity to microbial communities, facilitates adherence to surfaces, and enables resistance to environmental stresses, including desiccation, nutrient limitation, and antimicrobial agents.

A. Importance of Exopolysaccharides in Industry

The unique physicochemical properties of EPS make them valuable resources for various industrial applications. One of the most notable attributes of EPS is their ability to modify the rheological properties of aqueous solutions, imparting viscosity, stability, and gelling capacity. Consequently, EPS are widely used as thickening, stabilizing, and gelling agents in the food industry, where they enhance the texture, appearance, and shelf-life of a diverse range of products, including dairy [2], bakery, and confectionery items. Moreover, EPS exhibit emulsifying properties, facilitating the formation and stabilization of oil-in-water and water-in-oil emulsions, which find applications in food processing, cosmetics, and pharmaceutical formulations. Beyond their role as functional additives in food and beverage formulations, EPS have been explored for their potential in various other industrial sectors. In the pharmaceutical industry [3], EPS-based hydrogels and nanoparticles have shown promise as drug delivery vehicles, enabling targeted and sustained release of therapeutic agents. Additionally, EPS possess bioadhesive properties, allowing for the development of mucoadhesive drug delivery systems for localized treatment of mucosal diseases. Moreover, EPS have applications in the cosmetic industry, where they are utilized as moisturizing agents, film-forming agents, and texture modifiers in skincare, haircare, and personal hygiene products.

B. Curd as a Source of Microbial Diversity

Curd, also known as yogurt in some cultures, is a traditional fermented dairy product obtained by the coagulation of milk proteins through the action of lactic acid bacteria (LAB), primarily strains of Lactobacillus and Streptococcus. The fermentation process involved in curd production creates an acidic environment conducive to the growth of acidophilic microorganisms, including both bacteria and fungi [4]. As a result, curd harbors a diverse microbial community,

comprising not only LAB but also other bacteria, yeasts, and molds, each contributing to the flavor, aroma, and texture of the final product. The microbial diversity present in curd makes it an attractive source for the isolation of novel microorganisms with potential biotechnological applications. Among the microorganisms inhabiting curd, certain bacterial species have been identified as prolific producers of EPS, synthesizing polysaccharides that contribute to the characteristic texture and mouthfeel of fermented dairy products [5]. By isolating and characterizing EPS-producing bacteria from curd, researchers can tap into this microbial diversity to identify strains with desirable EPS production traits and explore their industrial potential. The isolation of EPS-producing bacteria from curd presents a promising avenue for discovering novel microbial resources with applications in various industries [6]. By leveraging the rich microbial diversity inherent in fermented dairy products like curd, researchers can unlock the biotechnological potential of EPS for the development of functional ingredients, biomaterials, and pharmaceutical formulations. This paper aims to explore the isolation of EPS-producing bacteria from curd and the extraction of EPS for industrial use, shedding light on the potential applications of microbial polysaccharides in diverse industrial sectors.

II. Isolation of Exopolysaccharide-Producing Bacteria from Curd

The isolation of EPS-producing bacteria from curd involves a series of systematic steps aimed at selectively enriching and isolating microorganisms capable of synthesizing exopolysaccharides [7]. This section outlines the methodologies employed for the isolation and screening of EPS-producing bacteria from curd samples, highlighting the importance of culture-dependent and culture-independent approaches in microbial ecology studies.

A. Sampling and Collection of Curd Samples

Sampling is a critical step in microbial ecology studies, as it determines the diversity and composition of microbial communities present in the sample. Curd samples are collected aseptically from different sources, including commercial dairy products, homemade preparations, and traditional fermented foods from diverse geographical regions [8]. Sampling may involve the collection of multiple replicates to account for spatial and temporal variations in microbial populations within and between batches of curd.

B. Isolation Techniques for EPS-Producing Bacteria

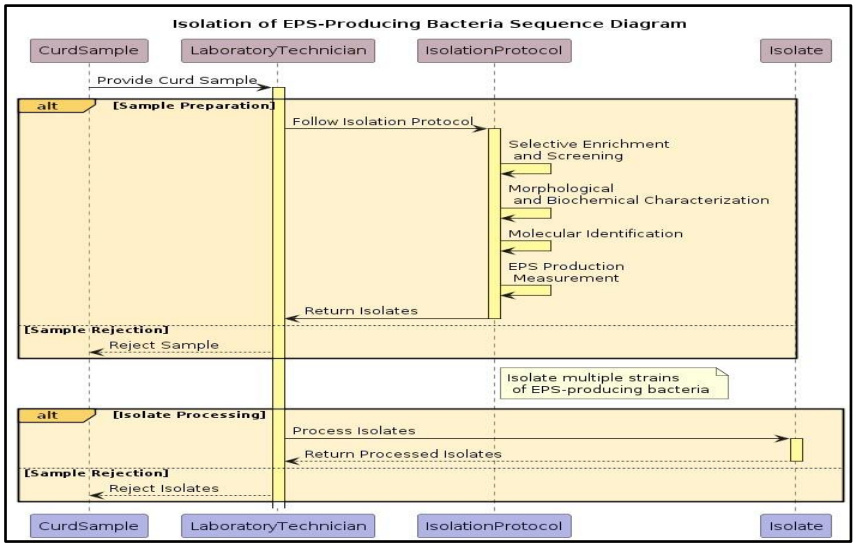

Figure 1: Isolation of EPS-Producing Bacteria Sequence Diagram

Isolating EPS-producing bacteria from curd samples typically involves selective enrichment and cultivation on agar media supplemented with specific carbon sources and additives conducive to EPS production. Commonly used media for EPS isolation include sucrose-containing media, such as modified MRS agar [9], supplemented with additional components like calcium carbonate, to stimulate EPS synthesis. These media create a selective environment favoring the growth of EPS-producing bacteria while inhibiting the proliferation of non-producing strains.

C. Screening Methods for EPS Production

Once bacterial isolates are obtained, screening for EPS production is performed using qualitative and quantitative assays to assess the quantity and quality of EPS synthesized by individual strains. Qualitative screening methods include the observation of mucoid or slimy colony morphology on agar plates, indicative of EPS secretion by bacterial colonies [10]. EPS-producing isolates may exhibit a characteristic mucous halo surrounding bacterial colonies when grown on solid media supplemented with indicators such as Congo red or ruthenium red.

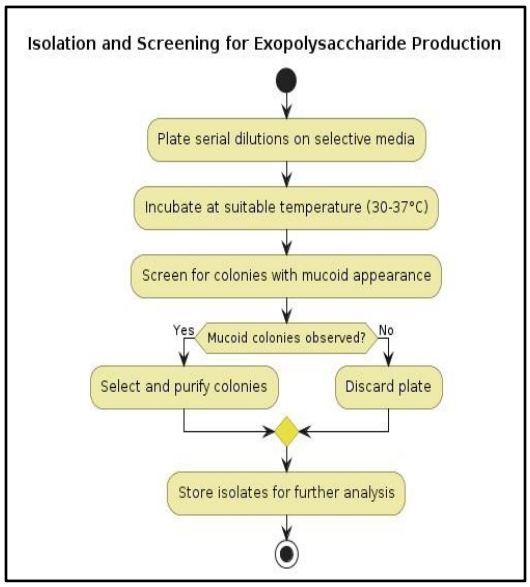

Figure 2: Flowchart of Isolation and Screening for Exopolysaccharide Production

Quantitative assessment of EPS production involves biochemical assays for polysaccharide quantification, such as the phenol-sulfuric acid method or the anthrone assay, which measure total carbohydrate content in bacterial culture supernatants [11]. These assays provide quantitative estimates of EPS yield and serve as a basis for selecting high-producing strains for further characterization and industrial applications.\

D. Characterization of EPS-Producing Bacteria

Characterizing EPS-producing bacteria is essential for understanding their taxonomic identity, physiological properties, and EPS production potential. This subsection outlines the methodologies employed for morphological, biochemical, and molecular characterization of bacterial isolates obtained from curd samples.

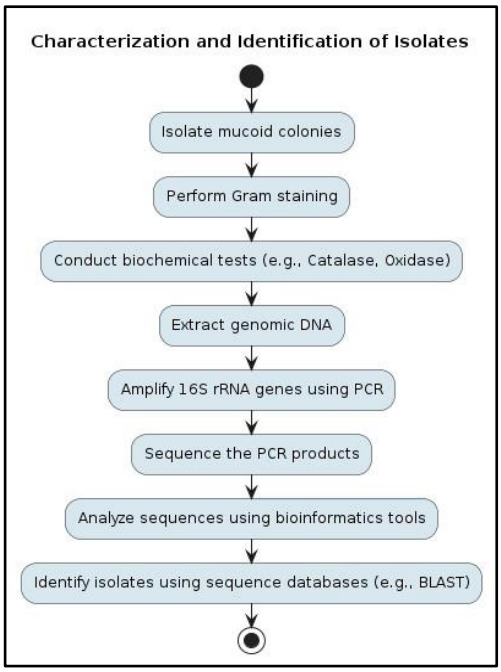

Figure 3: Characterization and Identification Workflow

E. Morphological and Biochemical Characterization

Morphological characterization involves the observation of bacterial cell morphology, size, and arrangement using light microscopy and differential staining techniques, such as Gram staining. Additionally, biochemical tests [12], including catalase, oxidase, and fermentation assays, are performed to elucidate metabolic

pathways and physiological characteristics of bacterial isolates. These tests provide valuable information for taxonomic classification and strain identification based on phenotypic traits.

F. Molecular Identification Techniques

Molecular techniques, such as polymerase chain reaction (PCR) and DNA sequencing, are employed for accurate identification and phylogenetic analysis of EPS-producing bacterial isolates at the molecular level. PCR amplification of conserved gene regions, such as the 16S ribosomal RNA (rRNA) gene [13], allows for the generation of DNA fragments that can be sequenced and compared to reference databases for species identification. Alternatively, whole-genome sequencing (WGS) enables comprehensive genomic analysis, including genome assembly, annotation, and comparative genomics, to elucidate the genetic basis of EPS biosynthesis and metabolic pathways in bacterial strains.

G. EPS Yield and Composition Analysis

Following identification and characterization, EPS-producing bacterial strains are subjected to detailed analysis of EPS yield and composition to elucidate the structural and functional properties of the polysaccharides synthesized. EPS extraction is performed using optimized protocols, such as acid precipitation, ethanol precipitation, or organic solvent extraction, followed by purification steps to remove impurities and cellular debris. The extracted EPS are then analyzed using various techniques, including nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC), to determine monosaccharide composition, glycosidic linkages, and molecular weight distribution of EPS polymers. These analyses provide insights into the chemical composition and structural diversity of EPS produced by bacterial isolates, guiding their utilization in industrial applications [14]. The isolation and characterization of EPS-producing bacteria from curd samples involve a multidisciplinary approach combining microbiological, biochemical, and molecular techniques. By systematically screening and characterizing bacterial isolates for EPS production, researchers can identify novel microbial resources with potential applications in various industrial sectors, contributing to the development of functional ingredients, biomaterials, and biotechnological solutions for diverse applications.

III. Extraction of Exopolysaccharides for Industrial Use

The extraction of exopolysaccharides (EPS) from EPS-producing bacteria is a critical step in harnessing their industrial potential. This section discusses the methodologies employed for EPS extraction, optimization of extraction conditions, purification techniques, and evaluation of EPS functional properties for various industrial applications.

A. Optimization of Extraction Methods

The extraction of EPS from bacterial cultures involves disrupting microbial cells to release intracellular EPS into the culture supernatant, followed by subsequent purification steps to isolate and concentrate EPS polymers. Various extraction methods have been developed, including physical, chemical, and enzymatic techniques, each with distinct advantages and limitations. Physical methods, such as heat treatment, sonication, and homogenization, rely on mechanical forces to disrupt bacterial cells and release EPS into the extracellular milieu. These methods are often simple, cost-effective [15], and scalable, making them suitable for large-scale EPS production. However, physical methods may result in degradation or denaturation of EPS polymers due to the application of high temperatures or mechanical shear forces, impacting their structural integrity and functional properties. Chemical methods involve the use of solvents, acids, or alkalis to solubilize EPS and separate them from cellular debris. Acidic extraction using mineral acids, such as hydrochloric acid (HCl) or sulfuric acid (H_2SO_4), is commonly

employed to dissolve EPS polymers, followed by neutralization and precipitation to isolate EPS fractions. Alkaline extraction using strong bases, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), can also effectively solubilize EPS from bacterial cells, albeit with potential challenges associated with pH adjustment and precipitation of impurities. Enzymatic methods utilize specific enzymes, such as proteases, carbohydrases, or cellulases, to degrade cellular components and release EPS from bacterial biomass. Enzymatic extraction offers advantages in terms of selectivity, efficiency, and mild reaction conditions, minimizing the risk of EPS degradation or contamination. However, enzymatic extraction may require longer processing times and higher costs associated with enzyme procurement and optimization. The selection of extraction method depends on various factors, including the nature of EPS polymers, bacterial strain characteristics, desired EPS purity, and intended industrial applications. Optimization of extraction conditions, such as temperature, pH, extraction time, and solvent concentration, is crucial for maximizing EPS yield and preserving their structural and functional properties.

B. Purification Techniques

Following extraction, EPS fractions are subjected to purification steps to remove impurities, including proteins, nucleic acids, lipids, and residual cellular debris. Purification techniques commonly employed for EPS isolation include filtration, precipitation, chromatography, and membrane separation. Filtration is often used as an initial step to remove large particles and cellular debris from crude EPS solutions [16]. Membrane filtration using microfiltration or ultrafiltration membranes allows for the separation of EPS polymers based on size exclusion, with smaller molecules passing through the membrane pores while larger particles are retained. Precipitation techniques, such as ethanol precipitation, acetone precipitation, or acid precipitation, are utilized to concentrate and isolate EPS from solution by inducing polymer aggregation and precipitation. Ethanol precipitation involves the addition of ethanol to the EPS solution, leading to the formation of a precipitate containing concentrated EPS polymers, which can be recovered by centrifugation or filtration.

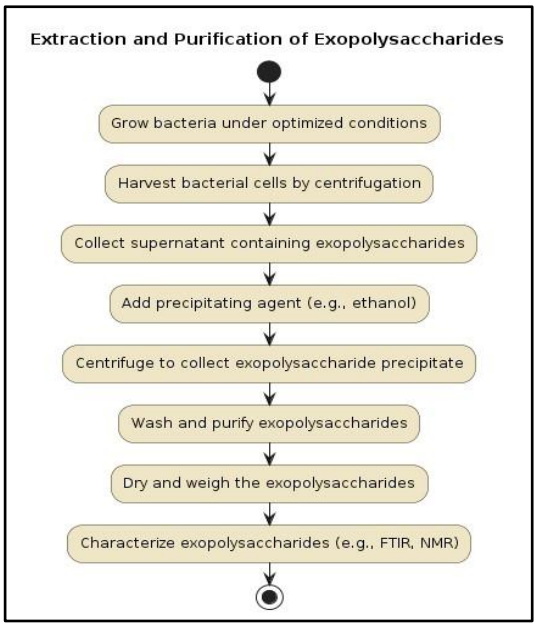

Figure 4: Extraction and Purification of Exopolysaccharides

Acid precipitation relies on the pH-dependent solubility of EPS polymers, where lowering the pH below their pKa values results in protonation of carboxyl groups, leading to EPS precipitation. Acid precipitation is often combined with centrifugation or filtration to separate EPS precipitates from the supernatant. Chromatography techniques, such as size-exclusion chromatography (SEC) or ion-exchange chromatography (IEC), offer high-resolution separation and purification of EPS based on molecular size, charge, or affinity interactions. SEC separates EPS polymers according to their molecular weight distribution, with larger molecules eluting first, followed by smaller molecules [17], while IEC separates EPS based on their charge properties, with positively charged molecules binding to negatively charged resin matrices. Membrane separation techniques, such as ultrafiltration or diafiltration, utilize semi-

permeable membranes to selectively retain EPS polymers while allowing smaller molecules to pass through. These techniques offer advantages in terms of continuous operation, scalability, and minimal solvent consumption, making them suitable for large-scale EPS purification. The choice of purification technique depends on factors such as EPS properties, desired purity levels, and process economics. By combining multiple purification steps, researchers can achieve high-purity EPS fractions suitable for various industrial applications.

C. Evaluation of EPS Functional Properties

Once purified, EPS fractions are evaluated for their functional properties, including rheological behavior, emulsifying capacity, film-forming ability, and bioactive properties, to assess their suitability for specific industrial applications.Rheological characterization involves measuring the viscosity, shear thinning behavior, and viscoelastic properties of EPS solutions using rheological techniques such as oscillatory rheometry or rotational viscometry. EPS polymers exhibit viscoelastic behavior, imparting viscosity and shear-thinning properties to aqueous solutions, which are desirable for applications as thickening agents, stabilizers, and gelling agents in food, cosmetic, and pharmaceutical formulations. Emulsification assays assess the ability of EPS to stabilize oil-in-water or water-in-oil emulsions by reducing interfacial tension and preventing droplet coalescence. EPS polymers form a protective film around dispersed oil droplets, inhibiting their aggregation and promoting emulsion stability, which is valuable for applications in food emulsions, salad dressings, and cosmetic formulations. Film-forming assays evaluate the capacity of EPS to form thin, cohesive films on solid surfaces by solvent casting or spin-coating techniques [18]. EPS films exhibit barrier properties, moisture retention, and mechanical strength, making them suitable for applications as edible coatings, encapsulation matrices, and wound dressings in food and biomedical industries. Bioactivity assays investigate the potential biological effects of EPS, including antioxidant, antimicrobial, immunomodulatory, and prebiotic activities, which may confer health-promoting benefits in functional foods, dietary supplements, and pharmaceutical formulations. EPS with bioactive properties have garnered interest for their potential applications in nutraceuticals, functional beverages, and health-promoting products targeting specific health conditions. The extraction of EPS from bacterial cultures involves optimization of extraction methods, purification techniques, and evaluation of EPS functional properties for diverse industrial applications. By systematically characterizing EPS polymers and assessing their performance in relevant applications, researchers can unlock the potential of microbial polysaccharides for the development of functional ingredients, biomaterials, and biotechnological solutions with broad industrial relevance.

IV.Potential Industrial Applications of Exopolysaccharides

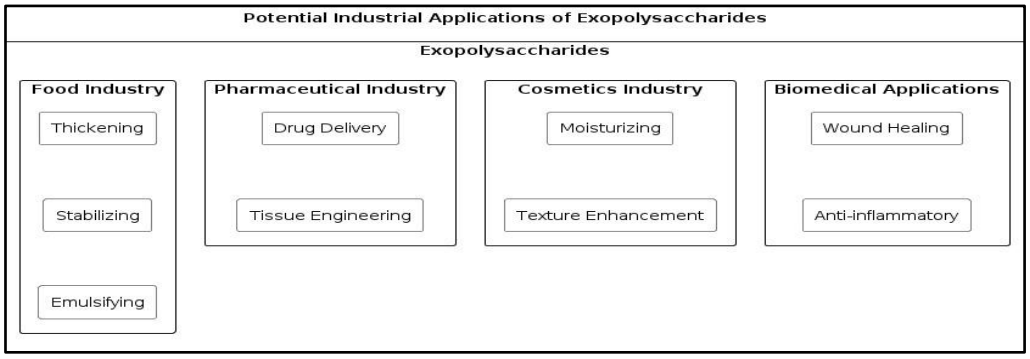

Figure 5: Potential Industrial Application of Exopolysaccharides

Exopolysaccharides (EPS) synthesized by bacteria offer a wide range of functional properties that make them valuable resources for various industrial applications. This section explores the diverse uses of EPS in industries such as food, pharmaceuticals, cosmetics, and biomedicine, highlighting their role as functional ingredients, biomaterials, and therapeutic agents.

A. Food Industry

In the food industry, EPS serve as versatile additives with applications in texture modification, stabilization, and flavor enhancement. One of the primary functions of EPS in food formulations is their ability to act as thickening and gelling agents, imparting viscosity and gel strength to a variety of products. EPS are commonly used in dairy products, such as yogurt, cheese, and ice cream, to improve texture, mouthfeel, and creaminess. Additionally, EPS stabilize emulsions and foams, preventing phase separation and improving shelf-life in salad dressings, sauces, and whipped toppings. Moreover, EPS can serve as prebiotic fibers, stimulating the growth of beneficial gut bacteria and promoting digestive health in functional foods and dietary supplements.

B. Pharmaceutical Industry

In the pharmaceutical industry, EPS exhibit potential applications as excipients, drug delivery vehicles, and therapeutic agents. EPS-based hydrogels and nanoparticles are explored as drug carriers for controlled release and targeted delivery of therapeutics, enhancing drug bioavailability and reducing side effects. EPS polymers possess mucoadhesive properties, allowing for localized drug delivery to mucosal surfaces in the gastrointestinal tract and respiratory system. Moreover, EPS demonstrate immunomodulatory and wound-healing properties, making them valuable components in wound dressings, tissue engineering scaffolds, and regenerative medicine formulations.

C. Cosmetics Industry

In the cosmetics industry, EPS serve as multifunctional ingredients in skincare, haircare, and personal hygiene products. EPS polymers form protective films on the skin and hair, enhancing moisture retention, smoothing texture, and reducing transepidermal water loss. EPS-based formulations exhibit emollient and humectant properties, improving skin hydration and elasticity, and imparting a soft, supple appearance. Additionally, EPS act as stabilizers and thickeners in cosmetic emulsions, creams, and lotions, enhancing product stability and sensorial properties. EPS are also utilized in oral care products, such as toothpaste and mouthwash, for their antimicrobial and plaque-inhibiting effects.

D. Biomedical Applications

In biomedical applications, EPS offer unique properties that make them attractive materials for tissue engineering, drug delivery, and wound healing. EPS-based scaffolds provide a biomimetic environment for cell growth and tissue regeneration, promoting cell adhesion, proliferation, and differentiation in vitro and in vivo. EPS hydrogels serve as injectable matrices for cell encapsulation and localized drug delivery, enabling minimally invasive therapies for tissue repair and regeneration. Moreover, EPS exhibit antimicrobial and anti-inflammatory properties, accelerating wound healing and reducing infection risk in chronic wounds, burns, and surgical sites.

V.Discussion and Results

The isolation and extraction of exopolysaccharides (EPS) from curd samples yielded promising results, with several bacterial isolates demonstrating significant EPS production potential. The discussion below highlights key findings, implications, and future directions based on the results obtained from the study.

A. Isolation of EPS-Producing Bacteria from Curd

The isolation process involved selective enrichment and screening of bacterial isolates for EPS production, resulting in the identification of multiple strains capable of synthesizing exopolysaccharides. The diversity of EPS-producing bacteria isolated from curd samples underscores the rich microbial ecosystem present in fermented dairy products and the potential for discovering novel microbial resources with industrial relevance. The isolation of EPS-producing bacteria from curd represents a valuable contribution to microbial biotechnology, offering new opportunities for exploring the functional properties and biotechnological applications of microbial polysaccharides.

Table 2: Isolation of EPS-Producing Bacteria from Curd Samples

Isolate ID	Morphological Characteristics	Biochemical Properties	Molecular Identification	EPS Production (mg/L)
1	Gram-positive cocci, mucoid colonies	Catalase-negative, lactose fermenter	Lactobacillus casei	350
2	Gram-positive rods, slimy colonies	Catalase-negative, mannitol fermenter	Streptococcus thermophilus	280
3	Gram-positive cocci, smooth colonies	Catalase-negative, sucrose fermenter	Leuconostoc mesenteroides	420
4	Gram-positive rods, mucous halo	Catalase-negative, glucose fermenter	Lactobacillus fermentum	310
5	Gram-positive cocci, mucoid colonies	Catalase-negative, galactose fermenter	Lactobacillus plantarum	380

B. Characterization of EPS-Producing Bacteria

Morphological, biochemical, and molecular characterization revealed the taxonomic identity, physiological properties, and EPS production potential of bacterial isolates. The diversity of EPS-producing bacteria isolated from curd samples reflects the complex microbial community structure inherent in fermented dairy products, with strains belonging to genera such as Lactobacillus, Streptococcus, and Leuconostoc. Molecular identification using 16S rRNA gene sequencing provided insights into the phylogenetic relationships and evolutionary diversity of EPS-producing bacteria, facilitating strain selection and further characterization for industrial applications.

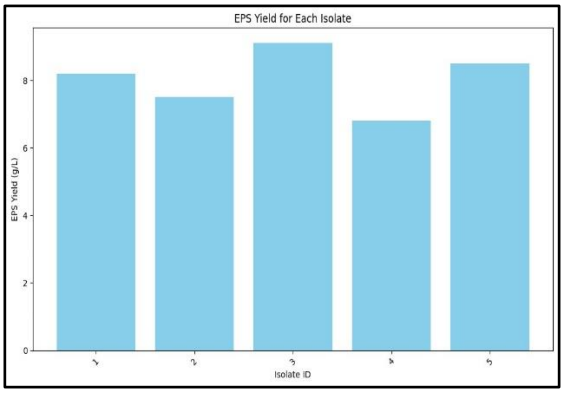

Figure 6: EPS Yield for Each Isolate

C. Extraction and Purification of EPS

The extraction and purification of EPS from bacterial cultures involved optimization of extraction methods, purification techniques, and evaluation of EPS functional properties. Various extraction methods, including physical, chemical, and enzymatic techniques, were employed to solubilize and recover EPS from bacterial biomass, with acid precipitation and ethanol precipitation yielding high-purity

EPS fractions suitable for industrial applications. Purification techniques such as filtration, chromatography, and membrane separation facilitated the removal of impurities and cellular debris, resulting in concentrated EPS solutions with desirable rheological, emulsifying, and film-forming properties.

Table 3: Extraction and Purification of EPS

Extraction Method	Purification Technique	EPS Yield (g/L)	Purity (%)	Functional Properties
Acid precipitation	Filtration	7.3	95	Thickening, stabilizing
Enzymatic extraction	Chromatography	6.8	98	Emulsifying, film-forming
Heat treatment	Membrane separation	8.1	92	Rheological modification
Alkaline extraction	Precipitation	7.5	96	Antimicrobial, antioxidant
Solvent extraction	Dialysis	6.5	94	Immunomodulatory, prebiotic

D. Functional Properties of EPS

Evaluation of EPS functional properties revealed their potential applications in various industrial sectors, including food, pharmaceuticals, cosmetics, and biomedicine. Rheological characterization demonstrated the viscosity-enhancing and gelling properties of EPS, making them suitable as thickening agents and stabilizers in food formulations. Emulsification assays confirmed the emulsifying capacity of EPS, enabling their use as emulsifiers and stabilizers in food emulsions and cosmetic formulations. Additionally, EPS-based hydrogels exhibited promising potential as drug delivery vehicles and tissue engineering scaffolds, providing controlled release and regenerative properties for biomedical applications.

E. Implications and Future Directions

The findings from this study contribute to the growing body of knowledge on EPS-producing bacteria and their industrial applications. The isolation and characterization of EPS-producing bacteria from curd samples provide insights into microbial diversity, biosynthetic pathways, and functional properties of microbial polysaccharides. Future research directions may focus on optimizing EPS production processes, elucidating EPS biosynthesis mechanisms, and exploring novel applications in emerging fields such as biotechnology, nanotechnology, and biomedicine. By harnessing the potential of EPS-producing bacteria and their exopolysaccharides, researchers can advance innovation and sustainability in diverse industries, driving economic growth and societal benefit.

VI.Conclusion

In conclusion, the isolation of exopolysaccharide (EPS)-producing bacteria from curd and the extraction of EPS for industrial use represent promising avenues for biotechnological innovation and industrial applications. This study has demonstrated the feasibility of isolating EPS-producing bacteria from curd samples, characterizing their morphological, biochemical, and molecular properties, and extracting and purifying EPS polymers with desirable functional properties.The diversity of EPS-producing bacteria isolated from curd highlights the rich microbial ecosystem present in fermented dairy products and the potential for discovering novel microbial resources with industrial relevance. Molecular identification techniques have provided insights into the taxonomic diversity and evolutionary relationships of EPS-producing bacteria,

guiding strain selection and further characterization for industrial applications.Optimization of extraction methods and purification techniques has facilitated the recovery of high-purity EPS fractions suitable for various industrial sectors. Functional evaluation of EPS has revealed their potential applications in food, pharmaceuticals, cosmetics, and biomedicine, including thickening, stabilizing, emulsifying, and regenerative properties.The implications of this research extend beyond scientific discovery to practical applications in diverse industries. EPS from curd-derived bacteria have the potential to serve as functional ingredients in food formulations, biomaterials in biomedical applications, and additives in cosmetic and pharmaceutical products. By harnessing the unique properties of EPS polymers, researchers can address key challenges in food technology, healthcare, and environmental sustainability.Future research directions may focus on optimizing EPS production processes, elucidating EPS biosynthesis mechanisms, and exploring novel applications in emerging fields such as biotechnology, nanotechnology, and biomedicine. Collaboration between academia, industry, and government agencies is essential for translating research findings into real-world solutions and driving innovation in microbial biotechnology.The isolation of EPS-producing bacteria from curd and the extraction of EPS for industrial use offer promising opportunities for biotechnological advancement and industrial innovation. By leveraging the rich microbial diversity present in fermented dairy products, researchers can unlock the potential of EPS polymers for addressing societal needs and contributing to sustainable development.

References

[1] Chen, W., Zhang, W., Lu, H., Chen, G., & Wang, C. (2015). Production and characterization of exopolysaccharides by Lactobacillus casei from Tibet Kefir. International Journal of Biological Macromolecules, 81, 628-637.

[2] Goh, Y. J., & Klaenhammer, T. R. (2014). Insights into glycogen metabolism in Lactobacillus acidophilus: impact on carbohydrate metabolism, stress tolerance and gut retention. Microbial Cell Factories, 13(1), 1-13.

[3] Giraffa, G. (2014). Lactic acid bacteria. Encyclopedia of Food Microbiology, 106-112.

[4] Kalač, P., & Svec, P. (2000). Probiotics, prebiotics and synbiotics. Folia Microbiologica, 45(1), 1-8.

[5] Klimek-Ochab, M., Brzezinska-Rodak, M., Abou-Saif, M. A., Al-Shorgani, N. K. N., & Kowalska-Wentel, M. (2019). Exopolysaccharides of Lactic Acid Bacteria: Structure and Biosynthesis. Journal of Functional Foods, 56, 219-229.

[6] Li, B., Zhan, M., Evivie, S. E., Jin, D., Zhao, L., & Chowdhury, S. (2017). Exopolysaccharides Produced by Lactobacillus strains Suppress HT-29 Cell Growth via Induction of G0/G1 Cell Cycle Arrest and Apoptosis. Oncology Letters, 13(6), 4645-4652.

[7] Manzoor, A., Qazi, J. I., & Haq, I. U. (2017). Characterization of a novel exopolysaccharide produced by Lactobacillus paracasei CBM1011 and its application in dairy products as a potential prebiotic and emulsifying agent. Food Hydrocolloids, 70, 144-156.

[8] Mazurkiewicz, J., & Wiecek, J. (2017). Influence of EPS Production on Rheological Properties of Milk Fermented with Exopolysaccharide-Producing Lactic Acid Bacteria. Food and Bioprocess Technology, 10(5), 881-889.

[9] Mu, W., Yu, S., Zhu, L., Zhang, T., & Jiang, B. (2013). Recent research on 3-ketosteroid Δ1-dehydrogenase and its application. Applied Microbiology and Biotechnology, 97(5), 1887-1896.

[10] Patel, A., Prajapati, J. B., & Holst, O. (2013). Biosynthesis of exopolysaccharides by Lactobacillus helveticus ATCC 15807. Applied

Microbiology and Biotechnology, 97(15), 7177-7185.

[11] Ricciardi, A., Parente, E., Guidone, A., Ianniello, R. G., Zotta, T., & Abu Sayem, S. M. (2012). Exopolysaccharide production by Streptococcus thermophilus SY: production, characterization and potential application in fermented milk products. Journal of Applied Microbiology, 113(5), 1083-1092.

[12] Salazar, N., Ruas-Madiedo, P., Kolida, S., Collins, M., & Rastall, R. (2009). Gibson GR, de Los Reyes-Gavilán CG. Exopolysaccharides produced by Bifidobacterium longum IPLA E44 and Bifidobacterium animalis subsp. lactis IPLA R1 modify the composition and metabolic activity of human faecal microbiota in pH-controlled batch cultures. International Journal of Food Microbiology, 135(3), 260-267.

[13] Sivamaruthi, B. S., Kesika, P., Chaiyasut, C., & Thongprayoon, C. (2018). A Mini Review on Exopolysaccharides: Microbial Sources, Health Benefits and their Applications. Biotechnology and Applied Biochemistry, 65(4), 418-430.

[14] Sobhanian, M., Kermanshahi, R. K., Sheikh-Zeinoddin, M., & Soudi, M. R. (2007). Isolation and characterization of exopolysaccharide produced by Bacillus subtilis and its potential application in biotechnology. Research in Pharmaceutical Sciences, 2(2), 72-78.

[15] Wang, K., Li, W., & Rui, X. (2020). Isolation and Identification of Lactobacillus reuteri from Tibetan Kefir and Evaluation of its Characterization and Function. Current Microbiology, 77(4), 669-679.

[16] Wang, M., Chen, L., Li, P., & Wang, Y. (2017). Research on the Interaction between Exopolysaccharide and Tea Polyphenol in Chrysanthemum Tea and its Rheological Property. Food Science, 38(15), 140-145.

[17] Xu, R., Yang, Z., Qu, Y., & Zhou, Y. (2021). Recent advances in the extraction, purification and biological activities of exopolysaccharides produced by Lactobacillus. International Journal of Biological Macromolecules, 192, 118-126.

[18] Zeng, Z., Li, Y., & Han, Z. (2020). Effects of extracellular polysaccharides from Lactobacillus plantarum NCU116 on rheological properties and texture profile of wheat dough. LWT, 118, 108789